

Absorption, Translocation, and Metabolism of Aminocyclopyrachlor in Tall Fescue (*Lolium arundinaceum*)

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Synthetic auxin herbicides are commonly used in forage, pasture, range, and turfgrass settings for dicotyledonous weed control. Aminocyclopyrachlor (AMCP) is a newly developed pyrimidine carboxylic acid with a chemical structure and mode of action similar to the pyridine carboxylic acids—aminopyralid, clopyralid, and picloram. Injury to sensitive dicotyledonous plants has been observed following exposure to monocotyledonous plant material previously treated with pyridine compounds. The absorption, translocation, and metabolism of AMCP has been documented in susceptible broadleaf weeds; however, no information is available, to our knowledge, regarding AMCP fate in tolerant Poaceae, which may serve as the vector for off-target plant injury. Based on this premise, research was conducted to characterize absorption, translocation, and metabolism of AMCP in tall fescue. ^{14}C -AMCP was applied to single tiller tall fescue plant foliage under controlled laboratory conditions at North Carolina State University (Raleigh, NC). Radiation was quantified in leaf wash, treated leaf, foliage, crown, roots, and root exudates at 3, 12, 24, 48, 96, and 192 h after treatment (HAT). ^{14}C -AMCP was rapidly absorbed by tall fescue, reaching 38 and 68% at 3 and 48 HAT, respectively. Translocation of ^{14}C -AMCP was limited to the foliage, which reached maximum translocation (34%) at 96 HAT. Most of the recovered ^{14}C -AMCP remained in the leaf wash, treated leaf, or foliage, whereas minimal radiation was detected in the crown, roots, or root exudates throughout the 192-h period. No AMCP metabolism was observed in tall fescue through the 192 HAT. These data suggest AMCP applied to tall fescue can remain bioavailable, and mishandling treated plant material could result in off-target injury.

Nomenclature: Aminocyclopyrachlor; tall fescue, *Lolium arundinaceum* (Schreb.) S.J. Darbyshire.

Key words: Absorption, metabolism, off-target movement, synthetic auxin, translocation.

Synthetic auxin herbicides are widely used for broadleaf weed control in forage, pasture, range, and turfgrass systems (Senseman 2007). The phenoxyacetic acids 2,4-D and MCPA were commercially released in 1945 and 1946, respectively, making them the first-available selective herbicides used for controlling dicotyledonous weeds in monocotyledonous crops (Cobb and Reade 2010). Pyridine carboxylic acids, such as aminopyralid, clopyralid, and picloram are widely used because of their broad-spectrum weed control at lower use rates compared with alternative phenoxyacetic herbicides (Senseman 2007). These synthetic auxin herbicides mimic the natural plant auxin indole-3-yl-acetic acid (IAA) but are not metabolized rapidly (Grossmann 2009). IAA is found in its highest concentrations within meristematic plant tissue, where it stimulates cell division, differentiation, and plant growth (Cobb and Reade 2010). Once absorbed through the cuticle, synthetic auxin compounds are systemically translocated via the phloem or the xylem or both to the meristematic plant regions, such as the shoots and roots, lending effective control against perennial species (Grossmann 2009). Synthetic auxin injury can be characterized by a loss in apical dominance, leaf cupping, epinastic curvature, and unregulated plant growth (Cobb and Reade 2010). The inactivity of these compounds in monocotyledonous plants is not fully understood but is thought to be due to compartmentalization, metabolism, target site insensitivity, or irreversible sequestration into cell wall constituents (Cobb and Reade 2010; Grossmann 2009).

Aminocyclopyrachlor (AMCP) is a synthetic-auxin herbicide belonging to the pyrimidine carboxylic acid herbicide family with a mode of action and chemical structure similar to pyridine herbicides (Claus et al. 2008; Senseman 2007).

Research indicates AMCP has low mammalian toxicity, minimal volatility potential, and broad-spectrum weed control at low application rates (70 to 315 g ae ha⁻¹) because of foliar and root absorption (Claus et al. 2008; Finkelstein et al. 2008; Strachan et al. 2010). Physicochemical and environmental properties indicate AMCP is highly water soluble ($K_{\text{SP}} = 4,200 \text{ mg L}^{-1}$), possesses minimal affinity to soil organic matter ($K_{\text{oc}} = 28 \text{ ml g}^{-1}$), and is moderately persistent in bare-ground ($t_{1/2} = 72 \text{ to } 128 \text{ d}$) and turfgrass ($t_{1/2} = 37 \text{ to } 103 \text{ d}$) systems (Claus et al. 2008; Finkelstein et al. 2008). AMCP received initial registration for broadleaf weed control in commercial and residential turfgrass in 2010 under the trade name Imprelis (DuPont Crop Protection, Laurel Run Building, Chestnut Run Plaza, Wilmington, DE 19898), and other markets are currently being pursued (Anonymous 2010; Claus et al. 2008; Finkelstein et al. 2008).

Research has characterized the absorption, translocation, and metabolism of phenoxyacetic, pyridine, and pyrimidine carboxylic acids in susceptible broadleaf weeds (Bukun et al. 2009, 2010; Lym and Moxness 1989; Valenzuela-Valenzuela et al. 2001). Leafy spurge (*Euphorbia esula* L.) absorbed 34 and 14% of applied ^{14}C -2,4-D and ^{14}C -picloram, respectively, by 72 h after treatment (HAT) with most of both herbicides remaining as parent compounds 96 HAT (Lym and Moxness 1989). Bukun et al. (2009) reported Canada thistle [*Cirsium arvense* (L.) Scop.] absorbed 72 and 34% ^{14}C -clopyralid and ^{14}C -aminopyralid, respectively, by 24 HAT. In the same study, translocation of ^{14}C -clopyralid and ^{14}C -aminopyralid from the treated leaf was 39 and 17%, respectively, by 192 HAT, with no metabolism reported for either compound (Bukun et al. 2009). Bukun et al. (2010) reported a maximum ^{14}C -AMCP absorption (57%) and translocation (~20%) in Canada thistle by 24 and 192 HAT, respectively, with no metabolism of the AMCP-free acid over the tested time course (Bukun et al. 2010). Interestingly, Valenzuela-Valenzuela et al. (2001) found yellow starthistle

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(*Centaurea solstitialis* L.) rapidly absorbed and metabolized ^{14}C -clopyralid by 2 HAT. These data indicate the absorption, translocation, and metabolism of synthetic auxin herbicides can vary depending on plant species and compound.

Off-target plant injury via livestock manure and contaminated compost has been linked to synthetic auxin compounds remaining in previously treated Poaceae plant material (Blewett et al. 2005; Burkhart and Davitt 2002; Davis et al. 2010; Miltner et al. 2003; Vandervoort et al. 1997). Kates (1965) first documented off-target synthetic auxin injury to cultivated tobacco (*Nicotiana tabacum* L.) from livestock manure, in which mules used for field cultivation had grazed on a right-of-way previously treated with picloram and then defecated in the furrows before transplanting. Recently, aminopyralid injury was reported in gardens fertilized with contaminated manure from livestock that had grazed on previously treated pastures (Davis et al. 2010). Clopyralid gained public attention because off-target plant injury was reported from compost containing previously treated turfgrass clippings (Blewett et al. 2005; Burkhart and Davitt 2002). Miltner et al. (2003) reported a waiting period of greater than 1 yr following a clopyralid application could be necessary for treated turfgrass to be used as compost feedstock. These reports indicate synthetic auxin residues can be persistent in Poaceae following applications and become bioavailable as plant materials decompose.

AMCP has the potential to be widely used for broadleaf weed control in various Poaceae systems as an alternative to currently labeled phenoxyacetic and pyridine herbicides. Particular to turfgrass systems, management practices often require multiple mowing events per week and can result in turfgrass clipping accumulation (Beard 1973). To avoid off-target plant injury, the Imprelis label recommended turfgrass clippings be returned within the treated area during mowing events after application (Anonymous 2010). Because of past issues with synthetic auxin bioavailability, it is critical to understand the fate of AMCP in treated Poaceae material to avoid potential off-target plant injury. To date, no published research, to our knowledge, has determined the fate of AMCP in tolerant monocotyledonous plants. Therefore, the objective of this research was to characterize the absorption, translocation, and metabolism of AMCP in tall fescue.

Materials and Methods

Plant Material. Research was conducted in 2011 at North Carolina State University in Raleigh, NC, to characterize the absorption, translocation, and metabolism of ^{14}C -AMCP in tall fescue. 'Confederate' tall fescue (Wyatt-Quarles Seed, Garner, NC 27529) was seeded in 66-ml containers (RLC4 Pine Ray Leach Cone-tainers, Stuewe & Sons, Inc., Corvallis, OR 97333) (2.5 cm in diameter by 16 cm depth) filled with a sand medium and thinned to 1 plant container⁻¹ following germination. Tall fescue seedlings were placed into a greenhouse at 24/15 C day/night temperatures with a 12-h photoperiod (490 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetic photon flux density at plant height) until establishment. Plants were irrigated once daily and fertilized weekly with a foliar-applied 20–20–20 (N–P–K) water-soluble fertilizer (Scotts-Sierra Horticultural Products Co., Marysville, OH 43041) to provide 1.2 g of N–P₂O₅–K₂O m⁻². Tall fescue plants remained in the greenhouse until reaching the five- to seven-leaf stage (single

tiller) and were then acclimated to a laboratory growth chamber for 3 d before experiment initiation.

Following acclimation, the third leaf from the crown on each tall fescue plant was covered with aluminum foil, and plants were moved outdoors, where the remaining foliage was oversprayed with commercially formulated AMCP (Imprelis) at 79 g ae ha⁻¹ plus nonionic surfactant (NIS; Induce surfactant, Helena Chemical Co., Memphis, TN 38137) at 0.25% v/v using a CO₂-pressurized sprayer boom (Spraying Systems Co., Wheaton, IL 60189) calibrated to deliver 304 L ha⁻¹. Following foliar overspray, plants were immediately returned to the laboratory and five 1- μl droplets of radiolabeled ^{14}C -AMCP with a specific activity of 1487 kBq mg⁻¹ (DuPont) plus NIS at 0.25% v/v were applied to the foil-covered leaf on the adaxial leaf surface for a total of 4.2 kBq plant⁻¹.

Absorption, Translocation, and Root Exudation. Plants were harvested 3, 12, 24, 48, 96, and 192 HAT and separated into treated leaf, treated leaf wash, remaining aboveground foliage, crown, roots, and root exudates. The treated leaf was excised into a 20-ml scintillation vial containing 10 ml of 50 : 50 v/v methanol : deionized water and shaken by hand for 1 min to remove any unabsorbed ^{14}C -AMCP from the leaf surface. Radioactive recovery from the leaf wash was determined by placing a 2-ml aliquot of leaf-wash solution into 15 ml of scintillation fluid (Ultima Gold LLT 6013371; PerkinElmer Life and Analytical Sciences, Inc., Waltham, MA 02451), which was subjected to liquid scintillation spectroscopy (LSS) using a Packard TRI-CARB 2100TR Liquid Scintillation Spectrometer (Packard Instrument Co., Downers Grove, IL 60515) to calculate total absorption relative to the percentage of total-applied radioactivity. Following the leaf wash, the treated leaf was removed from the scintillation vial, and plants were further dissected. All plant material was oven-dried for 48 h at 65 C, weighed, combusted using a biological oxidizer (OX-500 Biological Material Oxidizer, R. J. Harvey Instrument Co., Tappan, NY 10983), and was quantified using LSS. Root exudation was determined by dissecting container cells to remove the intact root system. Sand medium was removed from the roots by suspension into 130 ml of deionized water. Remaining growth media in the container cells were rinsed with 130 ml of methanol into shaker jars (Ball 16 oz canning jar, Jarden Home Brands, Daleville, IN 47334). Root-wash solution was combined with growth-media solution for a total of 260 ml 50 : 50 v/v methanol : deionized water solution. Growth solution was shaken and allowed to settle for 2 min; then, a 2-ml aliquot solution was combined with scintillation fluid for quantifying total ^{14}C radioactivity using LSS. These methods are similar to those used in previously described research evaluating AMCP absorption and translocation in Canada thistle (Bukun et al. 2010).

Experimental design was a randomized complete block in a six by six factorial arrangement (six plant parts by six harvest periods) with three replications and two experimental runs. Percentage of ^{14}C absorption was calculated by total amount of radioactivity applied minus radioactivity recovered in the leaf wash. Translocation was calculated by measuring the total amount of radioactivity in previously mentioned plant samples (other than treated leaf) and dividing by the total amount of radioactivity applied.

Table 1. Interaction of plant part and hours after treatment main effects on percentage of ¹⁴C-aminocyclopyrachlor recovered in tall fescue.^{a,b}

Hours after treatment	% radiation recovered within plant part (% of applied ^c)										Total recovery ^e		
	Leaf wash ^d		Treated leaf		Foliage		Crown		Roots			Root exudates	
3	49	a	25	efg	5	ij	4	ij	0	j	3	j	86
12	38	abc	35	b-e	11	hij	4	ij	0	j	1	j	89
24	37	bcd	41	ab	14	ghi	3	j	0	j	0	j	95
48	32	b-f	35	b-e	21	fgh	2	j	1	j	8	ij	99
96	29	c-f	30	b-f	34	b-e	2	j	1	j	1	j	97
192	26	efg	26	d-g	25	efg	1	j	2	j	3	j	83

^a Pooled analysis over two experimental runs.

^b Means within rows and columns with the same letter (a-j) are not significantly different according to Fisher's Protected LSD ($P < 0.01$).

^c Based on 4.2 kBq of ¹⁴C-aminocyclopyrachlor plant⁻¹.

^d Treated leaf washed in 50 : 50 v/v methanol : deionized water solution.

^e Total recovery based on the sum of radiation recovered in each plant part at the corresponding harvest period.

Metabolism. Only the treated leaf, foliage, and crown were analyzed for ¹⁴C-AMCP metabolism. Plant samples were oven-dried at 60 C for 48 h and were homogenized by placing plant material in a 1.5-ml microsampling vial with 0.1-g of glass beads and 1 ml of 90 : 10 v/v methanol : water plus 0.05% v/v formic acid solution. Plant material was pulverized using a dental capsule mixer (Silamat S5, Ivocar Vivadent, Amherst, NY 14228) at 450 × g for 60 s. Following maceration, homogenized plant material was then centrifuged for 5 min at 17,600 × g and supernatants were transferred to 10-ml auto-sampler vials. The previously mentioned processes were conducted three times per sample to collect sufficient supernatant. Remaining glass beads and pelleted plant material were oxidized, and radiation was quantified by LSS using previously described methods.

Supernatant samples were subjected to high-performance liquid chromatography (HPLC) to determine ¹⁴C-AMCP metabolism. The HPLC system included a gradient pump (Hitachi L-6200A Intelligent Pump, Hitachi, Ltd., Chiyodaku, Tokyo 100-8280, Japan), UV absorbance detector (Waters 486 Tunable Absorbance Detector; Waters Corporation, Milford, MA, 01757), and a C18 column (Adsorbosphere C18 Column; Alltech Associates, Inc., Deerfield, IL 60015) solid-phase extraction cartridge, coupled with a ¹⁴C radiation detector (Radiomatic FLO-ONE Beta flow scintillation analyzer; Packard Instrument). The samples were separated with a gradient, with mobile phases consisting of (1) 99.9 : 0.01 ultrapure water : acetonitrile plus 0.05% formic acid (v/v), and (2) 50 : 50 ultrapure water : acetonitrile plus 0.05% formic acid (v/v). The separation program included 10-min column equilibration with sample 1, followed by a linear gradient elution for 10 min from 100% sample 1 to 100% sample 2, which was then further eluted with sample 2 for 10 min and a column wash with 100% methanol for 10 min. The experimental design was identical to those described previously for absorption and translocation. Chromatograms from extracted samples were compared with those produced from analytical ¹⁴C-AMCP standards to determine whether metabolism had occurred.

Data Analysis. ANOVA was conducted with SAS software (SAS Institute Inc., Cary, NC 27513) using mixed-model methodology (SAS, 2004). Harvest period and plant sample were considered fixed variables in the model. Harvest period by plant sample was evaluated to determine if an interaction between the main effects was present. Replication, experimental run, and their interaction were considered random effects (Carmer et al. 1989). Treatment means were separated

using Fisher's Protected LSD ($P \leq 0.05$). Absorption and translocation means were plotted using nonlinear regression in SigmaPlot (Systat Software Inc., San Jose, CA 95110) to illustrate the effect of harvest period and plant sample on ¹⁴C-AMCP concentrations within tall fescue.

Results and Discussion

Absorption and Translocation. The harvest-period main effect was not significant ($P > 0.05$), indicating ¹⁴C-AMCP levels were homogeneous at all harvest timings. Percentage of ¹⁴C-AMCP recovery at 3, 12, 24, 48, 96, and 192 HAT was 86, 89, 95, 99, 97, and 83%, respectively, when radioactivity from the leaf wash was combined with radioactivity found within each plant part (Table 1). This recovery is well within acceptable levels and comparable to past research examining synthetic auxin absorption and translocation (Bukun et al. 2009, 2010).

The plant-part main effect was significant ($P < 0.01$) for ¹⁴C-AMCP, when pooled over harvest period (Figure 1). Leaf wash, treated leaf, and foliage contained 36, 30, and 19%, respectively, of applied ¹⁴C-AMCP. The crown, roots, and root exudates contained only 3, 1, and 3%, respectively. These results indicate that most of the AMCP can remain unabsorbed on tall fescue leaves or within the aboveground foliage during a 192-h period.

Foliar absorption of ¹⁴C-AMCP was rapid because 38% radioactivity was detected in tall fescue 3 HAT (Figure 2). Maximum absorption (68%) was achieved 48 HAT and remained unchanged through 192 HAT. The rate of absorption observed in tall fescue was similar to that of previous research with AMCP, aminopyralid, and clopyralid in Canada thistle, which reached maximum absorption at 24, 24, and 96 HAT, respectively, and total absorption ranged from 56 to 80% (Bukun et al. 2009, 2010). Unlike foliar absorption, ¹⁴C-AMCP in the leaf wash reached minimum levels by 48 HAT and remained unchanged until 192 HAT (Figure 2). These data illustrate the partitioning of ¹⁴C-AMCP from the leaf surface to absorption within the tall fescue plant because radioactivity collected within the leaf wash and plant material followed inverse regression patterns ($r^2 \geq 0.96$; $P < 0.001$) (Figure 2).

Translocated ¹⁴C-AMCP from the treated leaf attained highest concentration within foliage (34%) 96 HAT, following a nonlinear regression pattern ($r^2 = 0.94$; $P < 0.001$), similar to that observed for total ¹⁴C-AMCP absorption (Figure 3). Bukun et al. (2009, 2010) also reported similar foliar translocation of ¹⁴C-AMCP, ¹⁴C-

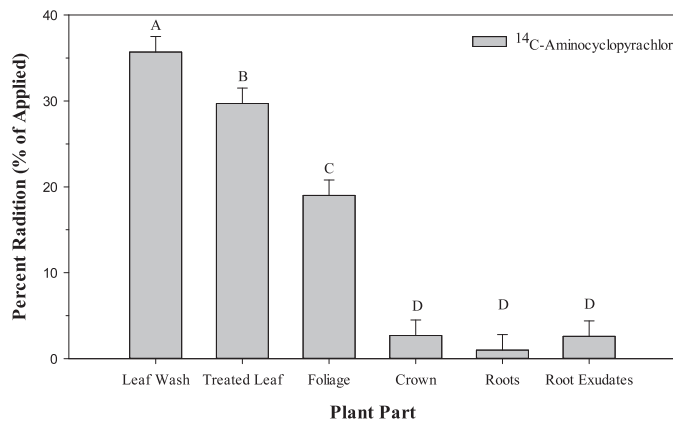


Figure 1. Main effect of plant part on percentage of ^{14}C -aminocyclopyrachlor concentration pooled over a 192-h period.

aminopyralid, and ^{14}C -clopyralid in Canada thistle. Minimal ^{14}C -AMCP translocation was detected in the tall fescue crown, roots, or root exudates during the 192-h period and did not follow a similar regression pattern, compared with foliar translocation. Interestingly, ^{14}C -AMCP detected in root exudates reached an 8% maximum at 48 HAT but was $\leq 3\%$ at all other harvest periods. Past research has also indicated minimal translocation of synthetic auxin herbicides to belowground plant tissue (Bukun et al. 2009, 2010; Lym and Moxness 1989).

A significant interaction between harvest-period and plant-part main effects ($P < 0.01$) was observed (Table 1). In general, ^{14}C -AMCP recoveries were greater in the leaf wash, treated leaf, and foliage than they were in the crown, roots, and root exudates throughout the 192-h period. At 3 HAT, the leaf wash and treated leaf contained 49 and 25% ^{14}C -AMCP, respectively, whereas recovery was $\leq 5\%$ in the foliage, crown, roots, and root exudates. No differences in ^{14}C -AMCP were detected between the leaf wash and the treated leaf at 12 and 24 HAT but radioactivity-recovered rates were greater than within foliage at the same respective times. At 48 HAT, the leaf wash, treated leaf, and foliage

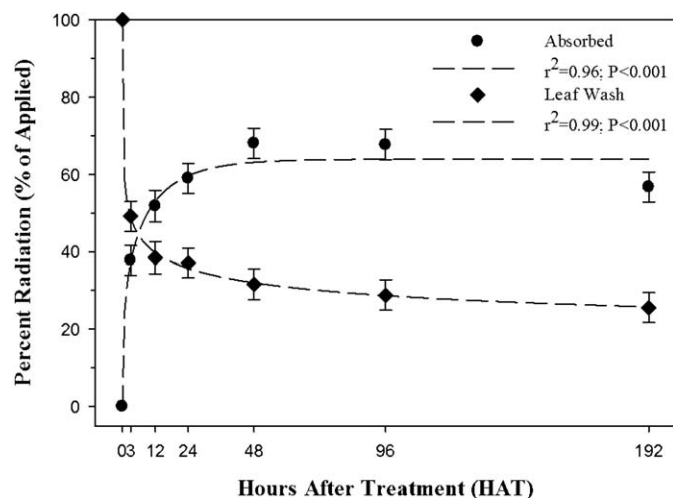


Figure 2. Percentage of ^{14}C -aminocyclopyrachlor absorbed in tall fescue and remaining in the leaf wash during a 192-h period. Data points are means and standard errors. Absorption regression is based on the nonlinear equation: $y = 63.9(1 - e^{-0.7x})^{0.3}$. Leaf wash absorption was based on the nonlinear equation $y = [11.2 + (100 - 11.2)]/[1 + (x/1.1)^{0.3}]$.

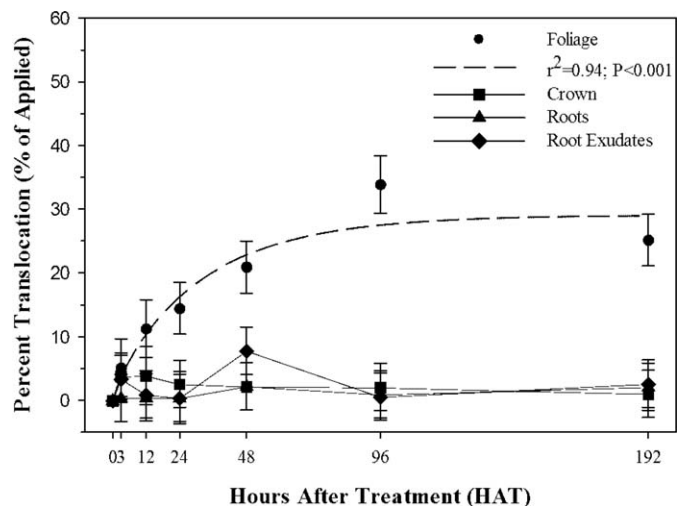


Figure 3. Percentage of ^{14}C -aminocyclopyrachlor translocated in tall fescue plant parts during a 192-h period. Data points are means and standard errors. Foliage regression was based on nonlinear equation $y = 3.5(1 - e^{-3.3x})$.

contained 32, 35, and 21% ^{14}C -AMCP, whereas the remaining 11% of applied ^{14}C -AMCP remained in the crown, roots, and root exudates (2, 1, and 8%, respectively). No differences were apparent between ^{14}C -AMCP concentrations in the leaf wash, treated leaf, and foliage (25 to 34%) at 96 or 192 HAT. It is unclear why minimal ^{14}C -AMCP was found in the crown throughout the study duration because translocation to the other foliage would require movement through the crown. It is speculated that this observation could be due to rapid ^{14}C -AMCP phloem mobility through the tall fescue crown to the other foliage, thereby not concentrating within that plant region.

Metabolism. HPLC analysis determined ^{14}C -AMCP remained intact as the parent compound for the duration of the 192-h period with no degradation. Pelleted plant material contained $\leq 2\%$ of ^{14}C -AMCP, indicating the sample-extraction methods were valid. Similarly, Bukun et al. (2010) and Roten (2011) reported no metabolism of AMCP in Canada thistle or loblolly pine (*Pinus taeda* L.) at 48 HAT. Other research has indicated minimal metabolism of pyridine carboxylic acids in susceptible plants (Bukun et al. 2009; Lym and Moxness, 1989). As previously mentioned, the chemical structure of AMCP is very similar to pyridine herbicides, so the lack of metabolism in tall fescue was expected.

Review of published literature suggests this is the first research characterizing ^{14}C -AMCP absorption, translocation, and metabolism in a tolerant Poaceae. Many synthetic auxin herbicide labels, including AMCP, suggest returning turfgrass clippings, if the turfgrass has been previously treated, to avoid unintended plant injury (Anonymous 2008a,b, 2010). Similarly, in forage, pasture, and rangeland settings, synthetic auxin labeling includes restrictions against composting previously treated plant material and manure from livestock that previously grazed on treated areas (Anonymous 2011a,b). Because of the high solubility, low soil adsorption, and relatively persistent half-life, it is speculated that unabsorbed AMCP could resuspend from tall fescue leaves following irrigation or rainfall events and relocate into the soil profile, where it could become bioavailable (Claus et al. 2008; Finkelstein et al. 2008). Furthermore, these results indicated

the aboveground foliage (including treated leaf) contained considerable ^{14}C -AMCP, and recommended practices of returning previously treated turfgrass clippings to the stand following a mowing event could release the herbicide into the soil as clippings decompose. Because AMCP is foliar and root absorbed, it is probable that tall fescue could reabsorb herbicide from the soil profile and translocate it back to the aboveground foliage. Future research should investigate this proposed “cyclic” AMCP movement within perennial Poaceae systems, such as turfgrass, forage, pasture, and rangeland settings, because that may contribute to the longevity associated with synthetic auxin residues in previously treated plant material.

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