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Physiological Basis for Differential Selectivity of Four Grass Species to Aminocyclopyrachlor

Ethan T. Parker, Glenn R. Wehtje, J. Scott McElroy, Michael L. Flessner, and Peter Panizzi*

Aminocyclopyrachlor (AMCP) is a synthetic auxin herbicide used for broadleaf weed control in pasture and rangeland. The tolerance and fate of AMCP within pertinent grass species is not well understood. Research was conducted to establish the tolerance of four grass species to AMCP application and observe their absorption, translocation, and metabolism. Results indicate that tall fescue is the most tolerant of AMCP at rates required for weed control. Bahiagrass and bermudagrass are marginally tolerant, and cogongrass is the most sensitive. Tall fescue and bahiagrass absorbed more AMCP than bermudagrass and cogongrass, but cogongrass absorption is the most rapid and complete within 2 days after treatment (DAT). Cogongrass and bermudagrass translocated the least amount out of the target area, whereas bahiagrass and tall fescue translocated the most. Radioisotope imaging revealed that tall fescue may sequester absorbed AMCP in leaf tips. This sequestering may be the basis of the greater tolerance to AMCP by tall fescue relative to the other species evaluated. No metabolism of AMCP was detected in any grass species out to 42 DAT.

Nomenclature: Aminocyclopyrachlor; bahiagrass, *Paspalum notatum* Flueggé; bermudagrass, *Cynadon dactylon* (L.) Pers.; cogongrass, *Imperata cylindrica* (L.) Beauv.; tall fescue, *Lolium arundinaceum* (Schreb.) S.J. Darbyshire.

Key words: Absorption, herbicide fate, metabolism, synthetic auxin, translocation.

Synthetic auxin herbicides are widely used in turfgrass, pasture, roadside, and grass cropping systems for selective control of broadleaf weeds. Although broadleaf weeds are typically the targets of synthetic auxin herbicides, many warm-season grasses can be injured by their application (Bell et al. 2000; Cudney et al. 1997; Doroh et al. 2009; Grossmann 2003; Johnson 1995; Kaufman 1955; McElroy et al. 2005; Patton et al. 2010). Synthetic auxins are typically applied for POST weed control and subsequently absorbed by foliage and roots (Bovey et al. 1983; Ross and Lembi 1999; Senseman 2007). Synthetic auxin compounds function by mimicking indole-3-yl-acetic acid (IAA), which stimulates cell division, differentiation, and ultimately plant growth at meristematic plant tissues (Grossmann 2010; Ross et al. 2002). Symptoms of these herbicides include leaf epinasty, uncontrolled root growth, changes in nucleic acid levels, and increased biosynthesis of ethylene (Anderson 1996; Deshpande and Hall 2000; Devine et al. 1993).

Currently, aminocyclopyrachlor (AMCP) is the only pyrimidine carboxylic acid herbicide to be registered and commercialized. It is structurally similar to the pyridine carboxylic acid herbicides, which include picloram, clopyralid, and aminopyralid (Senseman 2007). AMCP is characterized by comparatively low use rates and low mammalian toxicity (Claus et al. 2009; Strachan et al. 2010; Turner et al. 2008; U.S. Environmental Protection Agency [USEPA] 2010). AMCP exhibits the synthetic auxin herbicide mode of action (MOA) (Grossmann 2003; Woodward and Bartel 2005). Between fall 2010 and August 2011, the potassium salt formulation of AMCP was marketed as Imprelis® by E.I. du Pont de Nemours and Company (DuPont) for the selective control of broadleaf weeds in both cool-season and some warm-season turfgrasses. AMCP was labeled for use on lawns, golf courses, sod farms, and athletic fields. AMCP rates for select established warm-season grasses were 52.5 to 79.0 g ae ha^{-1}, whereas established cool-season grasses such as tall fescue were labeled as high as 105.0 g ae ha^{-1} (Anonymous 2010).

Despite previous issues of off-target damage, AMCP has the potential to be widely used for broadleaf weed control in various grass crops as an alternative to currently labeled phenoxyacetic and pyridine herbicides (Anonymous 2012, 2014; Lewis

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*First, second, and third authors: Graduate Research Assistant, Professor, and Professor, Department of Crop, Soil, and Environmental Sciences, Auburn University, 201 Funchess Hall, Auburn University, AL 36849; fourth author: Assistant Professor, Department of Plant Pathology, Physiology, and Weed Science, Virginia Tech, 435 Old Glade Road, Blacksburg, VA 24061; fifth author: Assistant Professor, Harrison School of Pharmacy, Auburn University, 4306 Walker Building, Auburn University, AL 36849. Corresponding author's E-mail: jsm0010@auburn.edu

et al. 2013; Patton et al. 2013). AMCP controls a number of troublesome and invasive weeds with minimal injury to desired grass species (Belcher and Walker 2010; Curtis et al. 2009; Flessner et al. 2011a; Gannon et al. 2009; Jenks and Walter 2012; Mansue and Hart 2010; Montgomery et al. 2009). Some grass species can be injured and exhibit differing levels of tolerance to AMCP application (Anonymous 2010; Belcher and Walker 2010; Brecke et al. 2010; Curtis et al. 2009; Flessner et al. 2011a, 2011b; Harmoney et al. 2012; Kniss and Lyon 2011; Reed et al. 2013). It is possible that AMCP could control some troublesome monocot species within grass crops (Enloe et al. 2012; Reed et al. 2013, Vargas et al. 2014). AMCP is currently being explored for use in pastures, roadsides, and forestry situations alone and in combination with other chemistries (Ezell and Self 2013; Isreal et al. 2013; Meredith et al. 2013; Sellers et al. 2013).

Previous research has revealed that AMCP is readily absorbed in tolerant and susceptible species (Bell et al. 2011; Bukun et al. 2010; Lewis et al. 2013; Lindenmayer et al. 2013, Vargas et al. 2014). Absorption of AMCP in broadleaf species has been classified as rapid, with complete absorption occurring within 24 to 48 h after treatment (HAT) (Bell et al. 2011; Bukun et al. 2010; Lindenmayer et al. 2013). Lewis et al. (2013) reported that absorption of AMCP into tall fescue, a tolerant Poaceae species, was also rapid with maximum absorption at 48 HAT. This study reported that maximum translocation away from the treated leaf occurred within 96 HAT, indicating limited but rapid movement throughout aboveground tissues. Vargas et al. (2014) reported that absorption of AMCP-methyl ester into large crabgrass [*Digitaria sanguinalis* (L.) Scop.], a sensitive Poaceae species, was rapid, with $> 90\%$ absorption at only 1 HAT. Studies indicate that AMCP movement in broadleaf species reached a peak between 24 and 192 HAT, depending on the species (Bell et al. 2011; Bukun et al. 2010; Lindenmayer et al. 2013). Thus, it appears that both susceptible and tolerant species exhibit similar absorption and translocation of AMCP, which did not correlate to susceptibility within broadleaf species (Bell et al. 2011). There is no documentation of AMCP metabolism in either susceptible or tolerant species out to 192 HAT (Bell et al. 2011; Bukun et al. 2010; Lewis et al. 2013; Lindenmayer et al. 2013). This correlates well with the lack of metabolism found with similar pyridine carboxylic acid herbicides (Bukun et al. 2009; Lym and Moxness 1989).

In light of the damage to off-target species, the tolerance of important grass species to AMCP and its fate within these plants must be examined. If AMCP is absorbed by grasses and remains as the active form, there is a possibility that it can be returned to the soil through mowing, grazing, or root exudation (Lewis et al. 2013). If AMCP remains as the active form within the plant where it is protected from microbial degradation and photolysis (USEPA 2010; Finkelstein et al. 2008), it is likely that AMCP activity can be prolonged.

A number of studies on grass tolerance to AMCP have been published. However, none directly compare tolerant and susceptible grass species (Flessner et al. 2011a, 2011b; Kniss and Lyon 2011; Lewis et al. 2013). Our first objective was to compare the tolerance of four grass species to AMCP. The four species were bahiagrass, bermudagrass, cogongrass, and tall fescue. The second objective was to utilize radiotracing techniques to determine the role of absorption, translocation, and metabolism as possible mechanisms of the observed variance in these species.

Materials and Methods

Tolerance Studies. Grasses selected for evaluation were 'Pensacola' bahiagrass (Pennington® Seed), common bermudagrass, cogongrass, and 'Kentucky 31' tall fescue (Pennington® Seed). Experiments use the free-acid formulation of AMCP (aminocyclopyrachlor, DPX-MAT28, E. I. du Pont de Nemours and Company). Experiments used a randomized complete block design with four replications per treatment. Two experiments each were conducted for bahiagrass, bermudagrass, cogongrass, and tall fescue for a total of eight experiments. All experiments were repeated in time and applied on February 3 and March 17, 2014. Herbicide-containing treatments also included a nonionic surfactant (NIS) (Activator 90 Non-Ionic Surfactant, Loveland Products Inc.) at 0.25% v v^{-1}. This adjuvant was included because previous research involving AMCP and other related synthetic auxins indicates it substantially increased herbicide absorption (Bell et al. 2011; Bukun et al. 2009; Lewis et al. 2013). The greenhouse was located in Auburn, AL. Greenhouse temperatures were maintained between 23 and 26 C for the duration of the experiments. Normal sunlight was supplemented with sodium-halide growth lamps producing 150 µmol m^{-2} s^{-1} at the grass canopy.

Total peak irradiance was < 800 µmol m^{-2} s^{-1} throughout the experiments. All grass species were grown simultaneously from seed in separate plastic flats containing a potting substrate (Scott's Miracle-Gro® Potting Mix, The Scott's Company, LLC). Grasses were then prepared for treatment by transplanting five individual plants into 700-cm^3 pots with the use of Marvyn loamy sand (native) soil. Grasses were allowed to resume active growth for 2 wk prior to treatment application. Grasses were maintained with weekly fertilizer applications (Miracle-Gro® All Purpose Plant Food, The Scott's Company, LLC), daily irrigation, and monthly insecticide applications. AMCP rates were 52.5 and 105.0 g ae ha^{-1}, and a nontreated check was included. These rates were based on the lowest and highest labeled AMCP rates for effective weed control (Anonymous 2010). Treatments were applied at a spray volume of 187 L ha^{-1} in an enclosed spray chamber with a single nozzle (TeeJet® TP8002EVS, Spraying Systems Co.).

Visual ratings of each grass species were taken at 10 and 20 d after treatment (DAT) and were chosen to bracket a typical synthetic auxin rating date of 2 wk after treatment (WAT) (Sciumbato et al. 2004). Ratings were not extended beyond 20 DAT because previous research has indicated that tall fescue is tolerant of AMCP, and the other species studied are injured, but recover from AMCP applications (Anonymous 2010; Enloe et al. 2012; Flessner et al. 2011b; Montgomery et al. 2009). It should also be noted that because absorption and translocation occurs rapidly (< 8 DAT), plant injury within that time was of primary interest. Visual injury ratings were based on a scale of 0 to 100, with 100 corresponding to dead plants and 0 indicating healthy plants. All plants were clipped to a uniform height of approximately 7.5 cm following the 10 DAT rating date to simulate mowing and allow for measurement of regrowth. Plant heights and both fresh and dry weights were then taken at 20 DAT. Plant foliage was then oven dried at 85 C for 1 wk and weighed. All species were analyzed together. Experimental units consisted of a single pot.

AMCP Fate Studies. The free-acid formulation of ^{14}C-AMCP (aminocyclopyrachlor DPX-MAT28) with a specific activity of 1522 kilobecquerels (kBq) mg^{-1} was used in both experiments. Greenhouse conditions were maintained as in previously mentioned studies. Grasses were grown from seed in plastic flats containing a potting substrate (Scott's Miracle-Gro® Potting Mix). Plants were prepared for treatment by transplanting single plants into 66-cm^3-cell pack pots with the use of Marvyn loamy sand (native) soil. Grasses were allowed to resume active growth for 2 wk prior to treatment. Grasses were maintained by subirrigation to prevent removal of any adsorbed herbicide from the leaf surface. AMCP at 52.5 g ha^{-1} was used for all studies (Anonymous 2010). All herbicide-containing treatments included a NIS as previously described.

Absorption and Translocation. Ten microliters of ^{14}C-AMCP was applied to the adaxial side of a fully mature leaf for a total of 1.67 kBq plant^{-1}. The herbicide was applied beginning at one end of the marked treated area on the leaf then extending the solution across the treated area. This created a thin film that increased surface area contact, allowed for rapid drying, and reduced the chance of losing droplets to runoff. The physiology of the AMCP was of primary interest, therefore no broadcast application was applied prior to treatment with the radiolabeled AMCP. Lack of broadcast application allowed the detection of movement absent the confounding effects produced by herbicide injury. For each species, four individual plants were harvested at 1, 2, 4, and 8 d after treatment (DAT) then divided into the following sections: leaf wash, target area (a 2-cm leaf section to which the herbicide had been directly applied), treated leaf, remainder foliage, and crown plus roots. The leaf wash was obtained by placing the target area in a 20-ml plastic vial containing 1 ml of a 90 : 10 v v^{-1} methanol : deionized water solution and agitating it for 30 s to remove adsorbed ^{14}C-AMCP. The leaf wash was combined with 15 ml of Universol$^{\text{TM}}$ fluid (MP Biomedicals, LLC) after harvest. Radioactivity of the leaf wash fluid was quantified via liquid scintillation spectroscopy (LSS) (Beckman Coulter$^{\text{TM}}$ LS-6500 Multipurpose Scintillation Counter, Beckman Coulter, Inc.). This allowed quantification of unabsorbed herbicide relative to the amount applied. Upon removing the washed target area from the scintillation vial, the remainder of the plant was dissected as indicated above. Plant material was oven dried at a temperature of 85 C, combusted with the use of a tissue oxidizer (OX501 Biological Oxidizer, R.J. Harvey Instrument Corporation), and quantified via LSS.

Percentage of ^{14}C-AMCP absorption was calculated by summing the total amount of radioactivity recovered in harvested plant parts minus the leaf wash. Translocation was calculated by dividing the radioactivity recovered in each plant part by the total radioactivity absorbed. Percent recovery was calculated as total radiation recovered across all plant parts and the leaf wash divided by total radiation applied. Radiation in the form of percent recovered of applied was the response variable for both absorption and translocation studies.

Metabolism. Two applications of 10 µl of ^{14}C-AMCP totaling 3.34 kBq plant^{-1} were applied to separate leaves in order to increase the likelihood of detecting potential metabolism. Only the target areas and remainder of aboveground foliage were analyzed for metabolism. Plants were harvested at 7, 14, 21, and 42 DAT to examine the possibility that persistence of the parent compound could lend itself to gradual metabolism by select grass species. Immediately after harvest, plant sections were placed in 7 by 12–cm plastic bags and stored at −20 C until processing. Root material was placed in aluminum trays for oxidizing and subsequent ^{14}C quantification for recovery calculation. Plant parts were ground with a mortar and pestle with liquid nitrogen. Ground material was next moved into a glass grinding tube with 3 ml of methanol in order to improve extraction. Ground material was then placed into 14-ml glass tubes and centrifuged at 2000 × *g* for 10 min. Supernatants were decanted into 20-ml scintillation vials and concentrated with the use of a nitrogen evaporator (N-EVAP^{TM}122, OA-SYS^{TM}, Organomation Associates, Inc.). The concentrated solution was immediately re-eluted in 1 ml of methanol for spotting onto silica gel chromatography plates (Silica gel G 20 by 20 cm TLC plates, Analtech, Inc.). The resulting pellet from centrifuging was oxidized along with harvested roots and the dried remainder of the 1 ml concentrated solution for recovery calculation.

Analysis of possible AMCP metabolites was done by normal-phase thin-layer chromatography (TLC). Silica gel plates were used as the stationary phase, and a solvent system of methanol, isopropanol, ethyl acetate, and acetic acid (7 : 1 : 1 : 1 v v^{-1}) was used as the mobile phase (Bell et al. 2011). Plates were manually scored into 19 individual 1-cm lanes, and a 100-µl aliquot of concentrated solution was applied 3 cm above the bottom of the plate, leaving a blank lane between each replicate. The area of application was maintained to ∼10 mm by successive spotting and drying. The first lane of each plate was left blank to use as a background. A 10-µl drop of ^{14}C-AMCP was spotted on the next lane for metabolite comparison. Plates were developed for approximately 75 min or until the mobile phase had moved 150 mm above the origin, then allowed to air dry prior to scanning.

Radioactive positions, percentages, and corresponding retardation factor (R_f) value of the parent compound and possible metabolites were determined by scanning TLC plates with the use of radio-chromatogram scanner (AR-2000 imaging scanner, Bioscan, Inc.). Radioactive trace peaks were manually integrated. Peaks below 5% of total radioactivity were rejected. Peaks were identified by comparing their R_f values with those from the corresponding ^{14}C-AMCP standard. Data collected consisted of the percentage of the parent herbicide, the percentage of all metabolites detected that were more polar than the parent herbicide, and the percentage of all metabolites that were less polar than the parent herbicide (Bell et al. 2011).

Radioisotope Imaging. To confirm the biodistribution of radiolabeled material within plants visually, radioisotope images (RI) were taken with the use of a multipurpose image analyzer (Fujifilm FLA-5100, Fujifilm Holdings Corporation). Plants used for imaging were treated identically to those in absorption and translocation experiments prior to harvest. Upon harvest, plant roots were washed and entire intact plants were manually pressed between layers of newspaper, and dried at 85 C for 2 wk. Plants were removed and glued onto 8 by 10–cm cardstock mounts. Mounts were covered in plastic wrap then placed in exposure cassettes (Amersham Biosciences) and pressed against phosphor imaging plates for approximately 24 h, then scanned with the use of the image analyzer, in a procedure described in depth by Wehtje et al. (2007).

Experimental Design and Data Analysis. Separate experiments were conducted to determine the absorption and translocation, and the metabolism of AMCP within four grass species. Each experiment was repeated in time between February 3 and March 17, 2014. A randomized complete block design in a five by four factorial arrangement (five plant parts across four harvest intervals) was used for absorption and translocation studies, and a three by four factorial (three plant parts across four harvest intervals) was used for metabolism studies. All studies had four replications and two experimental runs.

Data from tolerance studies were subjected to ANOVA in SAS (SAS® Institute Inc., v. 9.2) with PROC GLIMMIX to test for significance of replication-in-time, herbicide rate, and grass species, and allow for lack-of-fit testing. Experimental run

Table 1. Response of four graminaceous species to foliar-applied aminocyclopyrachlor at two rates

Grass species	Foliar height reduction[a,b]	Foliar weight reduction	Visual injury[c] 10 DAT[d]	Visual injury[c] 20 DAT
	%			
$52.5 \text{ g ae ha}^{-1}$				
Bahiagrass	17 a	59 c	7 bc	21 b
Bermudagrass	17 a	28 b	3 b	1 c
Cogongrass	67 b	85 d	13 c	72 a
Tall fescue	6 a	5 a	0 a	0 c
Mean	27 A	44 A	6 B	24 B
$105.0 \text{ g ae ha}^{-1}$				
Bahiagrass	44 b	65 bc	20 a	56 b
Bermudagrass	37 b	56 b	6 b	17 c
Cogongrass	66 c	85 c	22 a	73 a
Tall fescue	14 a	(+) 5 a	3 b	3 d
Mean	40 B	50 A	13 A	37 A

[a] Means within a common rate and column followed by similar letters are not significantly different according to Fisher's protected $LSD_{(0.05)}$ comparison. Rate main effect means within a column followed by different upper-case letters are statistically different according to $LSD_{(0.05)}$ comparison.

[b] Reduction relative to appropriate nontreated control.

[c] Visual injury rated on a scale of 0 to 100 with 0 equivalent to the nontreated control, and 100 corresponding to death.

[d] Abbreviation: DAT, days after treatment.

and replication were considered random effects with treatment, species, and harvest interval being considered fixed effects. All visual rating data were transformed for statistical analysis with the use of square-root transformation, then backtransformed for presentation. Means were separated with the use of Fisher's protected least significant difference (LSD) and considered significant if $P \leq 0.05$. Data from fate studies were analyzed with the use of PROC GLIMMIX and multivariate analysis techniques to test for significance of replication-in-time, DAT, plant part, grass species, and allow for lack-of-fit testing. Means of fate studies were separated with the use of Duncan's multiple range test at the 0.05 level.

Results and Discussion

Tolerance Studies. ANOVA revealed that repetition in time and replications within the experiment were not significant. Therefore data were pooled accordingly. An interaction between treatment and species was significant ($P < 0.005$) for height and weight reduction data, whereas a three-way interaction of DAT, treatment, and species was significant ($P < 0.005$) for visual-injury data; this indicates that injury to most species increases with time. Cogongrass was clearly the most sensitive species, as indicated by all four response variables collected (Table 1). Visual injury symptoms on cogongrass included stunting of growth and reddening and weakening of crown tissues, followed by necrosis of plant foliage. Conversely, tall fescue was the most tolerant species, with only temporary leaf epinasty regardless of rate. The tolerance of tall fescue is in agreement with previous research (Anonymous 2010; Lewis et al. 2013). Bahiagrass and bermudagrass were intermediate to these two extremes; but between these two grasses, bahiagrass was more sensitive than bermudagrass. Bahiagrass was significantly more sensitive at the high rate than the low rate, and was injured at an unacceptable level for use in either turfgrass or forage systems (Johnson 1995; Johnson and Murphy 1995). Symptoms of bahiagrass injury were brittle crowns, reddening of stems, leaf epinasty, and in some cases complete plant necrosis. Like bahiagrass, bermudagrass was more sensitive to the high rate, whereas the low rate had little effect. In time, AMCP had an effect on bermudagrass growth resulting in significant swelling at nodes and leaf epinasty. These symptoms, however, did not prove fatal to treated plants or compromise grass quality. Ranking sensitivity (highest to lowest) when all four response variables are taken collectively is as follows: cogongrass > bahiagrass > bermudagrass > tall fescue. The differences in sensitivity are sufficiently great that AMCP may have potential for the selective control of cogongrass within other grass species.

As previously mentioned, it has been documented that tall fescue is tolerant of AMCP application. Our results agree with previous research, as well as the general trend that warm-season grasses are more sensitive to synthetic auxin herbicides than cool-

Table 2. Absorption of foliar-applied aminocyclopyrachlor at 1, 2, 4, and 8 d after foliar application by selected grass species. Greenhouse experiment repeated in time and data pooled.[a]

Grass species	1 DAT[b]	2 DAT	4 DAT	8 DAT
	% of applied			
Bahiagrass	16 a	28 a	29 a	45 a
Bermudagrass	21 a	22 a	33 a	38 ab
Cogongrass	24 a	25 a	24 a	25 b
Tall fescue	25 a	33 a	42 a	46 a

[a] Means within a column followed by the same letter are similar according to Duncan's multiple range test at the 0.05 level.

[b] Abbreviation: DAT, days after treatment.

season grasses (Bell et al. 2000; Flessner et al. 2009, 2011b; Lewis et al. 2013; Senseman 2007; Wehtje 2008). Treatment data indicate the expected outcome that greater rates of AMCP application result in impaired plant growth across sensitive species.

Absorption and Translocation. ANOVA revealed that repetition in time and replications within the experiment were not significant. Therefore data were pooled accordingly. Absorption of foliar-applied AMCP was sufficiently variable at both the 1 and 2 DAT harvests such that neither meaningful differences of absorption over time, nor differences between the grass species were evident (Table 2). All species absorbed greater than 30% of the total uptake within 24 HAT. This rapid absorption is consistent with past research (Bell et al. 2011; Bukun et al. 2010; Lewis et al. 2013), as was total percent recovery, which averaged 89% across all four species and harvest intervals. At 4 DAT, the amount of applied AMCP that had been absorbed ranged from 24% with cogongrass to 42% in tall fescue. However, no differences between the species could be detected ($P > 0.05$). At 8 DAT, amount absorbed ranged from 25% (cogongrass), to at least 45% (tall fescue and bahiagrass), a difference that was statistically significant. Absorption by bermudagrass was intermediate to these extremes. The most sensitive of all species tested, cogongrass, absorbed the least ^{14}C-AMCP, likely because of rapid uptake and desiccation of plant tissues, hindering further absorption. Across all species, no more than half of the applied AMCP was absorbed. Consequently, the overall highest-to-lowest ranking of absorption for foliar-applied AMCP is as follows: tall fescue ~ bahiagrass > bermudagrass > cogongrass. This ranking is nearly the opposite of the ranking of overall of sensitivity presented above. Therefore, differential absorption is not likely a contributing factor in differential response between the four grass species.

Table 3. Distribution of absorbed aminocyclopyrachlor over time by four grass species after foliar application. Greenhouse experiment, repeated in time and data pooled.[a]

Tissue of grass plant	Bahiagrass	Bermudagrass	Cogongrass	Tall fescue
	% of absorbed			
1 DAT[b]				
Target area	41 a	60 a	28 a	40 a
Remainder of treated leaf	35 a	10 bc	29 a	36 a
All remaining foliage	18 ab	29 b	21 a	20 ab
Crown and roots	5 b	0 c	21 a	3 b
2 DAT				
Target area	21 a	40 a	27 a	29 ab
Remainder of treated leaf	28 a	50 a	26 a	50 a
All remaining foliage	30 a	9 b	26 a	18 b
Crown and roots	20 a	0 b	20 a	2 b
4 DAT				
Target area	15 a	23 cb	30 a	28 a
Remainder of treated leaf	40 a	40 a	20 a	42 a
All remaining foliage	30 a	27 ab	28 a	26 a
Crown and roots	16 a	9 c	21 a	3 b
8 DAT				
Target area	15 b	14 b	29 a	26 b
Remainder of treated leaf	23 ab	65 a	28 a	53 a
All remaining foliage	37 a	17 b	26 a	19 b
Crown and roots	25 ab	4 b	17 a	2 c

[a] Means within a column of a common exposure time and followed by the same letter are similar according to Duncan's multiple range test at the 0.05 level.

[b] Abbreviation: DAT, days after treatment.

For translocation data, radioactivity recovered within the tissue samples harvested were normalized to 100, and presented as a percentage of the total amount absorbed (Table 3). Translocation varied considerably between the species. Extensive translocation occurred in cogongrass. The percentage of the foliar-absorbed AMCP that was detected in all four tissue samples were equivalent at both the 4 and 8 DAT harvests (Table 3). Cogongrass crown and root tissue had 21 and 17% of the amount absorbed at 4 and 8 DAT, respectively. Therefore foliar-absorbed AMCP had been readily translocated basipetally out of the treated leaf and into other tissues including the crown and root. Crafts (1961) stated that translocation of foliar-absorbed 2,4-D to all meristem tissues in the plants, particularly the roots, was required for treated plant death. Thus the translocation pattern exhibited by cogongrass is in agreement with its comparative sensitivity to AMCP. Translocation of foliar-absorbed AMCP was completely different in tall fescue compared to what was observed in cogongrass. No more than 3% of absorbed AMCP was recovered in the crown and root tissue in tall fescue at both harvest intervals. This percentage was significantly less than the amount recovered in the foliar portions of the plant. Furthermore, the "remainder of treated leaf" sample consistently (i.e., both 4 and 8 DAT) had the highest numerical percentage, and statistically the highest percentage (8 DAT only) recovered. Thus, in tall fescue, the propensity for absorbed AMCP to remain in the treated leaf indicated either minimal translocation, or at least a translocation pattern that precludes basipetal movement and contact with meristem tissues.

Translocation in bahiagrass and bermudagrass are intermediate to the extremes exhibited by cogongrass and tall fescue. Between bahiagrass and bermudagrass, more basipetal translocation occurred in bahiagrass. In bahiagrass the percentage recovered in the crown and root tissues was also statistically equivalent to the other three tissues samples collected. However, at 8 DAT recovery of radioactivity in the target area was less compared to the other tissue samples. The overall highest-to-lowest ranking in translocation of foliar-absorbed AMCP is a follows: cogongrass > bahiagrass > bermudagrass > tall fescue. This ranking is in agreement the overall sensitivity of the grass species to foliar-applied AMCP, indicating that differential translocation is a possible mechanism of selectivity between these species.

Data indicate that the majority of all AMCP applied to grass species, whether tolerant or susceptible, remains on the leaf surface or within aboveground tissues. Herbicide remaining within aboveground tissues may be moved off target by mowing or grazing. Results are similar to previous reports involving many other synthetic auxin herbicides, which indicate that these herbicides may be released from treated tissues in quantities large enough to elicit plant injury (Branham and Lickfeldt 1997; Miltner et al. 2003).

It is clear that all species absorb and translocate ^{14}C-AMCP in amounts sufficient to elicit injury (Rensburg and Breeze 1990; Strachan et al. 2011). Although AMCP is moved to the crown and roots of susceptible species, it is unlikely to result in prolonged control of either cogongrass (Enloe et al. 2012) or bahiagrass (Anonymous 2010) in turf, pasture, or forestry systems.

Metabolism. TLC analysis revealed no metabolites of ^{14}C-AMCP out to 42 DAT across all four species tested. Comparisons of means determined that all peaks detected from treated plants had an R_f value not significantly different from the AMCP-only check (data not shown). Percent recovery in metabolism studies averaged 86% across species and harvest intervals. This recovery is within an acceptable range, considering losses due to sample harvesting and processing, and is consistent with previous research involving synthetic auxin absorption and fate (Bukun et al. 2009, 2010; Lewis et al. 2013). The absence of metabolism in our studies is similar to other findings regarding AMCP and synthetic auxin metabolism within plants. Previous research involving AMCP metabolism in field bindweed (*Convolvulus arvensis* L.), prickly lettuce (*Lactuca serriola* L.), rush skeletonweed (*Chondrilla juncea* L.), and yellow starthistle (*Centaurea solstitialis* L.) found no metabolites out to 8 DAT (Bell et al. 2011; Lindenmayer et al. 2013). Grossman and Kwiatkowski (2000) found that ~90% of quinclorac, another synthetic auxin, remained as the parent compound within treated grasses. They concluded that differences in susceptibility were not a result of this limited metabolism. Alternatively, dicamba was found to be rapidly metabolized in both wheat (*Triticum aestivum* L.) and bluegrass (*Poa* spp.) (Broadhurst et al. 1966). Studies reported that at 2 DAT, only 31% of dicamba remained as the parent material and that by 10 DAT, over 83% of dicamba was present as primary or secondary metabolites. The previous body of

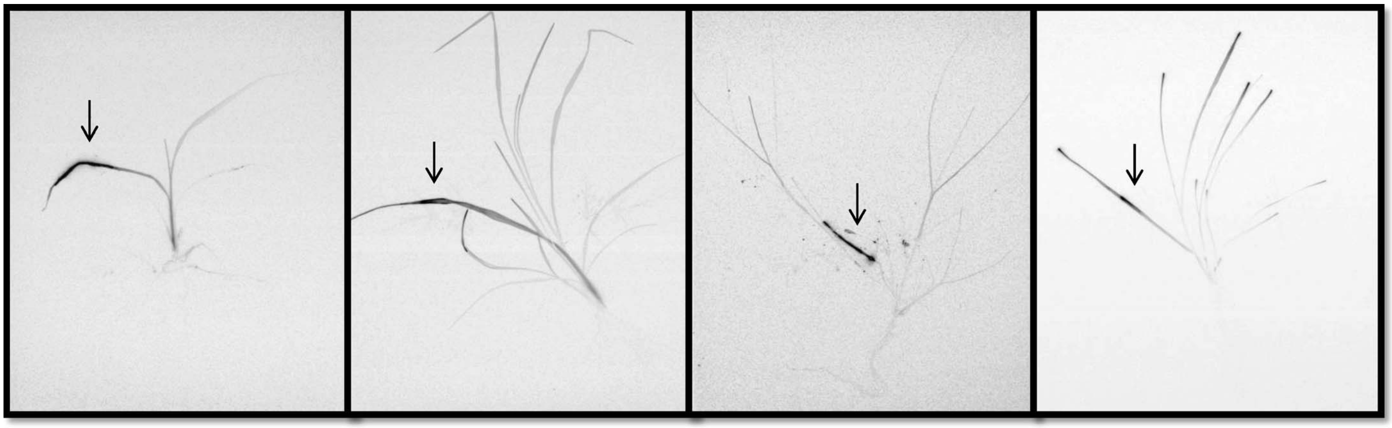

Figure 1. Images of four grass species treated with foliar-applied ^{14}C-aminocyclopyrachlor at 8 d after treatment. Arrows indicate the location at which the 10 µl of herbicide was applied. Images from left to right: cogongrass, bahiagrass, bermudagrass, and tall fescue.

research shows variable amounts of metabolism across synthetic auxins, but very limited or no metabolism of both pyridine and pyrimidine carboxylic acid herbicides (Bukun et al. 2009; Lewis et al. 2013; Lym and Moxness 1989).

Radioisotope Imaging. Radioisotope images (Figure 1) correlate well with absorption and translocation data. Both sets of data indicate that the majority of applied ^{14}C-AMCP remained in the target area and treated leaf for the duration of the experiment. It is interesting to note that tall fescue appears to be sequestering the radiolabeled material in leaf tips. Sequestering to this degree is not evident in the other three species. This could be a possible method that tolerant species, namely some grasses, use to protect vital meristematic tissues such as the crown and roots. The collection of ^{14}C-AMCP in leaf tips supports the argument that AMCP will likely experience "cyclic" movement in grass systems as treated material is mowed or grazed, returned to the soil profile, and then reabsorbed by plants. Future research may be in order to explore the role this sequestration of AMCP plays in plant tolerance and the ultimate fate of AMCP within grass weed control systems.

Our results indicate that the differential sensitivity of these four grass species to foliar-applied AMCP appears to be primarily related to differential translocation. Specifically, the tolerance of tall fescue is likely the result of minimal (less than 3% of applied AMCP) translocation. Furthermore, the limited translocation that does occur serves to transport the herbicide to leaf tips and away from herbicide-sensitive meristem tissues. Conversely, results indicate that neither the amount of absorption nor metabolism contribute to tolerance. It is interesting to note that the tolerance to foliar-applied AMCP appears to be the result of limited translocation in conjunction with translocation that serves to isolate the herbicide away from meristematic tissues. This effect was most clearly evident in tall fescue. Tall fescue is a C_3 species; the other three grasses are C_4 species. Leaves of C_4 species have a higher CO_2 exchange rate, a higher ratio of cross-sectional phloem area to leaf area, and greater translocation rates relative to C_3 species (Gardner et al. 1985). Leaves of C_4 species also export a larger percentage of their assimilate within a few hours than C_3 species. This improved export of assimilate by leaves of C_4 species may be because of their specialized anatomy, in which vascular sheath cells have chloroplasts, or the result of a greater cross-sectional phloem area. Our conjecture is that these differences may also inadvertently allow for greater symplastic and apoplastic translocation of foliar-absorbed AMCP, increasing herbicide–meristem contact, and thus increasing phytotoxicity. Absence of AMCP metabolism paired with a potentially long soil half-life and low soil sorption (Conklin and Lym 2013; Oliveira et al. 2011) is a double-edged sword. Prolonged exposure to AMCP may offer extended control of hard-to-eliminate weeds, such as cogongrass, with repeated applications, but there is also risk involved when a chemical remains in the soil profile for extended periods of time. Prolonged exposure to AMPC by bahiagrass will likely result in thinning of the stand and extensive plant injury. Because the majority of ^{14}C-AMCP remains in aboveground foliage as the parent compound, it is likely that AMCP will be returned to the soil profile in a cyclic manner, as proposed by Lewis et al. (2013). It has also been reported that AMCP exudes from grass roots (Lewis et al. 2013; Parker et al. 2014).

Altogether, these observations indicate that future AMCP use will be limited in some weed control systems, and highly effective in others. Applications near sensitive species should be avoided, as AMCP may be released deep into the soil profile via decaying roots or exudation from healthy tissues. In the future, the role of auxin binding proteins and their possible mutations should be explored as an additional mechanism of the observed tolerance of tall fescue to AMCP (Grossmann 2010).

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