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Biokinetics and Efficacy of Aminocyclopyrachlor-Methyl Ester as Influenced by Diflufenzopyr

Jose J. Vargas, James T. Brosnan, Thomas C. Mueller, Dean A. Kopsell, William E. Klingeman, and Gregory R. Armel*

Research studies evaluated effects of the auxin transport inhibitor, diflufenzopyr, on the biokinetics and efficacy of aminocyclopyrachlor-methyl ester (AMCP-ME) applications to black nightshade and large crabgrass. Absorption, translocation, and metabolism of ^{14}C-AMCP-ME was quantified with and without diflufenzopyr (35 g ai ha^{-1}). Diflufenzopyr had minimal effects on translocation of radioactivity in either species. Accumulation of radioactivity in aboveground plant sections of black nightshade was greater than or equal to that in large crabgrass by 72 h after treatment (HAT). In both species, metabolism of ^{14}C-AMCP-ME was rapid, as 60 to 78% of the extracted radioactivity was the free acid metabolite 8 HAT. In the greenhouse, black nightshade and large crabgrass were treated with AMCP-ME (9, 18, and 35 g ai ha^{-1}) alone and in combination with diflufenzopyr (35 g ha^{-1}). Mixtures of AMCP-ME plus diflufenzopyr did not increase large crabgrass control compared with AMCP-ME alone at any time. Diflufenzopyr (35 g ha^{-1}) increased black nightshade control with AMCP-ME (18 and 35 g ha^{-1}) 7 d after treatment (DAT). However, this increase in control was not observed 14 or 28 DAT. All treatments containing AMCP-ME controlled large crabgrass 70 to 79% 28 DAT compared with > 93% for black nightshade at the same time point.

Nomenclature: Aminocyclopyrachlor; aminocyclopyrachlor-methyl ester; diflufenzopyr; black nightshade, *Solanum nigrum* L.; large crabgrass, *Digitaria sanguinalis* (L.) Scop.

Key words: Absorption, auxin mimic herbicide, auxin transport inhibitor, metabolism, radiolabeled herbicide, translocation.

Auxin mimic herbicides are structurally similar to indole-3-acetic acid and high concentrations in sensitive species cause uncontrolled cell elongation leading to leaf epinasty and eventual necrosis (Grossmann 2007). Auxin mimic herbicides can also increase ethylene biosynthesis, resulting in reduced photosynthetic activity and increased leaf senescence (Bleecker and Kende 2000; Grossmann et al. 2002). Phenoxy carboxylic acids such as 2,4-D and MCPA were first commercialized as auxin mimic herbicides after World War II (Cobb and Kirkwood 2000).

Aminocyclopyrachlor (AMCP) is an auxin mimic herbicide registered for broadleaf weed control in pastures, as well as noncrop and rangeland areas (Rick and Meredith 2011). A pyrimidine carboxylic acid, AMCP contains a cyclopropyl substituent group on its heterocyclic ring, separating it from other auxin mimic herbicides (Armel and Hong 2008). AMCP controls many invasive weeds at rates lower than other auxin mimic herbicides such as 2,4-D. Minogue et al. (2011) reported effective control of kudzu [*Pueraria montana* var. *lobata* (Willd.) Maesen & S.M. Almeida] with aminocyclopyrachlor-methyl ester (AMCP-ME) at rates of 140 to 280 g ai ha^{-1}. Similarly, Koepke-Hill et al. (2012) reported 87 to 100% control of transplanted silk tree (*Albizia julibrissin* Durazz.) plants in a greenhouse 1 mo after treatment (MAT) with AMCP at 18 g ai ha^{-1}. In the field, Koepke-Hill et al. (2011) observed ≥ 95% control of mugwort (*Artemisia vulgaris* L.) 12 MAT with AMCP-ME at 280 g ha^{-1}. Similarly, Beeler et al. (2012) reported 77 to 93% control of trumpetcreeper [*Campsis radicans* (L.) Seem. ex Bureau] 12 MAT with AMCP-ME at rates of 70 to 280 g ha^{-1}. West et al. (2011) reported 99% control of bushkiller [*Cayratia japonica* (Thunb.) Gagnep.] by 10 MAT with AMCP at 350 g ha^{-1}.

Two formulations of AMCP have been researched for noncrop weed control: the methyl ester, AMCP-ME, and the free acid, AMCP. Bukun et al. (2010) reported that AMCP-ME is metabolized to AMCP within 6 h after application (HAT) in Canada thistle [*Cirsium arvense* (L.) Scop.] The researchers also reported greater foliar absorption and translocation of AMCP-ME in Canada thistle compared with AMCP. Similar responses have also

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* Research Associate II, Associate Professor, Professor, Professor, Professor, and Assistant Professor, respectively, Department of Plant Sciences, University of Tennessee, 252 Ellington Plant Science Building, 2431 Joe Johnson Drive, Knoxville, TN 37996. Corresponding author's E-mail: jvargas@utk.edu

been observed on several weed species including rush skeletonweed (*Chondrilla juncea* L.), yellow starthistle (*Centaurea solstitialis* L.), and prickly lettuce (*Lactuca serriola* L.) (Bell et al. 2011). Increased absorption of AMCP-ME was hypothesized to enhance translocation by generating greater source sink dynamics in Canada thistle (Bukun et al. 2010). However, de-esterification of AMCP-ME to AMCP has also been hypothesized to slow short-term (i.e., within 4 HAT) translocation of AMCP in yellow starthistle and rush skeletonweed (Bell et al. 2011).

Translocation of AMCP-ME and AMCP has been reported to be low. Maximum translocation (i.e., aboveground and belowground) of AMCP-ME did not occur until 96 HAT in Canada thistle compared with 192 HAT for AMCP (Bukun et al. 2010). Similarly, Lindenmayer et al. (2013) reported that only 14% of applied AMCP translocated out of treated field bindweed (*Convolvulus arvensis* L.) leaves by 192 HAT. Bell et al. (2011) reported that 19% of AMCP-ME translocated in yellow starthistle by 48 HAT; however, translocation in prickly lettuce and rush skeletonweed was $< 7\%$ at the same harvest timing. Bukun et al. (2010) reported no differences in belowground translocation in Canada thistle between formulations, with only 8.6% and 6.3% of AMCP-ME and AMCP translocating to root tissues, respectively, 192 HAT. Lindenmayer et al. (2013) surmised that belowground translocation in perennial species such as field bindweed may affect AMCP weed control efficacy. In a tolerant grass species, tall fescue [*Lolium arundinaceum* (Schreb.) S.J. Darbyshire] Lewis et al. (2013) reported limited translocation to foliage that did not occur until 96 HAT.

Several researchers have observed that auxin transport inhibitors can increase the activity of auxin mimic herbicides. Enloe and Kniss (2009) reported increased Russian knapweed [*Acroptilon repens* (L.) D.C.] control with a mixture of diflufenzopyr (0.14 kg ha^{-1}) plus dicamba (0.056 kg ha^{-1}) plus aminopyralid (0.05 kg ha^{-1}) than with aminopyralid applied alone 24 MAT. Russian knapweed control with this combination was greater than other auxin herbicides such as picloram, clopyralid alone, or in mixtures with 2,4-D or triclopyr. Lym and Deibert (2005) observed greater leafy spurge (*Euphorbia esula* L.) control with picloram plus diflufenzopyr than with picloram plus 2,4-D. In radiolabel experiments, the researchers surmised that greater control in the field may be the result of diflufenzopyr increasing absorption of ^{14}C-picloram.

Applications of auxin herbicides such as AMCP can also affect grassy weeds but often fail to provide commercially acceptable control. Reed et al. (2013) reported $\leq 78\%$ control of multileaf smooth crabgrass [*Digitaria ischaemun* (Schreb) Schreb. ex. Muhl.] with AMCP at 0.11 kg ha^{-1}. A structurally similar pyridine herbicide, aminopyralid, has little activity on most rangeland and pasture grasses (Masters et al. 2005). Aminopyralid applications controlling broadleaf weeds have been found to increase cover of grass species in prairies (Samuel and Lym 2008).

Diflufenzopyr may affect the absorption, translocation, and metabolism of AMCP-ME, leading to greater control of both broadleaf and grassy weeds. However, limited data have been published on the influence of auxin transport inhibitors on AMCP-ME biokinetics (i.e., absorption, translocation, metabolism) and weed control. Therefore, the objectives of this research were to (1) determine effects of diflufenzopyr on the biokinetics of AMCP-ME in black nightshade and large crabgrass; and (2) evaluate effects of diflufenzopyr on the efficacy of AMCP-ME for POST control of both species.

Materials and Methods

Plant Culture. Laboratory and greenhouse experiments were conducted from March through May 2009 at the University of Tennessee (Knoxville, TN; 35.96°N, 83.56°W E). Black nightshade and large crabgrass plants for these experiments were grown from seed (Herbiseed, New Farm Mire Lane West End, Twyford, U.K.; Azlin Seed Services, 112 Lilac Drive, Leland, MS, respectively) in 20 by 51 cm^2 germination trays (Dillen Greenhouse. Myers Industries, Inc. 1293 South Main Street, Akron, OH 44301) until developing at least three true leaves. These species were selected to evaluate effects of diflufenzopyr on AMCP-ME applications to an annual dicot and monocot weed species. Individual plants were transplanted into 10.2 by 10.2 cm^2 containers filled with peat-based growing media (ProMix BX Mycorrhizae, Premier Tech Horticulture, Quakertown, PA 18951) where they were supplied biweekly with nutrients using a complete water-soluble fertilizer (Peter's 20-20-20, JR Peters, Inc. Allentown, PA 18106) at a rate of 5 g N m^{-2} wk^{-1} and irrigated on an as-needed basis. Temperature in the greenhouse ranged from 22 to 28 C under conditions of natural light. Plants were cultured under these conditions until reaching heights ranging from 20 to 25 cm.

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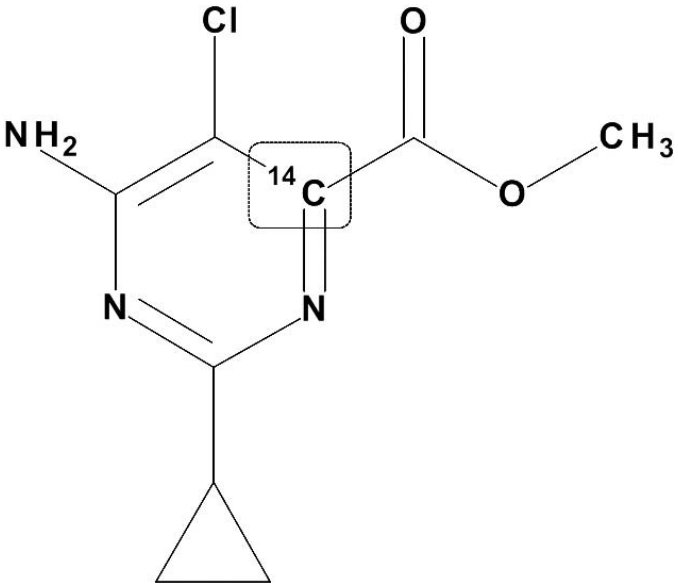

Figure 1. Molecular structure of ^{14}C-aminocyclopyrachlor-methyl ester (^{14}C-AMCP-ME), and its chemical International Union of Pure and Applied Chemistry nomenclature (methyl 6-amino-5-chloro-2-cyclopropyl-pyrimidine-4carboxylate). Radiolabeled carbon denoted ^{14}C.

Laboratory Research. Laboratory studies were conducted to evaluate the effect of diflufenzopyr on the absorption, translocation, and metabolism of ^{14}C-AMCP-ME (DuPont Crop Protection, Laurel Run Building, Chestnut Run Plaza, Wilmington, DE 19898) in black nightshade and large crabgrass. Black nightshade and large crabgrass plants were cultured under previously described conditions until developing a minimum of four internodes (black nightshade) or three tillers (large crabgrass). The specific activity of ^{14}C-AMCP-ME (Figure 1) used in these laboratory experiments was 1.64 MBq mg^{-1} with a total radiochemical purity of 99.8%.

Absorption Experiments. Before ^{14}C-AMCP-ME application, plants were treated with nonradiolabeled AMCP-ME (DPX-KJM44 80% WG, DuPont Crop Protection) at 35 g ai ha^{-1} alone or with diflufenzopyr at 35 g ai ha^{-1} using a CO_2-pressurized backpack sprayer calibrated to deliver 215 L ha^{-1} with an even fan nozzle (TeeJet 6504E even flat spray tip, Spraying Systems Co., 124 West Harrisburg Street, Dillsburg, PA 17019). These treatments were mixed with a methylated seed oil surfactant (Methylated Soybean Oil Plus, Universal Crop Protection Alliance, LLC., Eagan, MN 55121) at 1% v/v.

A ^{14}C-AMCP-ME solution was then applied to large crabgrass and black nightshade approximately 30 min after application of nonradiolabeled herbicides. This radiolabeled aqueous solution contained 2.5 mg of ^{14}C-AMCP-ME (which contained 4.1 Bq mg^{-1} of radioactivity), 4.6 ml of acetone (acetone, high-performance liquid chromatography [HPLC] grade, Fisher Scientific, 300 Industry Drive, Pittsburgh, PA 15275), 0.48 ml of deionized H_2O (Thermo Scientific type 1 reagent-grade deionized water, Fisher Scientific), and 0.02 ml of methylated seed oil. Eight 1-µl droplets of this ^{14}C solution were applied to the upper leaf surface of each large crabgrass and black nightshade plant, avoiding application on their midrib and lateral veins, using a micropipette (PB-600-1 repeating dispenser, Hamilton Co., 4970 Energy Way, Reno, NV 89502). A fully expanded black nightshade leaf at the second node distal from the apical bud received the ^{14}C solution, whereas the second youngest leaf from the bud leaf was treated on each large crabgrass plant. All plants were kept under high-intensity discharge lamps (Sun System III; Sunlight Supply, Inc., 5408 NE 88th Street, Building A., Vancouver, WA 98665) with a 16-h photoperiod after receiving the ^{14}C-solution.

To determine foliar ^{14}C-AMCP-ME absorption, each leaf treated with ^{14}C-solution was washed with 5 ml of a 50 : 50 solution of methanol and deionized water at 1, 4, 8, 24, 48, or 72 HAT. This 5-ml wash was dispensed as 1-ml aliquots to the treated leaf of each plant whereby the rinsate was captured in a scintillation vial (National Diagnostics, 305 Patton Drive, Atlanta, GA 30336). The rinsate was then mixed with 10 ml of scintillation fluid (Ecoscint H, biodegradable scintillation solution, National Diagnostics) and placed in a liquid scintillation counter (liquid scintillation analyzer 1900CA, Packard Instrument Company, 800 Research Parkway, Meriden, CT 06450). The total amount of radioactivity applied to each plant was determined by filling a scintillation vial with eight 1-µl droplets of the ^{14}C-solution and 10 ml of scintillation fluid. Radioactivity in all samples was determined using liquid scintillation spectroscopy (LSS) with total absorption calculated by determining the difference between the radioactivity applied to each plant and the radioactivity in rinsate collected at 1, 4, 8, 24, 48, and 72 HAT.

Absorption experiments were designed as a completely randomized 2 by 2 factorial with two replications. Factors included weed species (i.e., large crabgrass, black nightshade) and herbicide treatment (i.e., ^{14}C-AMCP-ME, ^{14}C-AMCP-ME + diflufenzopyr). Two experimental runs were con-

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ducted with data from each analyzed using PROC MIXED in SAS 9.3 (Version 9.3, SAS Institute, Cary, NC 27519). No treatment-by-experimental run interactions were detected; thus, data from each experimental run were combined. Nonlinear regression curves were used to evaluate differences in absorption due to applied treatments using GraphPad Prism software (Prism 5.0 for Mac OSX. GraphPad Software, San Diego, CA).

Translocation Experiments. Black nightshade and large crabgrass plants were dissected at 1, 4, 8, 24, 48, and 72 HAT and partitioned into four different plant sections: treated leaf (TL), above treated leaf (ATL), roots (R), and the rest of foliage including the main stem (ROF). Each plant section was stored in a sampling bag (Whirl-Pak, Nasco, 901 Janesville Avenue, Fort Atkinson, WI 53538) and kept frozen at −20 C for subsequent analysis. Samples were combusted in a biological oxidizer (biological oxidizer OX700, R. J. Harvey Instrument Corporation, 11 Jane Street, Tappan, NY 10983) that captured evolved $^{14}CO_2$ using a carbon dioxide absorbent (C^{14} Cocktail, UN2924, R. J. Harvey Instrument Corporation). A total of 10 ml of scintillation fluid was added to each combusted sample and radioactivity in all vials was determined using LSS. Recovery rate for applied ^{14}C was 99%. Translocation was calculated as the quotient of ^{14}C in each plant section by total ^{14}C radioactivity absorbed from all oxidized plant parts at 1, 4, 8, 24, 48, and 72 HAT.

Design of the translocation experiments was a 2 by 2 by 4 factorial arranged in a completely randomized design with two replications. Factors included weed species (i.e., large crabgrass, black nightshade), herbicide treatment (^{14}C-AMCP-ME, ^{14}C-AMCP-ME + diflufenzopyr), and plant section (i.e., TL, ATL, R, and ROF). Two experimental runs were conducted with data from each analyzed performing PROC MIXED in SAS 9.3. Treatment-by-experimental run interactions were detected; thus, data from each experimental run were analyzed and are presented separately. In each, Fisher's Protected LSD test was used to separate treatments at $P \leq 0.05$.

Metabolism Experiments. To determine ^{14}C-AMCP-ME metabolism, black nightshade and large crabgrass plants were cultured and treated as previously described and sectioned similarly to the translocation experiment. Each plant section was homogenized with liquid nitrogen and a solution containing 7 ml of acetonitrile (acetonitrile HPLC grade, mobile phase for HPLC applications, Fisher Scientific) and 3 ml of deionized water (Thermo Scientific). Each sample was then centrifuged (Fisher Scientific centrific model 225 benchtop centrifuge) for 1 min at 500 rpm. A 3-ml aliquot of this centrifuged ^{14}C mixture was then filtered and subjected to HPLC (Waters HPLC autosampler model 717, Waters Corporation, 34 Maple Street, Milford, MA) using an amino column measuring 300 by 4.6 mm with a 5-µm particle size (Phenomenex Luna NH2 100A column, 411 Madrid Avenue, Torrance, CA 90501). The HPLC injection volume was 100 µl. The mobile phase was 85% acetonitrile and 15% distilled water at a flow rate of 1 ml min^{-1} for 15 min. Two separate herbicide standards were prepared to calculate retention time peaks for the parent herbicide AMCP-ME and its free-acid metabolite, AMCP. These two nonradiolabeled standards were prepared at 20 ppm for AMCP-ME and 5 ppm for AMCP (DPX-MAT28 50% WG, Dupont Crop Protection). Retention times were 2.6 min for AMCP-ME and 7.9 min for AMCP. Samples from plants treated with ^{14}C-AMCP-ME and ^{14}C-AMCP-ME plus diflufenzopyr were analyzed using HPLC. Aliquots were removed at these two retention windows (2 to 4 min; 7 to 9 min), placed in two separate scintillation vials, and subjected to LSS. The amount of parent herbicide and metabolite in each plant was expressed as a percentage of the total radioactivity recovered.

Treatments for the metabolism experiments were arranged in a 2 by 2 factorial completely randomized design with two replications. Factors included weed species (i.e., large crabgrass, black nightshade) and herbicide treatment (^{14}C-AMCP-ME, ^{14}C-AMCP-ME plus diflufenzopyr). Two experimental runs were conducted with data from each analyzed using PROC MIXED in SAS 9.3. No treatment-by-experimental run interactions were detected; thus, data from each experimental run were combined. Differences in the amount of parent herbicide and metabolite in each plant were plotted from 1 to 72 HAT using GraphPad Prism 5.0 software with standard error bars presented as a means of statistical comparison.

Greenhouse Research. Research was conducted in a greenhouse at the University of Tennessee evaluating the effect of diflufenzopyr on AMCP-ME efficacy for black nightshade and large crabgrass control. Treatments included the factorial combination of AMCP-

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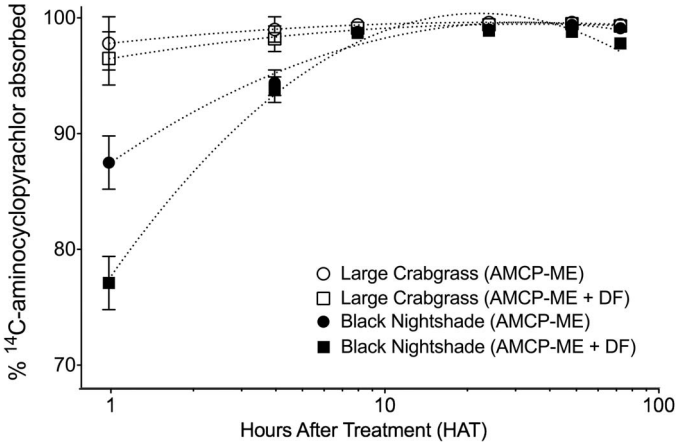

Figure 2. Effect of diflufenzopyr (DF) at 35 g ha^{-1} on absorption of ^{14}C-aminocyclopyrachlor-methyl ester (^{14}C-AMCP-ME) in large crabgrass and black nightshade at 1, 4, 8, 24, 48, and 72 h after treatment. Time intervals were $\log_{10}$ transformed to compare quadratic responses of each treatment. Regression equations for each treatment are presented in Table 1.

ME at 9, 18, and 35 g ha^{-1}, and diflufenzopyr at 9, 18, and 35 g ha^{-1}. All treatments included a methylated seed oil surfactant at a rate of 1% v/v. A nontreated check was included for comparison. Herbicides were applied using a CO_2-pressurized backpack sprayer calibrated to deliver 215 L ha^{-1} with an even fan nozzle. Black nightshade and large crabgrass control were evaluated visually at 7, 14, and 28 d after treatment (DAT) on a 0 (i.e., no control) to 100% (i.e., complete kill) scale relative to the nontreated check. Treatments were arranged in a 3 by 3 factorial, completely randomized design with three replications. Factors included three rates of AMCP-ME (9, 18, and 35 g ha^{-1}) and diflufenzopyr (9, 18, and 35 g ha^{-1}). Two runs of this experiment were conducted. Data from each experimental run were analyzed using PROC MIXED in SAS 9.3 with treatment means separated using Fisher's Protected LSD test at $P \leq 0.05$. No significant experimental run-by-treatment interactions were detected; thus, data from each experimental run were combined.

Results and Discussion

Absorption Studies. There was a species-by-herbicide treatment interaction in the absorption study (Figure 2, Table 1). The addition of diflufenzopyr did not change absorption of ^{14}C- AMCP-ME in large crabgrass; at 1 HAT, absorption of ^{14}C- AMCP-ME and ^{14}C-AMCP-ME plus diflufenzopyr was > 96% of the applied. However, diflufenzopyr reduced ^{14}C-AMCP-ME absorption in black nightshade. By 1 HAT, ^{14}C-AMCP-ME absorption in black nightshade was 88% without diflufenzopyr compared with only 77% with diflufenzopyr. Overall, ^{14}C absorption was greater in large crabgrass than in black nightshade from 1 to 4 HAT but no differences were detected between species regardless of treatment by 8 HAT (Figure 2). This response is similar to the findings of Bell et al. (2011) who observed greater ^{14}C-AMCP-ME absorption in weed species with lower sensitivity to ^{14}C-aminocyclopyrachlor (i.e., rush skeletonweed) than in those with higher sensitivity (i.e., prickly lettuce). Lewis et al. (2013) also reported rapid absorption of ^{14}C-AMCP in a tolerant monocot species, tall fescue. By 24 HAT in our study, absorption of ^{14}C-AMCP-ME in both large crabgrass and black nightshade was > 99%. This response differs from that reported by Bukun et al. (2010), who reported 84% absorption of ^{14}C-AMCP-ME in Canada thistle by 24 HAT. Lindenmayer et al. (2013) reported 48% absorption of ^{14}C-aminocyclopyrachlor in field bindweed by 48 HAT. Although the amount of ^{14}C- AMCP-ME absorption in the current study is greater than that reported by other researchers, our data are similar to previous reports that ^{14}C-AMCP-ME is rapidly absorbed in multiple weed species (Bell et al. 2011; Bukun et al. 2010; Lewis et al. 2013; Lindenmayer et al. 2013)

Translocation Studies. Significant experimental run-by-treatment interactions were present in the translocation study. Therefore, data from each

Table 1. Regression equations capturing variability in ^{14}C-aminocyclopyrachlor-methyl ester absorption in large crabgrass and black nightshade at 1, 4, 8, 24, 48, and 72 h after treatment when applied alone or in combination with diflufenzopyr at 35 g ha^{-1}. Time intervals were $\log_{10}$ transformed to compare quadratic responses of each treatment.

Treatment[a]	Large crabgrass	Black nightshade
^{14}C-Aminocyclopyrachlor-methyl ester	$y = 2.6x - 0.9x^2 + 97.8$, P = 0.0003	$y = 16.2x - 5.4x^2 + 87.4$, P = 0.013
^{14}C-Aminocyclopyrachlor-methyl ester + diflufenzopyr	$y = 3.9x - 1.3x^2 + 96.5$, P = 0.0038	$y = 34.4x - 12.6x^2 + 77.4$, P = 0.002

[a] ^{14}C-Aminocyclopyrachlor-methyl ester was applied in a radiolabeled aqueous solution containing 2.5 mg of ^{14}C-aminocyclopyrachlor-methyl ester at 4.1 Bq mg^{-1}, 4.6 ml of acetone, 0.48 ml of deionized water, and 0.02 ml of methylated seed oil.

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Table 2. Translocation of ^{14}C-aminocyclopyrachlor-methyl ester in partitioned large crabgrass and black nightshade plant parts at 1, 4, 8, 24, 48, and 72 h in the first run of a laboratory experiment.

Treatment[a]	Plant section	Large crabgrass						Black nightshade					
		1 HAT[b]	4 HAT	8 HAT	24 HAT	48 HAT	72 HAT	1 HAT	4 HAT	8 HAT	24 HAT	48 HAT	72 HAT
		% ^{14}C recovered											
^{14}C-Aminocyclopyrachlor-methyl ester	TL	93 a[c]	82 b	94 a	87 a	46 b	62 a	92 a	98 a	67 b	61 a	13 de	10 c
	ATL	1 b	4 d	2 b	4 b	13 de	11 bc	3 b	1 c	10 de	14 bc	22 cd	25 bc
	ROF	4 b	11 c	3 b	9 b	38 bc	27 b	5 b	1 c	2 c	22 b	62 a	59 a
	R	0 b	0 d	2 b	2 b	4 e	0 c	1 b	0 c	2 f	4 c	4 e	7 c
^{14}C-Aminocyclopyrachlor-methyl ester + diflufenzopyr	TL	95 a	96 a	97 a	86 a	71 a	67 a	88 a	92 b	80 a	70 a	38 bc	7 c
	ATL	0 b	0 d	0 b	4 b	3 e	17 bc	11 b	4 c	6 ef	6 c	15 de	51 a
	ROF	4.3 b	4 d	3 b	10 b	26 cd	16 bc	1 b	4 c	13 d	23 b	45 ab	40 ab
	R	0 b	0 d	0 b	0 b	0 e	0 c	0 b	0 c	1 f	2 c	2 e	2 c

[a] Translocation was calculated by dividing the amount of ^{14}C radioactivity of each respective plant section by the total ^{14}C radioactivity recovered from all oxidized plant parts.
[b] Abbreviations: ATL, above treated leaf; HAT, hours after treatment; ROF, rest of foliage including main stem; R, roots; TL, treated leaf.
[c] Means sharing the same letter are not significantly different from one another according to Fisher's Protected LSD test at $P \leq 0.05$.

experimental run were analyzed and are presented individually (Tables 2 and 3).

A significant treatment-by-weed species interaction was detected in the first experimental run. Translocation in large crabgrass during the first experimental run was slow as ≥ 94% of the absorbed radioactivity remained in the TL by 8 HAT (Table 2). Addition of diflufenzopyr had limited effect on translocation during the first experimental run; overall, translocation in large crabgrass occurred from 24 to 72 HAT with 62 and 67% of absorbed radioactivity remaining in the TL by 72 HAT for ^{14}C-AMCP-ME and ^{14}C-AMCP-ME plus diflufenzopyr, respectively. Lewis et al. (2013) also observed minimal translocation of ^{14}C-AMCP out of treated tall fescue leaves by 96 HAT. In our study, movement of radioactivity was out of the TL primarily to the ROF and ATL plant sections as 16 to 27% and 11 to 17% of absorbed radioactivity was detected in these plant section for ^{14}C-AMCP-ME and ^{14}C-AMCP-ME plus diflufenzopyr, respectively, by 72 HAT.

Translocation was more rapid in black nightshade than in large crabgrass during the first experimental run (Table 2). For ^{14}C-AMCP-ME and ^{14}C-AMCP-ME plus diflufenzopyr, ≤ 80% of absorbed radioactivity was detected in the TL by 8 HAT, with only 7 to 10% detected in the TL by 72 HAT. Similar to large crabgrass, translocation was primarily to the ROF and ATL plant sections by 72 HAT. The addition of diflufenzopyr increased translocation to ATL (51%) compared with when ^{14}C-AMCP-ME was applied alone (25%) by 72 HAT.

Significant treatment-by-weed species interactions were also detected in the second experimental run (Table 3). During this experiment, diflufenzopyr affected translocation in large crabgrass. By 8 HAT, 82% of absorbed radioactivity was detected in the TL with ^{14}C-AMCP-ME compared with only 54% for the mixture of ^{14}C-AMCP-ME plus diflufenzopyr. By 24 HAT, few significant differences in recovered radioactivity in the TL were detected between these treatments. Addition of diflufenzopyr to ^{14}C-AMCP-ME resulted in greater radioactivity recovered in the ROF plant section by 24 HAT and in the ATL plant section by 72 HAT.

Black nightshade results were similar between experimental runs as 74 to 89% of absorbed radioactivity remained in the TL by 8 HAT during the second experimental run, whereas only 9 to 19% was detected in the TL by 72 HAT (Table 3). Similar to the first experimental run, translocation

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Table 3. Translocation of ^{14}C-aminocyclopyrachlor-methyl ester in partitioned large crabgrass and black nightshade plant parts at 1, 4, 8, 24, 48, and 72 h in the second run of a laboratory experiment conducted in 2009.

Treatment[a]	Plant section	Large crabgrass						Black nightshade					
		1 HAT[b]	4 HAT	8 HAT	24 HAT	48 HAT	72 HAT	1 HAT	4 HAT	8 HAT	24 HAT	48 HAT	72 HAT
		% ^{14}C recovered											
^{14}C-Aminocyclopyr-achlor-methyl ester	TL	98 a[c]	94 a	82 a	50 ab	22 bc	40 ab	96 a	90 a	74 b	28 bc	9 cd	9 e
	ATL	1 b	1 c	4 ef	16 cd	7 c	13 e	2 b	3 c	6 de	10 cd	23 bcd	23 d
	ROF	1 b	5 bc	14 d	31 bc	71 a	46 a	2 b	6 c	18 c	56 a	67 a	62 a
	R	0 b	0 c	1 f	4 d	1 c	1 f	0 b	1 c	2 e	7 d	3 d	7 ef
^{14}C-Aminocyclopyr-achlor-methyl ester + diflufenzopyr	TL	98 a	90 a	54 b	42 ab	46 ab	26 d	93 a	84 a	89 a	41 ab	43 ab	19 d
	ATL	0 b	0 c	10 de	3 d	23 bc	36 bc	3 b	1 c	0 e	9 cd	34 bc	32 c
	ROF	2 b	7 b	32 c	54 a	30 bc	33 cd	4 b	14 b	10 d	49 a	23 bcd	48 b
	R	0 b	2 bc	4 ef	2 d	2 c	6 ef	0 b	1 c	1 e	2 d	2 d	1 f

[a] Translocation was calculated by dividing the amount of ^{14}C radioactivity of each respective plant section by the total ^{14}C radioactivity recovered from all oxidized plant parts.
[b] Abbreviations: ATL, above treated leaf; HAT, hours after treatment; ROF, rest of foliage including main stem; R, roots; TL, treated leaf.
[c] Means sharing the same letter are not significantly different from one another according to Fisher's Protected LSD test at $P \leq 0.05$.

was primarily to the ROF and ATL plant sections, with 48 to 62% and 23 to 32% of absorbed radioactivity detected in these plant sections, respectively, by 72 HAT. Diflufenzopyr had inconsistent effects on translocation in the second experimental run. Greater radioactivity was detected in the TL plant section at 8, 48, and 72 HAT with ^{14}C-AMCP-ME plus diflufenzopyr compared with ^{14}C-AMCP-ME alone, thus indicating reduced translocation. Black nightshade plants treated with ^{14}C-AMCP-ME plus diflufenzopyr had less radioactivity detected in the ROF plant section by 48 and 72 HAT.

In both experiments, translocation in large crabgrass was lower than in black nightshade. This finding supports the work of Bell et al. (2011) and Lewis et al. (2013) who documented that translocation of ^{14}C-AMCP-ME varied among species with variable susceptibility to AMCP. When applied alone, only 9 to 10% of radioactivity was detected in the TL of black nightshade by the end of the study, similar to previous reports on prickly lettuce (7%), rush skeletonweed (3%), Canada thistle (11%), and field bindweed (13%) (Bell et al. 2011; Bukun et al. 2010; Lindenmayer et al. 2013). When applied with diflufenzopyr, radioactivity in TL of black nightshade at the end of the study ranged from 7 to 19% (Tables 3 and 4).

Translocation in both large crabgrass and black nightshade was primarily to the ROF and ATL plant sections (Tables 2 and 3). By 72 HAT in the first experiment, radioactivity in the ATL and ROF sections of large crabgrass was 11 and 27%, with no differences due to diflufenzopyr detected. In the second experimental run, diflufenzopyr increased the amount of radioactivity detected in the ATL section (36%) compared with ^{14}C-AMCP-ME applied alone (13%); however, the opposite was true for the ROF plant section as less radioactivity (33%) was detected in plants treated with ^{14}C-AMCP-ME plus diflufenzopyr compared with those treated with ^{14}C-AMCP-ME alone (46%). In black nightshade, increased radioactivity was detected in the ATL plant section with ^{14}C-AMCP-ME plus diflufenzopyr (51%) than ^{14}C-AMCP-ME applied alone (25%) 72 HAT in the first experimental run. Concomitantly, radioactivity in the ROF plant section decreased with diflufenzopyr (40%) compared with ^{14}C-AMCP-ME alone (59%). In the second experimental run, addition of diflufenzopyr reduced the amount of radioactivity detected in the ATL and ROF plant sections of black nightshade compared with ^{14}C-AMCP-ME applied alone,

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Table 4. Large crabgrass and black nightshade response to aminocyclopyrachlor-methyl ester (AMCP-ME; 9, 18, and 35 g ha^{-1}) and diflufenzopyr (9, 18, and 35 g ha^{-1}) alone and in combination with one another in two combined greenhouse experiments conducted in Knoxville, TN during 2009.

Treatment[a]	Rate	Large crabgrass control			Black nightshade control		
		7 DAT[b]	14 DAT	28 DAT	7 DAT	14 DAT	28 DAT
	g ha^{-1}	%					
Aminocyclopyrachlor-methyl ester	9	11 cde[c]	25 de	73 ab	47 bcd	68 abcd	93 c
	18	12 cde	32 cde	72 ab	41 de	63 cd	99 ab
	35	20 a	41 abc	79 a	45 cde	75 ab	100 a
Diflufenzopyr	9	17 abc	34 bcde	67 b	24 f	26 f	76 d
	18	15 abcd	31 cde	67 b	33 ef	42 e	73 d
	35	8 e	24 e	51 c	35 ef	42 e	73 d
Aminocyclopyrachlor-methyl ester + diflufenzopyr	9 + 9	9 de	31 cde	70 ab	36 def	60 d	95 abc
	18 + 9	12 cde	39 abcd	75 ab	38 de	60 d	99 ab
	35 + 9	15 abc	39 abcd	77 ab	58 ab	78 a	100 a
	9 + 18	17 abc	31 cde	75 ab	47 bcd	70 abcd	96 abc
	18 + 18	19 ab	35 bcde	79 a	55 abc	72 abc	100 a
	35 + 18	13 bcde	40 abc	80 a	61 a	73 abc	100 a
	9 + 35	20 a	31 cde	77 ab	56 abc	67 bcd	94 bc
	18 + 35	19 ab	48 ab	73 ab	53 abc	73 abc	99 ab
	35 + 35	15 abc	51 a	77 ab	64 a	79 a	100 a

[a] All treatments contained methylated seed oil at 1% v/v.
[b] Abbreviations: DAT, days after treatment.
[c] Means followed by the same letter are not significantly different from one another according to Fisher's Protected LSD test at $P \leq 0.05$.

indicating reduced translocation. The overall amount of recovered radioactivity detected in these aboveground plant sections was greater than has been reported by other researchers evaluating the biokinetics of ^{14}C-AMCP-ME or ^{14}C-AMCP on other dicotyledonous species (Bell et al. 2011; Bukun et al. 2010; Lindenmayer et al. 2013).

Diflufenzopyr had no effect on translocation to R in either species. In large crabgrass, 0 to 6% of absorbed radioactivity was detected in the R plant section by 72 HAT during the both experimental runs (Tables 2 and 3). In black nightshade, 0 to 2% of absorbed radioactivity was detected in the R plant section by 72 HAT during both experimental runs. Accumulation of radioactivity in R after ^{14}C-AMCP-ME applications in these experiments is below 5%, which is similar to rush skeletonweed (3.6%), but less than reported with Canada thistle (8.6%) and field bindweed (13%) (Bell et al. 2011; Bukun et al. 2010; Lindenmayer et al. 2013). Reasons for this response are not clear but could explain variable sensitivity of these weed species and potential morphological responses to growth-regulating herbicides like AMCP applications when applications are made to different annual and perennial grass and broadleaf weeds (Lindenmayer et al. 2013).

Metabolism Studies. The free acid metabolite recovered from treated plants from 1 to 72 HAT varied because of weed species and applied treatment (Figure 3). In both species, metabolism of ^{14}C-AMCP-ME was rapid, as 60 to 78% of radioactivity detected by 8 HAT was the free acid metabolite in both weed species. This is similar to responses reported by other researchers evaluating de-esterification of auxin herbicides, inhibitors of acetyl CoA carboxylase, and inhibitors of protoporphyrinogen IX oxidase (Gershater and Edwards 2007; Gershater et al. 2007; Thompson and Nissen 2000). Bell et al. (2011) also reported that ^{14}C-AMCP-ME was rapidly metabolized in rush skeletonweed, with $<$ 60% of recovered radioactivity in the methyl ester form by 8 HAT. In the current study, a greater percentage of ^{14}C-AMCP-ME was metabolized to the free acid form in large crabgrass (74%) than in black nightshade (60%) by 72 HAT. Few differences in metabolism due to diflufenzopyr were detected in either species in the current study (Figure 3).

Greenhouse Studies. An AMCP-ME-by-diflufenzopyr interaction was detected in black nightshade control data (Table 4). No interactions were detected in large crabgrass data, as mixtures of

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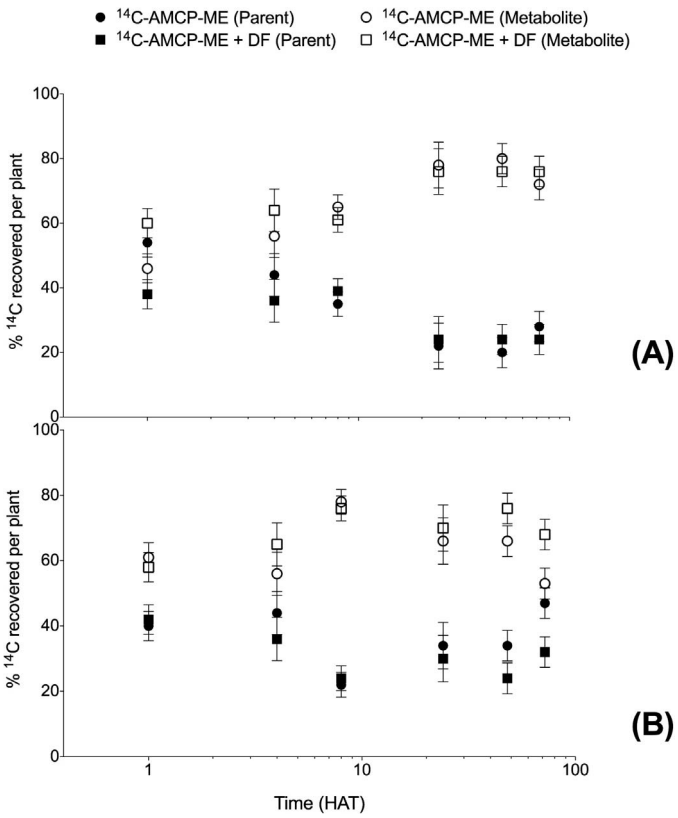

Figure 3. Effect of diflufenzopyr (DF) at 35 g ha^{-1} on metabolism of ^{14}C-aminocyclopyrachlor-methyl ester (^{14}C-AMCP-ME) in large crabgrass (A) and black nightshade (B) at 1, 4, 8, 24, 48, and 72 h after. The amount of ^{14}C-AMCP-ME and its free acid metabolite (AMCP) in each plant was expressed as a percentage of the total radioactivity recovered in each sample. Standard error bars are presented for large crabgrass (A) and black nightshade (B) data as a means of statistical comparison.

diflufenzopyr with AMCP-ME provided equivalent control of large crabgrass as AMCP-ME alone. AT 28 DAT, diflufenzopyr and AMCP-ME controlled large crabgrass 51 to 79%, respectively.

AMCP-ME applied alone at 18 or 35 g ha^{-1} controlled black nightshade 41 to 45%, respectively, by 7 DAT (Table 4). When AMCP-ME was applied at 9, 18, or 35 g ha^{-1} in combination with diflufenzopyr at 18 or 35 g ha^{-1}, black nightshade control increased from 53 to 64% 7 DAT. The addition of diflufenzopyr did not increase black nightshade control with AMCP-ME at 14 or 28 DAT. All treatments containing AMCP-ME provided ≥ 93% control of black nightshade regardless of rate (Table 4).

Our findings indicate that although diflufenzopyr has subtle, inconsistent effects on the biokinetics of ^{14}C-AMCP-ME in large crabgrass and black nightshade, these effects do not affect whole plant control in a greenhouse. ^{14}C-AMCP-ME absorption was greater in large crabgrass compared with black nightshade; however, the opposite relationship was true with regard to ^{14}C-AMCP-ME translocation. Accumulation of radioactivity in aboveground plant sections (ATL and ROF) in black nightshade was greater than or equal to large crabgrass by the end of the experiments, which could explain the increased control observed in greenhouse experiments. Moreover, the low amount of ^{14}C-AMCP-ME translocation in large crabgrass may explain the lack of antagonism reported with AMCP and fenoxaprop mixtures for selective control of smooth crabgrass in cool-season turfgrass (McCullough et al. 2011). Lack of whole plant effects in our greenhouse studies may also suggest that interactions of diflufenzopyr with a pyrimidine carboxylic acid such as AMCP may be different from previously published reports of diflufenzopyr interactions with auxin mimic herbicides of other chemical families.

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Literature Cited

Armel GR, Hong W (2008). Herbicidal mixtures. World Patent Application. WO08073369

Beeler JE, Armel GR, Brosnan JT, Vargas JJ, Klingeman WE, Koepke-Hill RM, Bates GE, Kopsell DA, Flanagan PC (2012) Trumpetcreeper control with various indole-3-acetic acid mimics and diflufenzopyr. HortTechnology 22:677–681

Bell JL, Burke IC, Prather TS (2011) Uptake, translocation and metabolism of aminocyclopyrachlor in prickly lettuce, rush skeletonweed and yellow starthistle. Pest Manag Sci 67:1338–1348

Bleecker AB, Kende H (2000) Ethylene: a gaseous signal molecule in plants. Ann Rev Cell Dev Biol 16:1–18

Bukun B, Lindenmayer RB, Nissen SJ, Westra P, Shaner DL, Brunk G (2010) Absorption and translocation of aminocyclopyrachlor and aminocyclopyrachlor-methyl ester in Canada thistle (*Cirsium arvense*). Weed Sci 58:96–102

Cobb AH, Kirkwood RC (2000) Herbicides and Their Mechanism of Action. Boca Raton, FL: CRC Press. 279 p

Enloe SF, Kniss AR (2009). Does a diflufenzopyr plus dicamba premix synergize Russian knapweed (*Acroptilon repens*) control with auxinic herbicides Invas Plant Sci Manag 2:318–323

Gershater MC, Edwards R (2007) Regulating biological activity in plants with carboxylesterases. Plant Sci 173:579–588

Gershater MC, Cummins I, Edwards R (2007) Role of a carboxylesterase in herbicide bioactivation in *Arabidopsis thaliana*. J Biol Chem 282:21460–21466

https://doi.org/10.1614/WS-D-13-00174.1 Published online by Cambridge University Press

Grossmann K (2007) Auxin herbicide action: lifting the veil step by step. Plant Signal Behav 2:421–423
Grossmann K, Caspar G, Kwiatkowski J, Bowe SJ (2002) On the mechanism of selectivity of the corn herbicide BAS 662H: a combination of the novel auxin transport inhibitor diflufenzopyr and the auxin herbicide dicamba. Pest Manag Sci 58:1002–1014
Koepke-Hill RM, Armel GR, Brosnan JT, Breeden GK, Vargas JJ (2012) Control of silk tree with aminocyclopyrachlor and other herbicides. Weed Sci 60:345–349
Koepke-Hill RM, Armel GR, Klingeman WE, Halcomb MA, Vargas JJ, Flanagan PC (2011) Mugwort control in an abandoned nursery using herbicides that mimic indole-3-acetic acid. HortTechnology 21:558–562
Lewis DF, Roten RL, Everman WJ, Gannon TW, Richardson RJ, Yelverton FH (2013) Absorption, translocation, and metabolism of aminocyclopyrachlor in tall fescue (*Lolium arundinaceum*). Weed Sci 61:348–352
Lindenmayer RB, Nissen SJ, Westra P, Shaner DL, Brunk G (2013) Aminocyclopyrachlor absorption, translocation and metabolism in field bindweed (*Convolulus arvensis*). Weed Sci 61:63–67
Lym RG, Deibert KJ (2005) Diflufenzopyr influences leafy spurge (*Euphorbia esula*) and Canada thistle (*Cirsium arvense*) control by herbicides. Weed Technol 19:329–341
Masters RA, Burch PL, Brueninger JM, Carrithers VF, Jachetta JJ, Kline WN, Hare DD, Chemello AA, Toth JL, Schultz RD (2005) Aminopyralid: a new herbicide for pasture vegetation management. Crop Sci Soc Am Abstr. https://scisoc.confex.com/crops/2005am/techprogram/P7003.htm. Accessed February 19, 2014
McCullough PE, Hart SE, Brosnan JT, Breeden GK (2011) Aminocyclopyrachlor enhances fenoxaprop efficacy for smooth crabgrass control. Weed Technol 25:506–510
Minogue PJ, Enloe SF, Osiecka A, Lauer DK (2011) Comparison of aminocyclopyrachlor to common herbicides for kudzu (*Pueraria montana*) management. Invas Plant Sci Manag 4:419–426
Reed TV, Yu J, McCullough PE (2013) Aminocyclopyrachlor efficacy for controlling Virginia buttonweed (*Diodia virginiana*) and smooth crabgrass (*Digitaria ischaemum*) in tall fescue. Weed Technol 27:488–491
Rick SK, Meredith JH (2011) Aminocyclopyrachlor: a new active for broadleaf weed control in range and pasture. Weed Sci Soc Am Abstr 51:316
Samuel LW, Lym RG (2008) Aminopyralid effects on Canada thistle (*Cirsium arvense*) and native plant species. Invas Plant Sci Manag 1:265–278
Thompson WM, Nissen SJ (2000) Absorption and fate of carfentrazone ethyl in *Zea mays*, *Glycine max*, and *Abutilon theophrasti*. Weed Sci 48:15–19
West AM, Richardson RJ, Monks DW, Shear TH (2011) Bushkiller (*Cayratia japonica*) response to selected herbicides. Invas Plant Sci Manag 4:73–77

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