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Aminocyclopyrachlor Efficacy for Non-Cropland Weed Control

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I am submitting herewith a thesis written by José Javier Vargas Almodóvar entitled "Aminocyclopyrachlor Efficacy for Non-Cropland Weed Control." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Plant Sciences.

James T. Brosnan, Major Professor

We have read this thesis and recommend its acceptance:

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Aminocyclopyrachlor Efficacy for Non-Cropland Weed Control

A Thesis Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

José Javier Vargas Almodóvar
August 2014

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DEDICATION

To my all:

Henry Javier Vargas

Annette Lynn Wszelaki

And…

ACKNOWLEDGEMENTS

I recognize how fortunate I am by being surrounded with outstanding family, friends, co-workers, colleagues, mentors and advisors. For all these people I am grateful and forever thankful. I want to give special thanks to Dr. Gregory Armel, for teaching and guiding me through the years, providing me with all the opportunities to develop myself professionally, and being a tremendous contributor to my education in Weed Science. Thank you very much for being a friend. I would also like to thank Dr. James T. Brosnan for his unconditional guidance, endless patience and motivation to complete my graduate studies. I would like to thank the rest of my graduate committee: Dr. Thomas C. Mueller, Dr. Dean A. Kopsell and Dr. William E. Klingeman for their support, advice and input on my graduate research.

There are many people who in one manner or another have encouraged and facilitated the execution of my graduate research including the entire team at the East Tennessee Research and Education Center, Plateau Research and Education Center, and Little River Animal and Environmental Unit. In addition I would like to thank the entire team in the Horticultural and Invasive Weed Management Program and the Turf and Ornamental Weed Science Program for their patience and full support. I also would like to thank the industry professional at DuPont Crop Protection and Land Management for their encouragement, support and providing me the opportunity to evaluate aminocyclopyrachlor on numerous studies through the years.

Most important I would like to thank my entire family for all the sacrifices they have made for me, in particularly my wife Annette Lynn Wszelaki for her genuine and unconditional love, support, and advice, and for always believing in me. I owe it all to you. *-JJVA*

ABSTRACT

The production of chemicals for crop protection purposes evolved after World War II with the commercialization of the auxin herbicides 2,4-D and MCPA. Their utility and effectiveness created an interest for North American and European companies to develop and research thousands of agrochemicals available today.

Recently discovered and introduced to the market by DuPont Crop Protection, aminocyclopyrachlor is the first broad spectrum synthetic auxin herbicide in this chemical class, and is structurally similar to the auxin herbicides: aminopyralid, clopyralid and picloram. Aminocyclopyrachlor has activity on broadleaf weed species with limited activity on monocot species. Aminocyclopyrachlor is absorbed via plant roots and foliage and translocates to meristematic areas. Affected plants exhibit epinasty, stem twisting, cupping of new leaves and damaged vascular systems typical of synthetic auxin herbicides.

Laboratory and greenhouse studies were established to evaluate biokinetics and efficacy of ^{14}C-aminocyclopyrachlor-methyl ester [Carbon-14 aminocyclopyrachlor-methyl ester] alone and in mixture with diflufenzopyr for control of black nightshade (*Solanum nigrum* L.) and large crabgrass [*Digitaria sanguinalis* (L.) Scop.]. Field studies were conducted to evaluate efficacy of aminocyclopyrachlor for Japanese honeysuckle (*Lonicera japonica* Thunb.) control in a pastures.

Overall, ^{14}C-aminocyclopyrachlor-methyl ester absorption was greater in large crabgrass than in black nightshade, while translocation in large crabgrass was lower than in black nightshade. Translocation in both large crabgrass and black nightshade was primarily to the rest of foliage and above treated leaf plant sections. In both species, metabolism of ^{14}C-

aminocyclopyrachlor-methyl ester was rapid, as 60 to 78% of radioactivity detected by 8 hours after treatment was the free acid metabolite in both weed species.

In the field, aminocyclopyrachlor demonstrated utility for managing Japanese honeysuckle in abandoned pasture fields when applied alone and in combination with other herbicides. For example, 2,4-D and metsulfuron controlled Japanese honeysuckle 42 to 68% by 52 weeks after treatment (WAT). Japanese honeysuckle control with aminopyralid and diflufenzopyr applied alone was ≤ 35%. Inclusion of aminocyclopyrachlor (70 g ha^{-1}) [grams of weight in hectare] increased Japanese honeysuckle control with 2,4-D to 83 to 92%, similar to aminocyclopyrachlor alone at 280 g ha^{-1} (85 to 90% control).

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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 at 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. by 10 MAT with AMCP at 350 g ha^{-1}. Several researchers have observed that auxin transport inhibitors can increase the activity of auxin mimic herbicides. Applications of auxin herbicides such as AMCP can also affect grassy weeds but often fail to provide commercially acceptable control. 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 (*Solanum nigrum* L.) (Figure 1.1) and large crabgrass [*Digitaria sanguinalis* (L.) Scop.] (Figure 1.2); and (2) evaluate effects of diflufenzopyr on the efficacy of AMCP-ME for POST control of both species.

Invasive plant species have morphological and physiological characteristics that allow them to quickly establish in a landscape, thrive outside their natural area of origin and out-compete native flora (Schweitzer and Larson 1999). Therefore, prioritizing detection, control, and eradication of invasive species is of critical concern to land managers (DiTomaso 2000; Maxwell et al. 2009).

Japanese honeysuckle (*Lonicera japonica* Thunb.) is an abundant and widespread invasive weed of non-cropland areas such as pastures. Japanese honeysuckle is highly adaptable which allows it to persist in an extensive range of habitats; it can be found in nearly all the contiguous 48 United States, as well as both Puerto Rico and Hawaii (Munger 2002). Proactive Japanese honeysuckle management can rely on both physical and chemical means of eradication including prescribed burning, grazing, mechanical removal, or herbicide applications (Drake et al. 2003; Nyboer 2002; Warchach 1953). The level of infestation and characteristics of surrounding vegetation will dictate the best control measure to implement. Mixtures with other herbicides labeled for non-crop use such as 2,4-D, aminopyralid, and metsulfuron-methyl may increase AMCP-ME efficacy for Japanese honeysuckle control. However, data describing Japanese honeysuckle control with AMCP-ME are minimal. Our hypothesis was that applications of AMCP-ME alone (or in mixtures with other herbicides) would control Japanese honeysuckle greater than applications of 2,4-D, metsulfuron, and aminopyralid alone. Thus, the

objective of this research was to evaluate Japanese honeysuckle control efficacy with applications of AMCP-ME alone and in mixtures with these herbicides.

CHAPTER I

BIOKINETICS AND EFFICACY OF AMINOCYCLOPYRACHLOR-METHYL ESTER AS INFLUENCED BY DIFLUFENZOPYR

A version of this chapter was originally published by José J. Vargas Almodóvar, James T. Brosnan, Thomas C. Mueller, Dean A. Kopsell, William E. Klingeman and Gregory R. Armel:

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My contributions to this paper include (i) conducting all experiments, (ii) collecting, processing and collaborating on data analysis and interpretation, (iii) review and literature examination, (iv) collaborating on the manuscript.

Abstract

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 were 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.

Introduction

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 at 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* (Lour.) Merr] 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 month 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.) 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.)

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.). The researchers also reported greater foliar absorption and translocation of AMCP-ME in Canada thistle compared with AMCP. Similar responses were 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 may 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 can 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 (*Festuca arundinacea* Schreb.), 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.) 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 ≤ 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 (*Solanum nigrum* L.) (Figure 1.1) and large crabgrass [*Digitaria sanguinalis* (L.) Scop.] (Figure 1.2); 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) where they were supplied biweekly with nutrients using a complete water-soluble fertilizer (Peter's 20-20-20, JR Peters, Inc. Allentown, PA) 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.

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) 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 1.3) 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). These treatments were mixed with a methylated seed oil surfactant (Methylated Soybean Oil Plus, Universal Crop Protection Alliance, LLC., Eagan, MN) 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 [high-performance liquid chromatography (HPLC) grade, Fisher Scientific, 300

Industry Drive, Pittsburgh, PA], 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). 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) 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). 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). 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 conducted with data from each analyzed using PROC MIXED in SAS 9.3 (Version 9.3, SAS Institute, Cary, NC). 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) 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) 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 ≤ 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). 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-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 ≤ 0.05. No significant experimental run-by-treatment interactions were detected; thus, data from each experimental run were combined.

Results and Discussion

Laboratory Research

Absorption Experiments

There was a species-by-herbicide treatment interaction in the absorption study (Figure 1.4). 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 1.2. 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 Experiments

Significant experimental run-by-treatment interactions were present in the translocation study. Therefore, data from each experimental run were analyzed and are presented individually (Tables 1.2 and 1.3).

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 1.2). 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 1.2). 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 1.3). 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 1.3). Similar to the first experimental run, translocation 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 1.2 and 1.3).

Translocation in both large crabgrass and black nightshade was primarily to the ROF and ATL plant sections (Tables 1.2 and 1.3). 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, 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 (1.2 and 1.3). 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 Experiments

The free acid metabolite recovered from treated plants from 1 to 72 HAT varied because of weed species and applied treatment (Figure 1.3). 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 1.3).

Greenhouse Research

An AMCP-ME-by-diflufenzopyr interaction was detected in black nightshade control data (Table 1.4). No interactions were detected in large crabgrass data, as mixtures of

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 1.4). 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 1.4).

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.

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

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

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

APPENDIX

FIGURES AND TABLES

Table 1.1 Regression equations capturing variability in ^{14}C-aminocyclopyrachlor-methyl ester absorption in large crabgrass [*Digitaria sanguinalis* (L.) Scop.] and black nightshade (*Solanum nigrum* L.) at 1, 4, 8, 24, 48, and 72 hours 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
	Regression equations	
^{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. Eight 1 µl droplets of this ^{14}C solution were applied to the upper leaf surface of black nightshade and large crabgrass. A 50:50 methanol-distilled water solution was applied to the treated leaf of each plant 1, 4, 8, 24, 48, and 72 HAT with rinsate captured and analyzed used liquid scintillation spectroscopy.

Table 1.2 Translocation of ^{14}C-aminocyclopyrachlor-methyl ester in partitioned large crabgrass [*Digitaria sanguinalis* (L.) Scop.] and black nightshade (*Solanum nigrum* L.) plant parts at 1, 4, 8, 24, 48, and 72 hours. First run of a laboratory experiment.

		Large crabgrass						**Black nightshade**					
	Plant Section	1	4	8	24	48	72	1	4	8	24	48	72
Treatment[a]		HAT[b]	HAT	HAT	HAT	HAT	HAT	HAT[b]	HAT	HAT	HAT	HAT	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: hours after treatment, HAT; treated leaf, TL; above treated leaf, ATL; roots, R; rest of foliage including main stem, ROF.

[c] Means sharing the same letter are not significantly different from one another according to Fisher's protected least significant difference test at $P \leq 0.05$.

Table 1.3 Translocation of ^{14}C-aminocyclopyrachlor-methyl ester in partitioned large crabgrass [*Digitaria sanguinalis* (L.) Scop.] and black nightshade (*Solanum nigrum* L.) plant parts at 1, 4, 8, 24, 48, and 72 hours. Second run of a laboratory experiment.

		Large crabgrass						Black nightshade					
	Plant Section	1	4	8	24	48	72	1	4	8	24	48	72
Treatment [a]		HAT[b]	HAT	HAT	HAT	HAT	HAT	HAT[b]	HAT	HAT	HAT	HAT	HAT
		% ^{14}C recovered											
^{14}C-aminocyclopyrachlor-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-aminocyclopyrachlor-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: hours after treatment, HAT; treated leaf, TL; above treated leaf, ATL; roots, R; rest of foliage including main stem, ROF.

[c] Means sharing the same letter are not significantly different from one another according to Fisher's protected least significant difference test at $P \leq 0.05$.

Table 1.4 Large crabgrass [*Digitaria sanguinalis* (L.) Scop.] and black nightshade (*Solanum nigrum* L.) 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

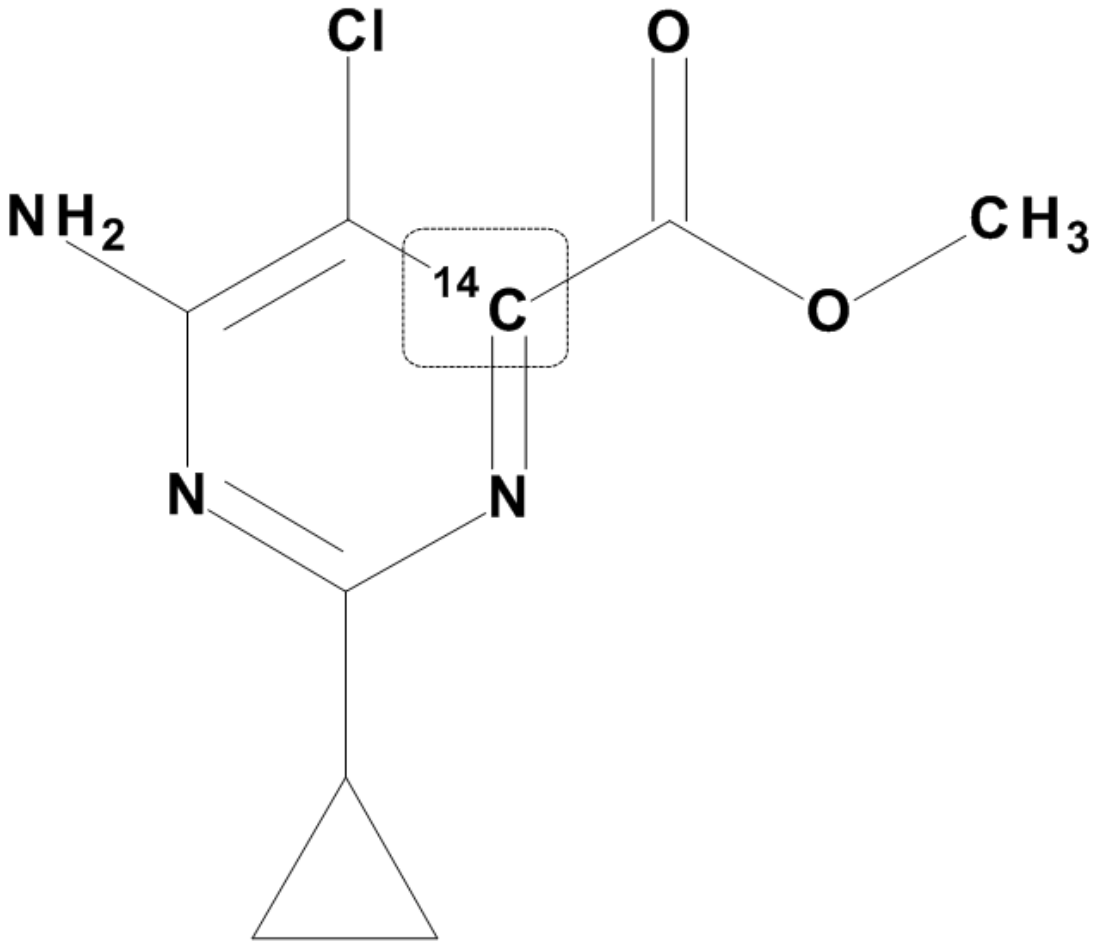
[a] All treatments contained methylated seed oil at 1% v/v

[b] Abbreviations: days after treatment, DAT

[c] Means followed by the same letter are not significantly different from one another according to Fisher's protected least significant difference test at $P \leq 0.05$.

Figure 1.1 Black nightshade (*Solanum nigrum* L.).

Figure 1.2 Large crabgrass [*Digitaria sanguinalis* (L.) Scop.]



methyl 6-amino-5-chloro-2-cyclopropyl-pyrimidine-4-carboxylate

Figure 1.3 Molecular structure of ^{14}C-aminocyclopyrachlor-methyl ester (^{14}C-AMCP-ME), and its chemical IUPAC nomenclature [methyl 6-amino-5-chloro-2-cyclopropyl-pyrimidine-4carboxylate]. Radiolabeled carbon denoted ^{14}C.

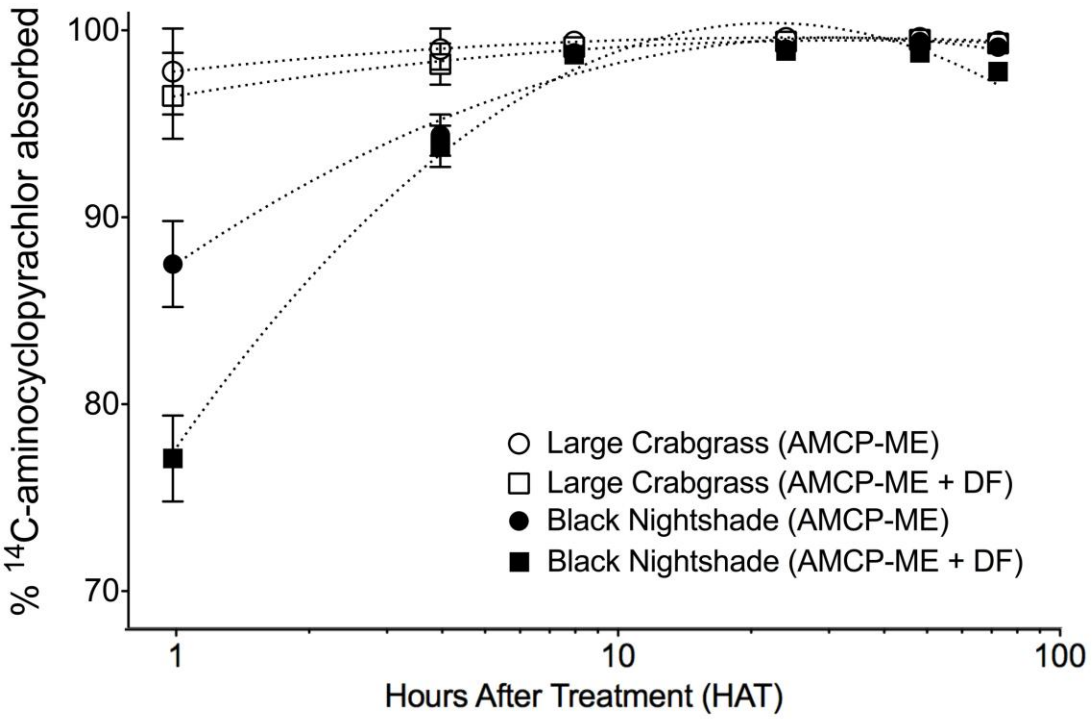


Figure 1.4 Effect of diflufenzopyr (DF) at 35 g ha^{-1} on absorption of ^{14}C-aminocyclopyrachlor-methyl ester (^{14}C-AMCP-ME) in large crabgrass [*Digitaria sanguinalis* (L.) Scop.] and black nightshade (*Solanum nigrum* L.) at 1, 4, 8, 24, 48, and 72 hours after treatment. Time intervals were log_{10} transformed to compare quadratic responses of each treatment. Regression equations for each treatment are presented in Table 1.1.

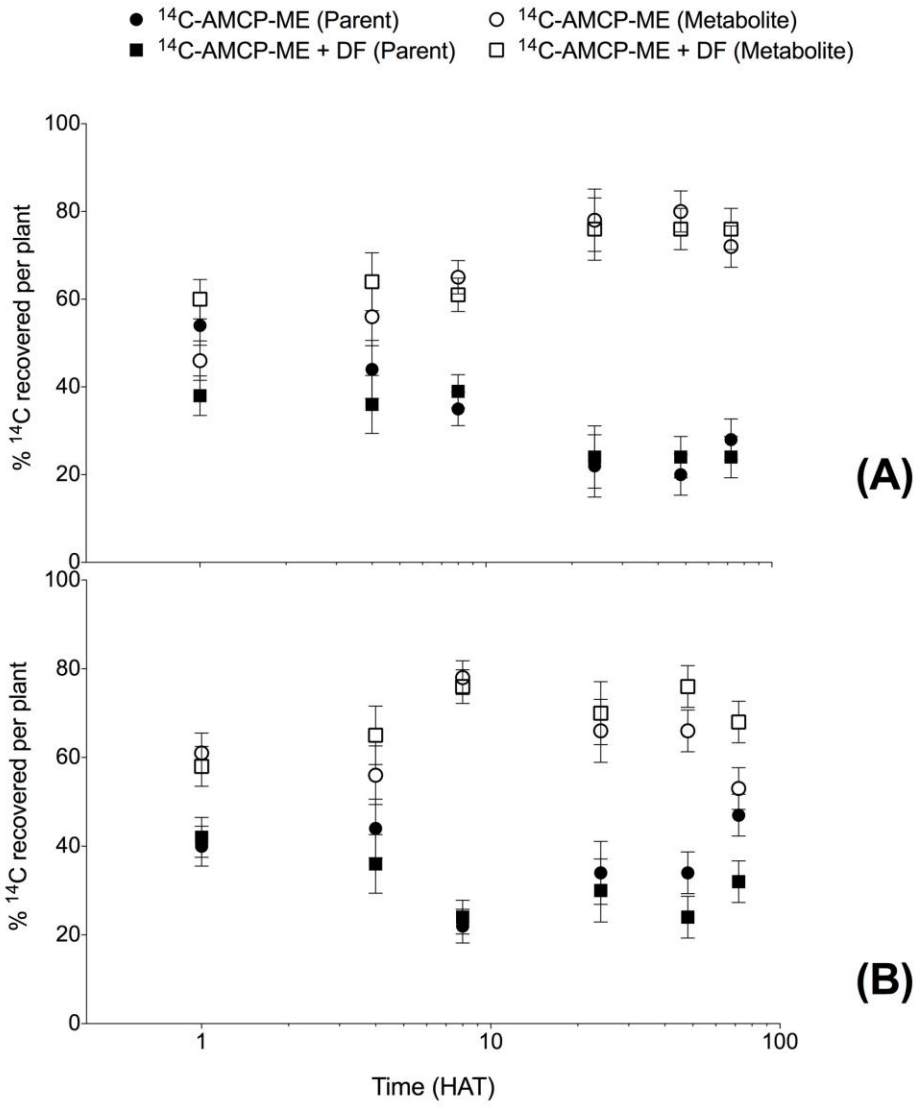


Figure 1.5 Effect of diflufenzopyr (DF) at 35 g ha^{-1} on metabolism of ^{14}C-aminocyclopyrachlor-methyl ester (^{14}C-AMCP-ME) in large crabgrass (A) and black nightshade (B) at 1, 4, 8, 24, 48, and 72 hours after. The amount of ^{14}C-AMCP-ME and its free acid metabolite (AMCP) in each plant was 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.

CHAPTER II

JAPANESE HONEYSUCKLE (*LONICERA JAPONICA*) CONTROL IN ABANDONED PASTURES WITH AMINOCYCLOPYRACHLOR ALONE AND IN MIXTURES

Jose J. Vargas, James T. Brosnan, Thomas C. Mueller, Dean A. Kopsell, William E. Klingeman, and Gregory R. Armel. Japanese Honeysuckle (*Lonicera japonica* Thunb.) Control in Abandoned Pastures with Aminocyclopyrachlor Alone and In Mixtures.

My contributions to this paper include (i) conducting all experiments, (ii) collecting, processing and collaborating on data analysis and interpretation, (iii) review and literature examination, (iv) and collaborating on the manuscript.

Abstract

Japanese honeysuckle (*Lonicera japonica* Thunb.) is an abundant and widespread invasive weed of non-cropland areas such as pastures. Aminocyclopyrachlor (AMCP) is a pyrimidine carboxylic acid herbicide with efficacy for invasive species control. Greenhouse and field research was conducted from 2008 to 2010 to evaluate Japanese honeysuckle control with applications of aminocyclopyrachlor-methyl ester (AMCP-ME) alone and in mixtures with other herbicides. Treatments included AMCP-ME alone (35, 70, 140, and 280 g ha^{-1}), aminopyralid (70 and 140 g ha^{-1}), 2,4-D (1080 g ai ha^{-1}), metsulfuron (42 g ha^{-1}), and diflufenzopyr (70 g ha^{-1}). When applied alone, 2,4-D and metsulfuron controlled Japanese honeysuckle 42 to 68% at 52 weeks after treatment (WAT). Japanese honeysuckle control with aminopyralid and diflufenzopyr applied alone was ≤ 35%. Inclusion of AMCP-ME (70 g ha^{-1}) increased Japanese honeysuckle control with 2,4-D to 83 to 92%, similar to AMCP-ME alone at 280 g ha^{-1} (85 to 90% control). Our studies demonstrate that AMCP-ME has utility for managing the invasive

weed Japanese honeysuckle in abandoned pasture fields. Future research is needed to determine if incorporating AMCP-ME applications with non-chemical management will improve efficacy for Japanese honeysuckle control.

Introduction

An invasive species is an alien or non-native organism that can cause economic loss, environmental damage, or harm to human health (Federal Register: Executive Order No. 13112, 3 C.F.R. 6183 1999). Invasive plant species have morphological and physiological characteristics that allow them to quickly establish in a landscape, thrive outside their natural area of origin and out-compete native flora (Schweitzer and Larson 1999). Therefore, prioritizing detection, control, and eradication of invasive species is of critical concern to land managers (DiTomaso 2000; Maxwell et al. 2009).

Japanese honeysuckle (*Lonicera japonica* Thunb.) (Figure 2.1) is a perennial woody vine native to eastern Asia that was introduced to North America for ornamental use in 1806 (Hardt 1986; Kaufman and Kaufman 2007). Japanese honeysuckle is highly adaptable which allows it to persist in an extensive range of habitats; it can be found in nearly all the contiguous 48 United States, as well as both Puerto Rico and Hawaii (Munger 2002). As a semi-evergreen woody vine, Japanese honeysuckle forms a dense canopy that depletes light, water, and nutrient resources available for desirable forbs and grassland species, which can negatively affect forage yield and quality even after deciduous surrounding vegetation becomes dormant (Sransky 1984). Japanese honeysuckle vines can grow to 12 m in length; moreover, underground and aboveground Japanese honeysuckle stems can be 1 to 5 cm in diameter and support new growth when in contact with soil (Figure 2.2) (Bravo 2003). Japanese honeysuckle also produces allelopathic organic compounds, such as hydroxy derivates of cinnamic acid, that reduce growth and development of surrounding species such as loblolly pine (*Pinus taeda* L.), shortleaf pine (*Pinus echinata* Mill.) and duckweed (*Lemma minor* L.) (Skulman et al. 2004; Vrhovšek 1998).

These characteristics have resulted in classifying Japanese honeysuckle as an invasive weed of non-crop land plant communities including prairies, savannas, forest understories, and pasture fields (Bravo 2003; Gross and Olin 2011; Nyboer 2002).

Proactive Japanese honeysuckle management can rely on both physical and chemical means of eradication including prescribed burning, grazing, mechanical removal, or herbicide applications (Drake et al. 2003; Nyboer 2002; Warchach 1953). The level of infestation and characteristics of surrounding vegetation will dictate the best control measure to implement. Controlled burning for Japanese honeysuckle control is challenging because consecutive spring burnings are needed prior to seed formation for complete control (Munger 2002). Animal grazing and mechanical removal (e.g., hand pulling or mowing) of plants can temporarily reduce small infestations; however, these practices are economically costly, time consuming and impractical (Little and Somes 1967).

Selective herbicide applications can improve Japanese honeysuckle control, specifically when combined with non-chemical management methods. The most common herbicides utilized for Japanese honeysuckle control include glyphosate, imazapyr, and triclopyr (Gries 2012). Applications of these herbicides when surrounding vegetation is dormant can reduce threat of injury to desirable species; however drought or moisture stress conditions can decrease herbicide efficacy (Taylorson 1967; Younce and Skroch 1989). Regehr and Frey (1988) reported that a fall application of 2,4-D plus dichlorprop at 1.8 + 1.8 g ae/L or glyphosate at 0.75 % v/v within two days of frost provided 79 and 95% Japanese honeysuckle control, respectively, by 30 months after treatment (MAT). Additionally, the researchers reported ≤ 58% control of Japanese honeysuckle when these herbicides were applied during winter conditions. Late summer

broadcast or spot applications of metsulfuron (84 g ai ha^{-1}) or tebuthiuron (4500 g ai ha^{-1}) have been used to control Japanese honeysuckle when established as a cover crop with no adjacent desirable species present (Miller 1998; 2003). Munger (2002) suggests that an effective practice for control of Japanese honeysuckle would be a prescribed burn followed by spot herbicide application to control newly sprouted plants after burning.

Aminocyclopyrachlor is a synthetic auxin mimic herbicide belonging to the pyrimidine carboxylic acid chemical family (Armel and Hong 2008; Finkelstein et al. 2008). Two formulations of aminocyclopyrachlor have been researched: a free acid form (AMCP) and a methyl ester (AMCP-ME). Aminocyclopyrachlor was developed for annual and perennial broadleaf weed control in industrial vegetation management, non-crop markets such as right-of-ways, roadsides, and rangeland (Rick and Meredith, 2011; Turner et al. 2009). Mammalian toxicity of aminocyclopyrachlor is low; oral and dermal LD_{50} values are > 5000 mg kg^{-1} (Moore 2008). AMCP-ME efficacy on invasive species has been previously reported. Applications of AMCP-ME at 70 to 280 g ha^{-1} have controlled mugwort (*Artemisia vulgaris* L.), trumpetcreeper (*Campsis radicans*), silk tree (*Albizia julibrissin* Durazz.), and bushkiller (*Cayratia japonica* Thunb.) greater than other synthetic auxin herbicides including aminopyralid, dicamba, clopyralid and picloram (Beeler et al. 2012; Koepke-Hill et al. 2011, 2012; West et al. 2011).

Preliminary field experiments conducted at The University of Tennessee determined that AMCP-ME efficacy for controlling invasive species such as Japanese honeysuckle increased with rates $\geq$ 70 g ha^{-1}. Mixtures with other herbicides labeled for non-crop use such as 2,4-D, aminopyralid, and metsulfuron-methyl may increase AMCP-ME efficacy for Japanese honeysuckle control. However, data describing Japanese honeysuckle control with AMCP-ME

are minimal. Our hypothesis was that applications of AMCP-ME alone (or in mixtures with other herbicides) would control Japanese honeysuckle greater than applications of 2,4-D, metsulfuron, and aminopyralid alone. Thus, the objective of this research was to evaluate Japanese honeysuckle control efficacy with applications of AMCP-ME alone and in mixtures with these herbicides.

Materials and Methods

Greenhouse Experiments

Greenhouse experiments were conducted in spring 2009 at the University of Tennessee Urban Landscape and Nursery Research Facility (Knoxville, TN; 35.96 N, 83.94 W) to evaluate Japanese honeysuckle vine response to various herbicide treatments. Japanese honeysuckle in these experiments was grown from field collected plants. Vines averaged 15 to 20 cm in length containing mature leaves and root tissue. A single vine was transplanted into each 10.2 x 10.2 cm nursery container (Myers Industries, Inc. Akron, OH) filled with 100% pine bark growing media (T.H. Blue Inc. Eagle Springs, NC). Vines were kept under natural light conditions and supplied with nutrients biweekly using a complete water-soluble fertilizer (Peter's 20-20-20. JR PETERS, Inc. Allentown, PA) at a rate of 453 g of N per 93 m^2. Irrigation was applied on an as-needed basis to prevent the onset of soil moisture stress. Average day/night air temperature in the greenhouse during these experiments was 31/19° C. Plants were cultured under these conditions until vines grew to lengths ranging from 20 to 25 cm.

Herbicides treatments applied to Japanese honeysuckle included: AMCP-ME (DPX-KJM44 80% WG, DuPont Crop Protection. Wilmington, DE) at 35, 70, 140, and 280 g ai ha^{-1}; triclopyr (Garlon 3A Specialty Herbicide. Dow AgroSciences LLC. Indianapolis, IN) at 5050 g ai ha^{-1}; AMCP-ME + 2,4-D (2,4-D Lo-V Ester. Universal Crop Protection Alliance, LLC. Eagan, MN) at 70 +1080 g ai ha^{-1}, respectively; and AMCP + metsulfuron (Escort XP. DuPont Crop Protection. Wilmington, DE) at 70 + 42 g ai ha^{-1}, respectively. All treatments included a methylated seed oil surfactant (Methylated Soybean Oil Plus. Universal Crop Protection Alliance, LLC. Eagan, MN) at a rate of 1% v/v. A non-treated check was included for comparison. Treatments were applied using a CO_2 pressurized backpack sprayer calibrated to deliver 215 L ha^{-1} with an even-fan nozzle (TeeJet 6504E even-fan nozzle, Spraying Systems Co. Dillsburg, PA). Herbicides treatments were applied on May 6, 2009.

Japanese honeysuckle control was evaluated 1, 2, and 4 weeks after treatment (WAT) on a 0 (i.e., no control) to 100% (i.e., complete kill) scale relative to the non-treated check. At 4 WAT, aboveground biomass and vine length data were also collected to quantitatively assess treatment responses. Aboveground biomass in each container was harvested at the soil surface, dried for 48 h at 51 C, and then weighed. Vine length data were determined by measuring the length of each vine from the base of the stem to the vine apex.

Experiments were arranged in a randomized complete block design with three replications and repeated in space. Data from each experimental run were analyzed using PROC MIXED in SAS (Version 9.3, SAS Institute, Cary, NC 27519) with treatment means separated using Fisher's protected least significant difference (LSD) test at $\alpha < 0.05$. No significant

experimental run-by-treatment interactions were detected; thus, data from each experimental run were pooled.

Field Experiments

Field experiments were conducted at the East Tennessee Research and Education Center - Little River Animal and Environmental Unit (Walland, TN; 35.45 N, 83.50 W). A field trial was initiated in June 2008 and repeat again in June 2009. Both trials were conducted in two abandoned pasture sites. These pastures had been abandoned due to the severity of the Japanese honeysuckle infestation present. The soil series in both pasture sites was a Hamblen silt loam (fine-loamy, siliceous, semiactive, thermic Fluvaquentic Eutrudepts).

Herbicide treatments evaluated in field experiments included: AMCP-ME at 35, 70, 140, and 280 g ai ha^{-1}; aminopyralid (Milestone, Dow AgroSciences LLC, Indianapolis, IN) at 70 and 140 g ai ha^{-1}; 2,4-D at 1080 g ai ha^{-1}; metsulfuron at 42 g ai ha^{-1}; diflufenzopyr at 70 g ai ha^{-1}; AMCP-ME + 2,4-D at 70 +1080 g ha^{-1}, respectively; AMCP-ME + diflufenzopyr at 70 + 70 g ha^{-1}; AMCP-ME + metsulfuron at 70 + 42 g ha^{-1}, respectively; aminopyralid + 2,4-D at 70 +1080 g ha^{-1}, respectively; aminopyralid + diflufenzopyr at 70 + 70 g ha^{-1}; and aminopyralid + metsulfuron at 70 + 42 g ha^{-1}. Diflufenzopyr was included in these experiments because previous researchers demonstrated increase efficacy of indole-3-acetic acid mimic herbicide for selective broadleaf weed control (Lym and Christianson 1998; Lym and Deibert 2005). All herbicide treatments included a methylated seed oil surfactant at 1% v/v, and a non-treated check was included for comparison. Treatments were applied using a CO_2 pressurized backpack

sprayer calibrated to deliver 215 L ha^{-1} with four flat fan nozzles (TeeJet 8002 flat-fan nozzle. Spraying Systems Co. Dillsburg, PA). Herbicides where applied on June 6, 2008 and June 10, 2009 for each experimental run, respectively.

Japanese honeysuckle control was evaluated 52 WAT on a 0 (i.e., no control) to 100% (i.e., complete kill) scale relative to the non-treated check. Aboveground biomass and stem density data were also collected at 52 WAT to quantitatively assess Japanese honeysuckle responses to herbicide treatment. Quantitative measurements were made within two 0.25 m^2 areas selected at random on each plot. The number of Japanese honeysuckle stems present within this area was recorded and all biomass harvested at the soil surface, dried for 48 h at 51 C, and weighed.

Experiments were arranged in a randomized complete block design with three replications with plot size measuring 6 x 3 m. This experiment was initiated in 2008 and repeated again in 2009. Data were subjected to analysis of variance using PROC MIXED in SAS (Version 9.3, SAS Institute, Cary, NC) with treatment means separated using Fisher's LSD test at $\alpha < 0.05$. Significant year-by-treatment interaction was detected; thus data from each year were analyzed and are presented individually.

Results and Discussion

Greenhouse Experiments

All treatments controlled Japanese honeysuckle ≥ 84 % by 4 WAT and reduced aboveground biomass compared to the non-treated check (Table 1). AMCP-ME applied alone at rates > 70 g ai ha^{-1} controlled Japanese honeysuckle 91 to 98% suggesting that AMCP-ME efficacy for Japanese honeysuckle control warranted investigation in field experiments. At 70 g $ha^{-1,}$ mixtures of AMCP-ME with metsulfuron or 2,4-D controlled Japanese honeysuckle greater than AMCP-ME alone, suggesting that mixtures warrant field investigation as well.

Field Experiments

Similar to our findings in the greenhouse, Japanese honeysuckle control increased with AMCP-ME rate (Table 2). AMCP-ME at 280 g ha^{-1} provided 85 to 90% control by 52 WAT (Table 2). Reductions in stem density and aboveground biomass with AMCP-ME at rates ≥ 140 g ha^{-1} were greater than with AMCP-ME at rates ≤ 70 g ha^{-1} in 2009 as well (Table 2).

When applied alone, 2,4-D and metsulfuron-methyl controlled Japanese honeysuckle 42 to 68 %. Japanese honeysuckle control with aminopyralid and diflufenzopyr applied alone was ≤ 35% in each year as well. Inclusion of AMCP-ME at 70 g ha^{-1} increased Japanese honeysuckle with 2,4-D to 83 to 92%, similar to AMCP-ME alone at 280 g ha^{-1}. This mixture of AMCP-ME + 2,4-D also provided greater Japanese honeysuckle control than AMCP alone at 70 g ha^{-1} in both years. Mixtures of AMCP-ME (70 g ha^{-1}) with 2,4-D, metsulfuron, or diflufenzopyr

reduced stem density and aboveground biomass similar AMCP-ME alone at rates ≥ 140 g ha^{-1}. Beeler et al. (2012) reported similar responses with AMCP-ME and AMCP-ME + 2,4-D mixtures applied to trumpetcreeper; applications at AMCP-ME (70 g ha^{-1}) with 2,4-D controlled trumpetcreeper and reduced stem density and aboveground biomass similar to AMCP alone at rates ranging from 70 to 280 g ha^{-1}.

Mixtures of aminopyralid with 2,4-D also increased Japanese honeysuckle control compared to aminopyralid alone in our field experiments (Table 2); however, these treatments did not provide commercially acceptable Japanese honeysuckle control by 52 WAT (≤ 67% control). The addition of diflufenzopyr or metsulfuron to AMCP-ME did not improve Japanese honeysuckle control. This response is similar to reports by Beeler et al. (2012) who observed no increases in trumpetcreeper control with mixtures of AMCP-ME + diflufenzopyr compared to AMCP-ME alone. Similarly, Enloe and Kniss (2009) observed that diflufenzopyr did not improve Russian knapweed (*Acroptilon repens* L.) control with dicamba.

Our studies demonstrate that AMCP-ME has utility for managing the invasive weed Japanese honeysuckle in abandoned pasture fields. AMCP-ME mixtures with a phenoxy-carboxylic acid such as 2,4-D will provide more effective control of Japanese honeysuckle than with mixtures containing a pyridine herbicide such as aminopyralid and or the semicarbazone diflufenzopyr. This supports previously published reports of AMCP-ME efficacy for invasive species management (Beeler et al. 2012; Koepke-Hill et al. 2011, 2012; West et al. 2011; Westra et al. 2008). Mixtures of AMCP-ME with 2,4-D can be used to effectively control Japanese honeysuckle and reduce aboveground biomass and stem density in place of increasing AMCP-ME application rate. Future research is needed to determine if incorporating AMCP-ME

applications with non-chemical management such as prescribed burning or mechanical mowing will improve efficacy for Japanese honeysuckle control.

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. HortTech 22:677-681

Bravo, MA (2003) Japanese honeysuckle, *Lonicera japonica* Thunb. Alien Plant Working Group, National Park Service, Washington, DC. http://www.nps.gov/plants/alien/fact/loja1.htm 6p.

Cobb AH, Reade PH (2010) Herbicides and Plant Physiology. Second Edition, Wiley-Blackwell, Oxford, UK. p.148

DiTomaso JM (2000) Invasive weeds in rangelands: species, impacts, and management. Weed Sci. 48:255-265

Drake SJ, Weltzin JF, Patricia DP (2003) Assessment of non-native invasive plant species on the United States Department of Energy Oak Ridge National Environmental Research Park. Castanea. 68:15-30

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

Federal Register (1999) Executive Order No. 13112, *Invasive Species* Federal Register 64:6183-6186.

Finkelstein BL, Armel GR, Bolgunas SA, Clark DA, Claus JS, Crosswicks RJ, Hirata CM, Hollingshaus GJ, Koeppe MK, Rardon PL, Wittenbach VA, Woodward MD (2008) Discovery of aminocyclopyrachlor (proposed common name) (DPX-MAT28): a new broad-spectrum auxinic herbicide. *In* Proceedings of the 236th ACS National Meeting in Philadelphia, PA. Washington, DC: American Chemical Society

Greis AL (2012) Characterization and Evaluation of Aminocyclopyrachlor on Native and Invasive Species in Florida. MS Thesis. University of Florida. Gainesville, Florida. 103 p.

Gross S, Olin E (2011) Development of a management ranking system for terrestrialnoxious weeds/Invasive Plant Species. USDA Forest Service, Lake Tahoe Basin Management Unit. 18p.

Hardt, RA (1986) Japanese honeysuckle: from "one of the best" to ruthless pest. Arnoldia 25 (3): 27–34.

Kaufman, SR, and W Kaufman (2007) Invasive plants: Guide to identification and the impacts and control of common North American species. Stackpole Books, Mechanicsburg, PA. 458 pp.

Koepke-Hill RM, Armel GR, Brosnan JT, Breeden GK, Vargas JJ, Mueller TC (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. HortTech 21:558-562.

Little S, Somes HA (1967) Results of herbicide trials to control Japanese Honeysuckle. U.S. Forest Service Research Note NE-62. Washington, DC: United States Forest Service

Lym, R. G. and K. M. Christianson. 1998. Diflufenzopyr increases perennial weed control with auxin herbicides. Proc. West. Soc. Weed Sci 51:59–61.

Lym RG, Deibert KJ (2005) Diflufenzopyr influences leafy spurge (*Euphorbia esula*) and Canada thistle (*Cirsium arvense*) control by herbicides. Weed Technol. 19:329-341

Maxwell BD, Lehnhoff EA, Rew LJ (2009) The rationale for monitoring invasive plant populations as a crucial step for management. Invas Plant Sci. Manag. 2:1–9

Miller JH (1985) Testing herbicides for kudzu eradication on a Piedmont site. Southern Journal of Applied Forestry 9:128-132

Miller JH (2003) Nonnative invasive plants of southern forests: a field guide for identification and control. Gen. Tech. Rep. SRS–62. Asheville, NC: U.S. Department of Agriculture, Forest Service, Southern Research Station. 93 p.

Moore G (2008) Aminocyclopyrachlor methyl (DPX-KJM44) Manufacturing Use Product: Acute Oral Toxicity-Up-and-Down Procedure in Rats. Project Number: 24511, 25264, 17799. Study prepared by Eurofins / Product Safety Laboratories. 18 p. MRID 47560112.

Munger GT (2002) *Lonicera japonica*. *In*: Fire Effects Information System. http://www.fs.fed.us/database/feis/plants/vine/lonjap/all.html. Accessed: January 10, 2011.

Nyboer R (2002) Vegetation management guideline, Japanese Honeysuckle (*Lonicera japonica* Thunb.) Illinois Nature Preserves Commission. Division of Natural Heritage. Illinois Department of Natural Resources. Vol. 1 No.11.

Regehr DL, Frey DR (1988) Selective control of Japanese honeysuckle (*Lonicera japonica*) Weed Technol. 2:139-143

Rick SK, Meredith JH (2011) Aminocyclopyrachlor: A new active for broadleaf weed control in range and pasture. Weed Sci. Soc. Am. Abstr. 51:316

Skulman BW, Mattice JD, Cain MD, and Gbur EE (2004) Evidence for allelopathic interference of Japanese honeysuckle (*Lonicera japonica*) to loblolly and shortleaf pine regeneration. Weed Sci 52: 433-439.

Stransky, JJ (1984). Forage yield of Japanese honeysuckle after repeated burning or mowing. J. of Range Manag. 37: 237–238

Schweitzer JA, Larson KC (1999) Greater morphological plasticity of exotic honeysuckle species may make them better invaders than native species. J. of the Torrey Botanical Soc. 126:15-23

Taylorson RB (1967) Seasonal variation in sprouting and available carbohydrate in yellow nutsedge tubers. Weeds 15:22-24

Turner RG, Claus JS, Hidalgo E, Holliday MJ, Armel GR (2009) Technical introduction of the new DuPont vegetation management herbicide aminocyclopyrachlor. Weed Sci. Soc. Am. Abstr. 49:405.

Vrhovšek U (1998) Extraction of hydroxycinnamoyltartaric acids from berries of different grape varieties. J. Agric. Food Chem.46:4203–4208

Warbach, O (1953) Control of Japanese Honeysuckle in Wildlife Borders. J. of Wildlife Manag. 17(3): 301-304.

West AM, Richardson RJ, Monks DW, Shear TH (2011) Bushkiller (*Cayratia japonica*) response to selected herbicides. Invas. Plant Sci. and Manag. 4:73-77

Westra P, Nissen S, Gaines T, Bekun B, Lindenmayer B, Shaner D (2008) Aminocyclopyrachlor for invasive weed management and restoration grass safety in the central great plains. Proc. North Central Weed Sci. Soc. 63:203.

Younce, MH and WA Skroch (1989) Control of selected perennial weeds with glyphosate. Weed Sci. 37:360-364

APPENDIX

FIGURES AND TABLES

Table 2.1 Japanese honeysuckle (*Lonicera japonica* Thunb.) control following herbicide treatments 1, 2, 4 weeks after treatment (WAT). Aboveground biomass data were collected 4 WAT. Means were combined from two experimental runs conducted in a greenhouse in Knoxville, TN (35.96 N, 83.94 W) in 2009.

		Japanese honeysuckle control			
Herbicide[a]	Rate	1 WAT	2 WAT	4 WAT	Aboveground biomass
	g ha^{-1}		%		g
AMCP-ME[b]	35	59	83	84	0.90
	70	72	85	91	0.74
	140	78	87	94	0.89
	280	83	95	98	1.18
AMCP-ME + metsulfuron	70 + 42	88	98	98	0.72
AMCP-ME + 2,4-D	70 + 1080	84	98	98	1.11
Triclopyr	5050	78	90	98	1.18
Non – Treated	-	0	0	0	2.13
$LSD_{0.05}$		3	3	2	0.78

[a]Herbicide treatments included methylated seed oil surfactant (Methylated Soybean Oil Plus. Universal Crop Protection Alliance, LLC. Eagan, MN) at 1% v/v.

[b]Abbreviations: AMCP-ME, aminocyclopyrachlor-methyl ester (DPX-KJM44 80% WG, DuPont Crop Protection. Wilmington, DE); WAT, weeks after treatment.

Table 2.2 Japanese honeysuckle (*Lonicera japonica* Thunb.) control, stem density and biomass at 52 weeks after applying herbicide treatments (WAT) on an abandoned pasture field in Walland, TN in 2008 and 2009, respectively.

		Japanese honeysuckle					
		2008			2009		
Herbicide[a]	Rate	Control	Stem Density[b]	Biomass[b]	Control	Stem Density[c]	Biomass
	g ha^{-1}	%	#/0.5 m^2	g/0.5m^2	%	#/0.5 m^2	(g/0.5m^2)
AMCP-ME[d]	35	13	15	18	42	16	22
	70	42	15	12	65	15	18
	140	68	3	6	87	7	9
	280	85	8	6	90	4	5
aminopyralid	70	3	23	24	22	19	21
	140	10	18	53	35	13	16
metsulfuron-methyl	42	53	11	6	42	6	7
2,4-D	1080	67	4	4	68	5	5
diflufenzopyr	70	22	8	12	5	17	16
Table 2.2 Continued.							
AMCP-ME + metsulfuron	70 + 42	62	17	26	53	9	11
aminopyralid + metsulfuron	70 + 42	43	10	10	50	17	18
AMCP-ME + 2,4-D	70 + 1080	83	1	0.25	92	2	3
aminopyralid + 2,4-D	70 + 1080	50	22	15	67	20	24

Table 2.2 Continued.

		Japanese honeysuckle					
		2008			2008		
Herbicide[a]	Rate	Control	Herbicide[a]	Rate	Control	Herbicide[a]	Rate
AMCP-ME + diflufenzopyr	70 + 70	48	11	13	72	6	5
aminopyralid + diflufenzopyr	70 + 70	38	19	14	30	14	19
Non – Treated	-	0	12	12	0	20	23
$LSD_{0.05}$		23	NS	NS	9	5	6

[a]Herbicide treatments included methylated seed oil surfactant (Methylated Soybean Oil Plus. Universal Crop Protection Alliance, LLC. Eagan, MN) at 1% v/v.

[b] Aboveground biomass and stem density were made within two 0.25 m^2 areas selected at random on each plot. The number of Japanese honeysuckle stems present within this area was recorded and all biomass harvested at the soil surface, dried for 48 h at 51 C, and weighed.

[c]1 gha^{-1} = 0.0143 oz/acre, 1 g = 0.0353 oz, 1 cm = 0.3937 inch, 1 stem/m^2 = 0.0929 stem/ft^2

[d]Abbreviations: AMCP-ME, aminocyclopyrachlor-methyl ester (DPX-KJM44 80% WG, DuPont Crop Protection. Wilmington, DE).

Table 2.3 Evaluation of aminocyclopyrachlor-methyl ester efficacy for control of various invasive weeds at 4 weeks after treatment (WAT) and stem density at 52 WAT after applying herbicide treatments on an abandoned pasture field in Walland, TN. Data were combined from studies conducted in 2008 and 2009.

		Allegheny blackberry		Canadian goldenrod		Poison hemlock	
		% Control	Stem Density[bc]	% Control	Stem Density	% Control	Stem Density
Herbicide[a]	Rate	4 WAT	52 WAT	4 WAT	52 WAT	4 WAT	52 WAT
AMCP-ME[d]	35	31	2	40	2	43	0
	70	40	2	58	1	32	0
	140	43	2	70	0	30	0
	280	60	0	75	0	80	0
aminopyralid	70	33	1	33	2	30	0
	140	42	0	40	0	48	0
metsulfuron-methyl	42	58	1	82	2	75	1
2,4-D	1080	37	3	65	0	79	0

Table 2.3 Continued.

		Allegheny blackberry		Canadian goldenrod		Poison hemlock	
		% Control	Stem Density[bc]	% Control	Stem Density	% Control	Stem Density
Herbicide[a]	Rate	4 WAT	52 WAT	4 WAT	52 WAT	4 WAT	52 WAT
AMCP-ME + metsulfuron	70 + 42	62	0	63	0	89	1
aminopyralid + metsulfuron	70 + 42	63	1	88	0	81	0
AMCP-ME + 2,4-D	70 + 1080	55	4	78	0	80	0
aminopyralid + 2,4-D	70 + 1080	53	1	57	1	67	0
AMCP-ME + diflufenzopyr	70 + 70	77	1	73	1	80	0
aminopyralid + diflufenzopyr	70 + 70	52	1	40	2	45	1
Non – treated	-	0	2	15	9	0	1
$LSD_{0.05}$		18	2	16	6	38	1

[a]Herbicide treatments included methylated seed oil surfactant (Methylated Soybean Oil Plus. Universal Crop Protection Alliance, LLC. Eagan, MN) at 1% v/v.

[b] Aboveground stem density were made within two 0.25 m^2 areas selected at random on each plot. Numbers of stems reflect above soil surface.

[c]1 g ha^{-1} = 0.0143 oz/acre, 1 g = 0.0353 oz, 1 cm = 0.3937 inch, 1 stem/m^2 = 0.0929 stem/ft^2

[d]Abbreviations: AMCP-ME, aminocyclopyrachlor-methyl ester (DPX-KJM44 80% WG, DuPont Crop Protection. Wilmington, DE).

Table 2.4 Evaluation of aminocyclopyrachlor-methyl ester efficacy for control of various invasive weeds at 4 weeks after treatment (WAT) and stem density at 52 WAT after applying herbicide treatments on an abandoned pasture field in Walland, TN. Data were combined from studies conducted in 2008 and 2009.

		Tall Ironweed		American sweetgum		Wingstem	
		% Control	Stem Density[bc]	% Control	Stem Density	% Control	Stem Density
Herbicide[a]	Rate	4 WAT	52 WAT	4 WAT	52 WAT	4 WAT	52 WAT
AMCP-ME[d]	35	63	1	45	0	83	0
	70	62	0	55	0	85	0
	140	67	0	62	1	94	0
	280	70	0	68	0	87	0
aminopyralid	70	48	1	25	0	50	0
	140	60	0	42	1	85	0
metsulfuron-methyl	42	35	2	38	1	68	0
2,4-D	1080	53	0	52	0	77	0
diflufenzopyr	70	25	0	20	1	32	0

Table 2.4 Continued.

		Tall Ironweed		American sweetgum		Wingstem	
		% Control	Stem Density[bc]	% Control	Stem Density	% Control	Stem Density
Herbicide[a]	Rate	4 WAT	52 WAT	4 WAT	52 WAT	4 WAT	52 WAT
AMCP-ME + metsulfuron	70 + 42	77	0	58	0	85	0
aminopyralid + metsulfuron	70 + 42	43	0	45	1	83	0
AMCP-ME + 2,4-D	70 + 1080	85	0	53	0	90	0
aminopyralid + 2,4-D	70 + 1080	60	0	57	2	72	0
AMCP-ME + diflufenzopyr	70 + 70	72	0	58	1	88	0
aminopyralid + diflufenzopyr	70 + 70	43	0	53	0	72	0
Non – treated	-	0	1	0	0	0	0
$LSD_{0.05}$		12	2	13	2	15	0

[a]Herbicide treatments included methylated seed oil surfactant (Methylated Soybean Oil Plus. Universal Crop Protection Alliance, Eagan, MN) at 1% v/v.

[b] Aboveground stem density were made within two 0.25 m^2 areas selected at random on each plot. Numbers of stems reflect above soil surface.

[c]1 g ha^{-1} = 0.0143 oz/acre, 1 g = 0.0353 oz, 1 cm = 0.3937 inch, 1 stem/m^2 = 0.0929 stem/ft^2

[d]Abbreviations: AMCP-ME, aminocyclopyrachlor-methyl ester (DPX-KJM44 80% WG, DuPont Crop Protection. Wilmington, DE).

Figure 2.1 Japanese honeysuckle (*Lonicera japonica* Thunb.) white and yellow inflorescence.

Figure 2.2 Japanese honeysuckle (*Lonicera japonica* Thunb.) semi-evergreen foliage, stem and leaves.

CONCLUSION

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. 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. . 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). Translocation in large crabgrass during the first experimental run was slow as ≥ 94% of the absorbed radioactivity remained in the TL by 8 HAT. 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. 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. Translocation in both large crabgrass and black nightshade was primarily to the ROF and ATL plant sections. 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. 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). 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).

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.

Similar to our findings in the greenhouse, Japanese honeysuckle control increased with AMCP-ME rate (Table 2). AMCP-ME at 280 g ha^{-1} provided 85 to 90% control by 52 WAT. When applied alone, 2,4-D and metsulfuron-methyl controlled Japanese honeysuckle 42 to 68 %. Japanese honeysuckle control with aminopyralid and diflufenzopyr applied alone was ≤ 35% in each year as well. Inclusion of AMCP-ME at 70 g ha^{-1} increased Japanese honeysuckle with 2,4-D to 83 to 92%, similar to AMCP-ME alone at 280 g ha^{-1}. This mixture of AMCP-ME + 2,4-D also provided greater Japanese honeysuckle control than AMCP alone at 70 g ha^{-1} in. Our studies demonstrate that AMCP-ME has utility for managing the invasive weed Japanese honeysuckle in abandoned pasture fields. AMCP-ME mixtures with a phenoxy-carboxylic acid such as 2,4-D will provide more effective control of Japanese honeysuckle than with mixtures containing a pyridine herbicide such as aminopyralid and or the semicarbazone diflufenzopyr. This supports previously published reports of AMCP-ME efficacy for invasive species

management (Beeler et al. 2012; Koepke-Hill et al. 2011, 2012; West et al. 2011; Westra et al. 2008). Mixtures of AMCP-ME with 2,4-D can be used to effectively control Japanese honeysuckle and reduce aboveground biomass and stem density in place of increasing AMCP-ME application rate. Future research is needed to determine if incorporating AMCP-ME applications with non-chemical management such as prescribed burning or mechanical mowing will improve efficacy for Japanese honeysuckle control.

VITA

José Javier Vargas Almodóvar was born on September 25, 1979 in San Germán, Puerto Rico to José Dolores Vargas Lucena and Carmen Luisa Almodóvar Rivera. While living his entire childhood in historical San Germán, PR, he graduated from the college preparatory high school Colegio San Jose in 1998. Later he went on to attend The University of Puerto Rico at Mayagüez, where he received a Bachelor's degree in Agronomy in 2007. Upon completion of his undergraduate degree he pursued an opportunity at The University of Tennessee in Knoxville under the tutelage of Dr. Gregory R. Armel in the Horticultural and Invasive Weed Management Program. He began his Master of Science Degree with a major in Plant Sciences and emphasis in Weed Science in 2010.