DISSERTATION

UNDERSTANDING AMINOCYCLOPYRACHLOR BEHAVIOR IN SOIL AND

PLANTS

Submitted by

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ABSTRACT

UNDERSTANDING AMINOCYCLOPYRACHLOR BEAHVIOR IN SOIL AND
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Many noxious and invasive weeds are perennial species that are inherently
difficult to control. Canada thistle (*Cirsium arvense*) and field bindweed (*Convolvulus
arevensis*) are two species of particular interest as they are capable of spreading quite
rapidly through creeping underground reproductive structures and are able to continually
regenerate from carbohydrate reserves stored in the roots. These weed species infest both
cropland and non-cropland, including rangeland, pasture, natural areas, and rights-of-
way, causing yield loss in crops from competition for soil resources and by harboring
crop insect and disease pests as well as reducing ecosystem diversity in natural areas by
displacing desirable or native vegetation with monocultures. Based on long-term weed
control observed in the field with aminocyclopyrachlor (Lindenmayer et al. 2009), a
better understanding of the herbicide's behavior in soil as well as within the plants was
necessary. The objectives of this research were to (1) compare soil and foliar activity of
aminocyclopyrachlor on Canada thistle to that of aminopyralid; (2) determine the
dissipation rates of aminocyclopyrachlor, aminopyralid, and clopyralid under field
conditions as well as evaluate their adsorption in six North American soils; and (3)
evaluate aminocyclopyrachlor absorption, translocation, and metabolism in field
bindweed.

Results of the first study indicated that aminocyclopyrachlor was just as effective
when applied to the soil as it was when applied to Canada thistle foliage and was similar

to aminocyclopyrachlor for up to one year after treatment. The study also revealed that Canada thistle biomass was reduced to a far greater extent when either aminocyclopyrachlor or aminopyralid was absorbed via root tissue than by emerging shoot tissue.   Overall, these results suggest that Canada thistle control can be achieved even through dormant season applications, reversing the tradition of spring or fall applied herbicides to actively growing foliage and that xylem mobility throughout Canada thistle plants from root absorption may contribute to more effective weed control.

Results of the second study revealed that aminocyclopyrachlor, aminopyralid, and clopyralid all had similar dissipation rates under field conditions with soil half-lives of 32.5, 28.9, and 26.6 d, respectively. Mobility of aminocyclopyrachlor and aminopyralid was limited for the first 14 d with some downward movement after 28 d, while clopyralid had more significant leaching by 14 d. Adsorption in the six soils tested was greatest with aminocyclopyrachlor, followed by aminopyralid, and clopyralid had the least soil adsorption with average K_d values across the six soils of 0.503, 0.378, and 0.236 mL g^{-1}, respectively. Adsorption was generally correlated with soil organic matter or texture, but not with pH. These results agreed with previously published information about aminopyralid and clopyralid and shed new light on aminocyclopyrachlor soil behavior.

Results of the third study showed that aminocyclopyrachlor absorption in field bindweed was maximized at 48.3% of the applied radioactivity by 48 hours after treatment (HAT). A translocation pattern of movement out of the treated leaf into the other plant tissues was revealed, with nearly equivalent aminocyclopyrachlor distribution between the treated leaf, above-ground tissue, and below-ground tissue at 192 HAT. Over the 192 h, no soluble metabolites were observed, but an increasing portion of the

radioactivity was found in the fraction bound to the plant tissue. These results indicate that aminocyclopyrachlor has greater translocation to below-ground tissue in field bindweed compared with other herbicides and other weed species and aminocyclopyrachlor is not rapidly metabolized in any field bindweed plant tissue.

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INTRODUCTION

Canada thistle (*Cirsium arevense*) is a deep-rooted, perennial forb native to Europe, western Asia, and northern Africa (Dersheid & Shultz 1960). Before its introduction to North America, Canada thistle was already a problematic weed throughout southern Europe as early as the 16th century and, by the mid 18th century, it had spread throughout Europe (Dewey 1901). Canada thistle was first thought to have been introduced into Canada by French settlers in the early 17th century as a crop seed contaminant. Around the same time, it is believed that it was independently introduced to the American colonies (Dewey 1901; Hansen 1918). Canada thistle had become so common that legislation to control it was enacted in Vermont as early as 1795 and New York in 1831 (Moore 1975). By the turn of the 20th century, Dewey (1901) reported the presence of Canada thistle in India, Australia, New Zealand and every U.S. state bordering on or north of the 37th parallel.

Canada thistle is currently classified as a noxious weed in 43 U.S. states and much of Canada as far north as the 59° N and is the most frequently listed noxious weed infesting both cropland and wild lands in the United States (Moore 1975; Skinner et al. 2000). Canada thistle has earned its noxious classification mostly through to its ability to

rapidly invade ecosystems. For example, in 1901, Canada thistle was reported in only five small patches in Montana (Blankenship 1901); however, by 1956, it was reported to cover 625,000 acres (Heikes 1956). Canada thistle is a major crop pest causing significant yield reductions due to competition for soil resources and acting as an alternate host for other crop pests (Moore 1975). Hodgson (1968a,b) reported spring wheat yield reductions due to Canada thistle of 15%, 35%, and 60% for Canada thistle densities of 2.4, 14.3, and 29.8 shoots m^{-2}. Canada thistle has also been shown to cause a significant yield reduction in alfalfa grown for seed (Moyer et al. 1991) or forage (Schreiber 1967) and barley (O'Sullivan et al. 1982). Forage availability (Haggar et al. 1986) and production (Reece and Wilson 1983) can be limited by Canada thistle infestations in range and pasture. Canada thistle can severely limit species diversity in natural areas (Stachion and Zimdahl 1980) by displacing native diversity with a monoculture; however, the larger ecological and economic impacts are still largely undocumented (Lym and Duncan 2005).

The invasive nature of Canada thistle is a direct result of its extensive, creeping root system. Hayden (1934) and Rogers (1928) reported that Canada thistle roots can extend horizontally by up to 6 m in a single season. Root segments as small as 3 – 6 mm thick and 8 mm long are capable of producing new shoots (Hayden 1934; Prentiss 1889). Rogers (1928) also found that a root fragment older than six weeks and younger than two years can also generate an entire plant. This impressive regenerative capacity is due to the large reserve of carbohydrates stored in Canada thistle roots. These reserves vary seasonally, similar to other perennial species, with a low point in the spring and

increasing until the fall (Arny 1932; McAllister and Haderlie 1985; Rogers 1928; Welton et al. 1929).

Invasive perennial weeds, such as Canada thistle, which primarily spread vegetatively and regenerates from root segments, require a dedicated and integrated approach in order to achieve control. Early tillage studies (Hansen, 1918; Rogers 1928) indicated that cultivation immediately following Canada thistle shoot emergence was effective in starving the roots of carbohydrates, a practice known as "black fallow". Later work observed greater root reserve depletion with fewer cultivations when the new Canada thistle shoots were cultivated eight to ten days after emergence, corresponding to a shallow tillage of six to eight cm every 21 days (Seely 1952). However, intense tillage over an extended period of time would be detrimental due to depletion of soil moisture, and organic matter oxidation. Tillage may also serve to spread Canada thistle infestations.

Mowing has also been investigated as a means to control Canada thistle (Beck and Sebastian 2000; Welton et al. 1929) in non-cropland. Mowing before seed set during the early bloom growth stage will both deplete the reserves of carbohydrates in the roots as well as the soil seed bank. Repeated mowing at various intervals can also slowly starve the roots of the photoassimilates. Beck and Sebastian (2000) found that mowing three times per growing season at one month intervals controlled 85% of a Canada thistle infestation after two years. Control can be improved by combining with other cultural or chemical control options.

Seeding competitive plant species is another method that can be used in an integrated approach to control Canada thistle. Planting competitive grasses has also been shown to be effective in competing with Canada thistle (Wilson and Kachman 1999) and, in fact, after three years, Canada thistle control was greater than 90% where perennial grasses were established, which was comparable to chemical control. Alfalfa has also demonstrated a competitive ability with Canada thistle in several studies (Cox 1913; Detmers 1927; Schreiber 1967).

Some biological control efforts have been undertaken in the United States and Canada. In Canada, a survey of Canada thistle identified 84 insect species believed to damage thistle plants with another 44 species described as visitors or predators (Maw 1976). However, 38% of the listed Canada thistle pests also attacked other plants of economic importance. Three species have been identified as good biological control agents. *Altica caruorum* Guer. defoliates leaves of only certain *Cirsium* species while refusing foliage of related genera (Harris 1964). *Ceutorhynchus litura* (F.) (Coleoptera) adults eat young Canada thistle shoots, but are not the main control agent. Eggs laid in leaf veins during the rosette stage hatch larvae that mine from the leaf veins into the stem and root collar (Peschken and Beecher 1973). *Urophora cardui* L. (Diptera) also appears to be a promising biological control agent. Females lay eggs in the terminal buds of Canada thistle, where a gall later develops. From this gall, adult insects emerge in the following spring (Peschken 1971). While several species of fungi attack Canada thistle, they do not cause significant enough damage to be considered for biological control (Moore 1975).

There are a number of herbicide options for Canada thistle control. For Canada thistle infestations in small grains, 2,4-D, MCPA, dicamba, clopyralid, metsulfuron, triasulfuron, tribenuron, and various combinations have been used, with clopyralid plus 2,4-D providing the best results (Lym and Zollinger 2000). Clopyralid is commonly used to control Canada thistle in flax, sugarbeets, and corn, but has rotational restrictions for crops like peas, lentils, potatoes, and other broadleaf crops for up to 18 months after treatment (Lym and Zollinger 2000). Dicamba has also been used to control Canada thistle in corn. With the advent of glyphosate-tolerant crops, glyphosate has become a popular and effective chemical option for Canada thistle control. Herbicides are typically applied in the rosette stage of plant growth before the plant bolts. Research has shown that translocation of several herbicides is preferential to root tissue during the rosette growth stage, resulting in better long-term control (Armel et al. 2005; Hunter 1995; Miller and Lym 1998).

For Canada thistle control in range, pasture, and non-cropland areas, products containing picloram, clopyralid, aminopyralid, dicamba, dicamba + diflufenzopyr, 2,4-D amine, chlorsulfuron, glyphosate, and various combinations of the above herbicides have been recommended (Dewey et al. 2006; University of Nebraska 2006). Herbicide applications to range, pasture, and non-croplands are typically made during two periods: spring/early summer when the plants are in the late rosette/bolting/early bud growth stage or in the fall to shoot regrowth of new rosettes. This is equivalent to the practice in crops where greater translocation to roots was found during the rosette growth stage. Enloe et al. (2007) found little difference between spring and fall applications of aminopyralid, picloram, or clopyralid but suggested that the fall timing with these herbicides gives land

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managers more flexibility and allows them to conduct other weed control efforts in the spring.

Aminopyralid was the first herbicide in the range and pasture market granted reduced-risk classification due to its favorable toxicological, ecotoxicological, and environmental fate profile (Jachetta et al. 2005). It can be applied in riparian areas up to the water's edge where herbicides like clopyralid and picloram are not labeled for use. Recently, the new auxinic herbicide, aminocyclopyrachlor, was introduced with similar characteristics as aminopyralid. Aminocyclopyrachlor is the first pyrimidine carboxylic acid herbicide and has been formulated as both the carboxylic acid as well as with an additional methyl-ester group to facilitate foliar absorption. Aminocyclopyrachlor has a proposed use pattern in non-cropland and rangeland to control broadleaf weeds and shrubs (Turner et al. 2009). Several species in numerous dicot families are sensitive to aminocyclopyrachlor, including Asteraceae, Fabaceae, Chenopodiaceae, Convolvulaceae, and Euphorbiaceae (Armel et al. 2009; Claus et al. 2008; Jenks 2010; Turner et al. 2009). Aminocyclopyrachlor has selectivity on many monocot species and has great potential for use in ecosystem restoration work (Edwards 2008; Vassios et al. 2009). Effective control of perennial weeds is paramount in these situations.

Observations from the field indicate that aminocyclopyrachlor is a very effective herbicide for Canada thistle. Aminocyclopyrachlor provided excellent control for up to 14 months after treatment with use-rates as low as 35 g ai ha^{-1}, which was similar to aminopyralid, but outperformed chlorsulfuron (Lindenmayer et al. 2009). Interestingly, laboratory studies have shown limited below-ground translocation of both aminopyralid and aminocyclopyrachlor in Canada thistle (Bukun et al. 2009; Bukun et al. 2010).

Despite limited translocation from foliar to root tissue, both herbicides seem to be very

effective against Canada thistle, suggesting that soil residual activity plays a role in long-

term weed control. It is hypothesized that perhaps the herbicide forms a boundary layer

in the soil, preventing the emergence of new shoots from lateral roots. To better

understand the mechanism by which Canada thistle is controlled by

aminocyclopyrachlor, the objectives of these field and greenhouse studies were to 1)

determine the efficacy of aminocyclopyrachlor when soil- or foliar-applied to Canada

thistle, compare to equivalent applications of aminopyralid, and 2) determine whether

root or foliar absorption of aminocyclopyrachlor or aminopyralid is more effective in

controlling Canada thistle.

MATERIALS AND METHODS

Field Evaluation of Soil and Foliar Herbicide Activity.

The first experiment was conducted to determine the efficacy of

aminocyclopyrachlor when applied to foliage or to soil and compare to equivalent

treatments of aminopyralid. Two sites were selected for this study; one was rain-fed only

(Kerbel), while the other was supplemented with 50 cm of sprinkler irrigation from May

to September (ARDEC). Both sites received 26 cm of rainfall the year following

herbicide application. Additionally, both sites had similar soil types, with ARDEC

having a Fort Collins loam (fine-loamy, mixed, superactive, mesic aridic haplustalf)

(1.5% OM, 44% sand, 41% silt,15% clay) and Kerbel having a Garrett loam (fine-loamy,

mixed, superactive, mesic pachic argiustoll) (2% OM, 44% sand, 41% silt, 15% clay).

Both sites had dense populations of Canada thistle at the beginning of the study, with an

average of 21 ± 4 and 19.5 ± 8.2 plants m^{-2} at ARDEC and Kerbel, respectively. Canada thistle plants were at the bud to early flowering growth stage at the time of herbicide treatment application.

The study area was prepared by shallowly tilling (2 cm deep) half of the area in strips to defoliate half of the existing Canada thistle plants using a tractor-mounted roto-tiller implement, while the remaining strips were undisturbed. A completely randomized design with randomization restricted to the tillage treatments was used to assign herbicide treatments to the individual plots. Therefore, the five herbicide treatments were applied so that they appeared in each of the two tillage treatments, for a total of ten herbicide and tillage treatment combinations. Each herbicide and tillage combination was replicated three times at each site. Plots measuring 3.05 x 4.57 m were established in the study area and were oversprayed with a CO_2 backpack sprayer calibrated to deliver 187 L ha^{-1} at 206 kPa on Sept. 30 at ARDEC and Oct. 2, 2008 at Kerbel. Aminocyclopyrachlor (MAT28) (DuPont, Wilmington, DE 19898) was applied at 140 g ai ha^{-1} in two formulations; a soluble liquid (SL) and a soluble granule (SG). Aminocyclopyrachlor methyl-ester (KJM44) (DuPont, Wilmington, DE 19898) was also applied at a rate of 140 g ai ha^{-1} as a SG. Aminopyralid (Dow AgroSciences LLC, Indianapolis, IN 46268) was applied at 126 g ai ha^{-1} as a SL. An untreated check was also included for each tillage treatment. Percent control for each of the herbicide and tillage treatment combinations was measured one year after treatment (YAT) based on biomass samples. These biomass samples were collected by clipping the above-ground biomass at the soil level in a 1.0 m^2 area in each plot. The biomass was then oven-dried for one week at 60 C and weighed.

Greenhouse Evaluation of Herbicide Site of Absorption.

Canada thistle roots were collected Oct. 4, 2010 from untreated adjacent plots at the ARDEC site previously used for the evaluation of soil and foliar herbicide activity. The roots were stored at 1 C for two weeks to promote root bud initiation. After the cold storage period, roots with visible bud initiation were selected and cut to 8 cm segments, each with at least one visible bud. Root segments were then planted in a Fort Collins loam soil sieved to 6 mm. Three root segments were planted per plastic pot (American Clay Works and Supply Co., Denver, CO 80204) measuring 13 cm tall x 13 cm wide x 6 cm deep.

A two by three factorial design was used to evaluate the effects of both herbicide and site of herbicide absorption. Both aminocyclopyrachlor and aminopyralid were applied at rates of 70 g ai ha^{-1} to their respective pots. An untreated control pot was also included for comparison. To determine the site of absorption, an approach similar to that taken by Enloe et al. (1999) was used to create a shoot zone and root zone layered herbicide application (Fig. 1.1).

For shoot zone treatments meant to simulate shoot absorption, each pot was filled with 2.5 cm of soil and the three root segments were placed evenly in the pot. Another 0.5 cm layer of soil was added, followed by a thin layer of activated charcoal (Norit Americas, Inc., Atlanta, GA 30338). The charcoal was added to prevent any herbicide from leaching below the level of the roots during application and incorporation. The pots were then filled with a final 2 cm layer of soil and the herbicides were applied to the soil surface and incorporated into the shoot zone with 0.5 cm of simulated rainfall using a

single-nozzle, overhead track sprayer (DeVries Manufacturing Corp., Hollandale, MN 56045) calibrated to deliver 187 L ha^{-1} at 206 kPa. To simulate root absorption only, each pot was filled with a 2 cm layer of soil. The herbicides were then applied and incorporated as previously described. A 1.0 cm layer of soil was then placed on the treated surface. The root segments were then placed in the pot and covered with a final 2 cm layer of soil.

All pots were sub-irrigated as needed and allowed to grow for 28 days. Sub irrigation of the pots was used to prevent downward movement of the herbicide in the surface-applied treatments. The amount of water each pot received from sub-irrigation (50 mL every two to three days) was not thought to be enough to cause significant upward movement of the herbicides and provided a more controlled and even moisture level in the pots.   After the 28 day study period, the above-ground biomass was clipped at the soil surface, oven-dried for one week at 60 C, and then weighed. The roots were also extracted from the soil and new root growth was removed from the original root segments, washed, oven-dried, and weighed. There were three replicates of each treatment combination and the study was repeated.

Data Analysis.

Data were analyzed using analysis of variance (ANOVA) (SAS Institute Inc., Cary, NC 27519) and treatment means were compared using Fisher's Protected Least Significant Difference (LSD) test (SAS Institute Inc., Cary, NC 27519) (P ≤ 0.05). The ANOVA for the field evaluation of foliar and soil herbicide activity was analyzed with site, tillage, and herbicide treatments as class variables. On the other hand, the ANOVA

for the greenhouse evaluation of herbicide site of absorption was analyzed with herbicide and soil layer treatments as class variables. An α of 0.05 was used, unless otherwise noted. The greenhouse experiments were repeated.

RESULTS AND DISCUSSION

Field Evaluation of Foliar and Soil Herbicide Activity.

Biomass data taken one YAT for herbicide treatments were normalized by transforming the data to a percent of the untreated control biomass for each respective tillage treatment to allow for a more even comparison. No significant site difference and no significant tillage effect were detected at an α = 0.05 level. Therefore, comparisons between herbicide treatments were combined over site and tillage treatments. It is very interesting that site was not a significant factor, despite the differences in irrigation. The fact that the reduction in Canada thistle biomass relative to the biomass of the untreated controls was similar between both sites indicates that these herbicides can perform well across a range of environments that may span from more arid upland to wetter lowland.

Perhaps most interesting is the fact that the tillage treatment was not significant, indicating that soil activity alone was just as effective in controlling Canada thistle as applying the herbicides to actively growing foliage. This finding has two important implications. First, it indicates that aminocyclopyrachlor and aminopyralid soil residual activity may make a major contribution to long-term control of perennial weed species, such as Canada thistle. This seems to agree with the fact that both sites had similar relative Canada thistle control, possibly due to soil residual activity. It could be speculated that maintaining a lethal level of herbicide concentration in the soil may

deplete energy reserves in the roots faster than repeated stress to above-ground plant tissue, especially since root tissue especially sensitive to auxinic herbicides.

Herbicide treatment means indicate that biomass reduction relative to the untreated control biomass was statistically similar regardless of herbicide or herbicide formulation 1 YAT (P = 0.06). The aminocyclopyrachlor formulations provided an average of 97% (± 2%), 93% (± 8%), and 93% (± 5%) relative Canada thistle biomass reduction for the MAT 28 SL and SG, and KJM44 SG, respectively, while aminopyralid provided an average of 76% (± 17%) relative Canada thistle biomass reduction. These results would suggest that all of the formulations of aminocyclopyrachlor provided equivalent Canada thistle control and that they performed similarly to aminopyralid one YAT, although aminopyralid control was more variable. The fact that Canada thistle control with the methyl ester form of aminocyclopyrachlor was equivalent to the free acid form, indicating that the active form of the herbicide is the free acid and that, while the methyl ester group may facilitate absorption, control is equivalent in the long-run. This is supported by Bukun et al. (2010) who found a rapid conversion of the methyl ester form to the free acid form in Canada thistle plants. Esterase activity is present in soil (Pancholy and Lynd 1971) and would convert the methyl ester to the free acid.

These data suggest that effective herbicide applications to perennial weeds, like Canada thistle, do not need to be confined to the fall. Fall applications provide more flexibility and opportunity for application timing for Canada thistle control with these herbicides. Herbicide translocation to root tissue is maximized when herbicide application timing is synchronized with seasonal carbohydrate flow to plant roots (Wilson et al. 2006). Additional research (McAllister and Haderlie 1985) has shown that

root bud growth and carbohydrate root reserves are greatest in the late fall, indicating a basipetal flux of plant photoassimilates. Miller and Lym (1998) found that clopyralid translocation to Canada thistle roots was greatest when applied during the fall rosette stage compared to the spring bolting stage and a similar observation was made by Hunter (1995) with glyphosate. However, more recent research would suggest that aminopyralid, clopyralid, and picloram are just as effective when applied in the spring as in the fall (Enloe et al. 2007; Sebastian et al. 1992). Fall herbicide applications to foliage usually require multiple re-treatments over several years to deplete root reserves at a high cost to land managers. Also, selectivity of traditional herbicides, such as picloram, at rates required to control Canada thistle may become an issue due to carryover concerns (Senseman 2007). In range and pasture situations, native species and desirable grass sensitivity is of concern. The combination of low use rates, selectivity, and soil residual activity of aminocyclopyrachlor and aminopyralid may be attractive to land managers as the herbicides provide greater flexibility in perennial weed management programs. Based on these results, applying either of these herbicides to dormant Canada thistle plants, even when there is no above-ground biomass, would be just as effective as applications made to actively growing plants, presumably due to the soil residual activity. This would allow land managers more time to focus management efforts on other weed species that require a spring or summer treatment regime.

Greenhouse Evaluation of Herbicide Site of Absorption.

Both above- and below-ground biomass were collected 28 DAT and data were normalized by transforming them to a percent of their relative untreated control for a more even comparison. ANOVA analysis showed that there was no significant

difference between repeats of the experiment (P = 0.67 and 0.17 for above- and below-ground biomass, respectively); therefore, the datasets were combined across repeats for analysis. The above-ground biomass response to herbicide treatments was highly significant (P = 0.0002, α = 0.05), while the below-ground biomass response to herbicide treatments was less significant (P = 0.08, α = 0.10). Based on Fisher's Protected LSD, there was a significant effect of herbicide layer location in the soil on both above- and below-ground biomass (Fig. 1.2). Canada thistle above-ground biomass reduction was greatest when the herbicide-treated layer of soil was located in the root zone and was similar for both aminocyclopyrachlor and aminopyralid (8 ± 4 % and 0.2 ± 0.2 % of the untreated control, respectively). Above-ground biomass reduction was least when the herbicide-treated layer of soil was positioned in the shoot zone and was, again, similar for both aminocyclopyrachlor and aminopyralid (85 ± 14 % and 60 ± 18 % of the untreated control, respectively).

The Fisher's Protected LSD for the relative below-ground biomass was similar to above-ground biomass in trend. The greatest Canada thistle below-ground biomass reduction was observed when the aminocyclopyrachlor-treated layer of soil was in the root zone (14 ± 7 % of the untreated control), while the least below-ground biomass reduction was found when the aminopyralid-treated layer of soil was located in the shoot zone (117 ± 50 % of the). When aminocyclopyrachlor-treated soil was positioned in the shoot zone and when the aminopyralid-treated soil was positioned in the root zone resulted in intermediate below-ground biomass (90 ± 18 % and 43 ± 26 %, respectively).

Visually, the Canada thistle plants that grew in the shoot zone treatments showed symptoms of herbicide absorption, such as twisting of the stems, epinasty of the leaves,

and inhibited growth of apical meristems (Fig. 1.3). Roots of these plants showed little inhibition of growth compared to the untreated controls. On the other hand, plants that grew in the root zone treatments rarely emerged and, if they did, had severe inhibition of root and shoot growth, thickening of the stem tissue, and necrosis (Fig. 1.3).

The differences in both above- and below-ground biomass production, as well as visual symptomology, clearly indicate herbicide absorption via root tissue was most inhibitory to Canada thistle growth. The initial hypothesis that Canada thistle root buds were emerging through a layer of soil where residual herbicide was concentrated and absorbing herbicide through shoot tissue was invalidated as the mechanism for long-term weed control. Instead, the results would indicate that Canada thistle roots are capable of directly absorbing both aminocyclopyrachlor and aminopyralid, contributing to control of up to a year after treatment. It is possible that constant root tissue exposure to herbicides in the soil is more disruptive of biological function.

Given the high water solubility of aminocyclopyrachlor and aminopyralid, (4.2 g L^{-1} at pH 7, 20 C and 2.48 g L^{-1} at 18 C, respectively) (Finkelstein et al. 2008; Senseman et al. 2007), the herbicides should stay solubilized in the soil solution and be readily absorbed by the roots. Additionally, the pK_a (4.65) and low log K_{ow} (-2.48 at pH 7, 20 C) (Finkelstein 2008; Bukun et al. 2010) of aminocyclopyrachlor provide an ideal situation in western U.S. soils where very little of the herbicide is adsorbed to the soil, enhancing its bioavailability. Similarly, the pK_a (2.56) and log K_{ow} (-2.96 at pH 7 and 20 C) (Senseman et al. 2007) of aminopyralid result in little soil binding. Low lipophilicity is not an issue for root absorption since there is no cuticle surrounding root tissue, but can present an issue to translocation due to plasma membranes and the Casparian strip.

Bukun et al. (2009; 2010) demonstrated limited phloem mobility for both aminopyralid and aminocyclopyrachlor from treated Canada thistle leaves to roots (7 and 6% at 192 hours after treatment HAT for aminopyralid and aminocyclopyrachlor, respectively) or throughout above-ground biomass (10 and 15% at 192 hours after treatment for aminopyralid and aminocyclopyrachlor, respectively). For long-distance phloem transport these herbicides may not be lipophilic enough to cross plasma membranes and move symplastically to the vasucular steele in the roots. At pH 4, the carboxylic acid on aminocyclopyrachlor protonates and the log K_{ow} increases slightly to -1.12, slightly decreasing the water solubility to 3.13 g L^{-1} (Finkelstein et al. 2008). Similarly, aminopyralid has a log K_{ow} of -1.76 at pH 5 and remains quite water soluble (2.48 g L^{-1}) (Senseman et al. 2007).

The more acidic conditions would mimic the apoplast in plant tissue and would indicate that the undissociated form remains relatively hydrophilic. The water soluble nature of aminocyclopyrachlor at physiological pH levels has implications on its ability to permeate plasma membranes and accumulate in cells and the phloem. The unifying weak acid theory (Kleier 1998) has been found to accurately predict phloem mobility as a function of both pK_a and log K_{ow} under experimental conditions (Hsu et al. 1988; Hsu and Kleier 1990; Grayson and Kleier 1990). The model has also led other researchers to propose that there is no single pK_a or single log K_{ow} that imparts optimum phloem mobility, but rather the two parameters are co-dependent in predicting symplastic transport (Bromilow 1990). Thus, the low log K_{ow} of these herbicides may negate the ideal pK_a for both aminocyclopyrachlor and aminopyralid, reducing acid trapping and limiting phloem mobility. Additionally, it has been proposed that the second nitrogen

atom in the pyrimidine ring of aminocyclopyrachlor may provide polarity to the molecule despite the neutral carboxylic acid (Bukun et al. 2010).

With limited phloem mobility, these herbicides will remain in the apoplast and move through intercellular space and cell walls. Normally, access to the vascular stele requires crossing the Casparian strip, forcing molecules to move symplastically through the endodermal cells of the root. One measure of the magnitude of xylem translocation developed by Shone and Wood (1974) is the transpiration stream concentration factor (TSCF). Experiments have shown that TSCF is directly related to the log K_{ow} of an herbicide (Hsu et al. 1990) and that herbicides with a log K_{ow} value between 2.0 and 4.0 would have optimal xylem mobility. Hsu et al. (1990) also considered the effects of soil on the TSCF/log K_{ow} relationship and found that this shifted the optimum log K_{ow} range to between 0.0 and 1.0. Neither aminocyclopyrachlor nor aminopyralid fit in this range and should not have excellent xylem mobility. However, weak acid herbicide TSCF values are more closely related the root concentration factor (RCF), also developed by Shone and Wood (1974), which have also been related to log K_{ow} values (Briggs et al. 1982). This relationship would still indicate that at log K_{ow} values as low as either aminocyclopyrachlor or aminopyralid, the RCF response would be flat and that the external solution is simply in equilibrium with the internal water in the root cells at a RCF value of about 0.90, since roots are roughly 90% water, and the herbicide is only passively diffusing.

Fortunately, there is a route that bypasses the endodermis and Casparian strip, providing direct access to the xylem and phloem without having to cross any membranes. Lateral roots, which develop from the pericycle, a layer of cells just interior of the

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endodermis, provide an entirely apoplastic route directly to the steele. Once in the steele, these herbicides could continue moving apoplastically to the xylem allowing them to be translocated with the water column. Another possibility not tested in these experiments is the presence of an active transport system that facilitates auxin absorption by the roots.

The hypothesis of root absorption may help explain the effective weed control observed for these herbicides when applied to the soil. The ability of both aminocyclopyrachlor and aminopyralid to be absorbed by the roots with the soil solution and translocated apoplastically to actively growing portions of the plant provides for an effective strategy for weed control. Long-term control of perennial weed species, consistent with observations made in the field (Lindenmayer et al. 2009), may be derived from constant exposure of the roots to lethal concentrations of residual herbicide in the soil. Perhaps, by depleting carbohydrate reserves in the roots of perennial species, such as Canada thistle, the ability to vegetatively regenerate from lateral root buds is greatly diminished and weed control is achieved. Taken together, the results of both experiments would indicate the importance of soil residual activity of both aminocyclopyrachlor and aminopyralid. The low use-rates, selectivity, and apparent flexibility in application timing gives land managers more options for perennial weed control with these herbicides and make them a very attractive option as part of an integrated weed management plan.
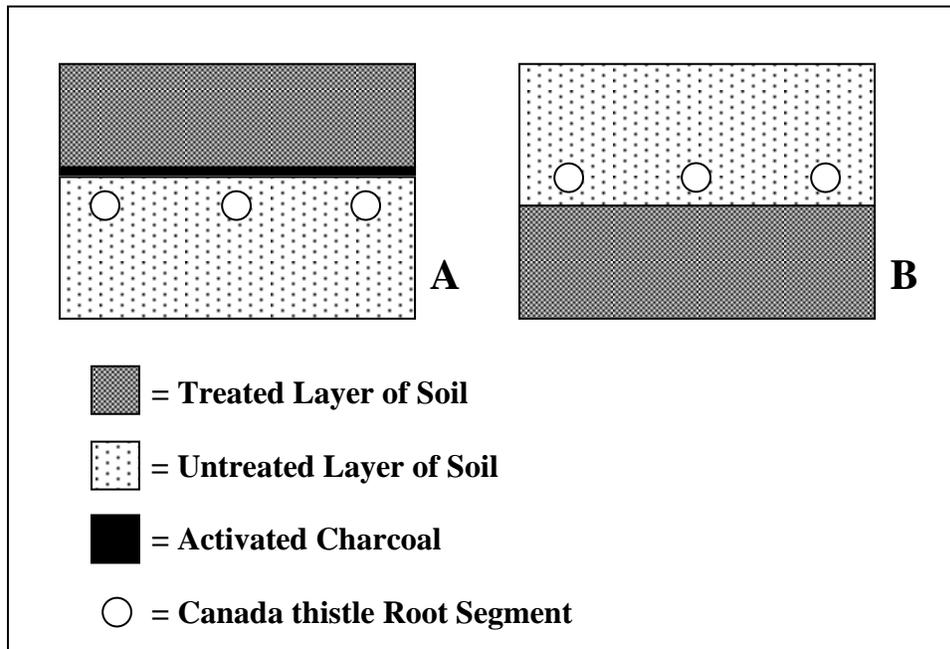
Figure 1.1. Potting schematic for layered herbicide application. Pane A illustrates a surface herbicide application while pane B illustrates a sub-surface herbicide application.
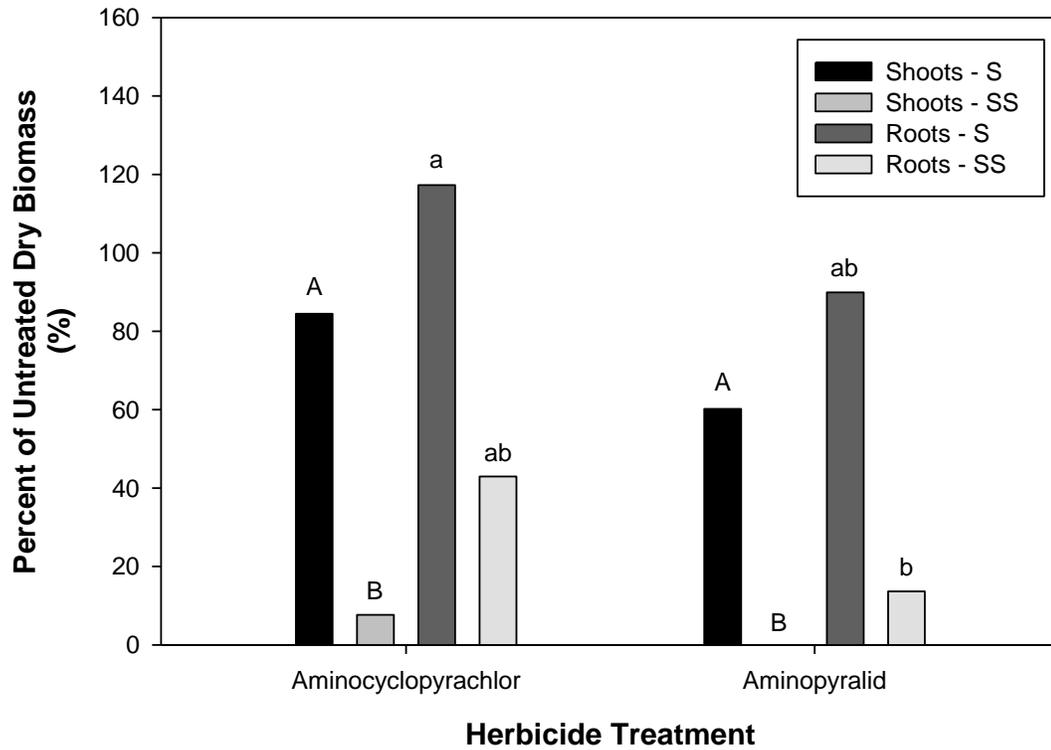
Figure 1.2. Canada thistle root and shoot biomass response to layered application of aminocyclopyrachlor and aminopyralid. Herbicide applications were made to the surface (S) and to the sub-surface (SS) layers of soil. Different letters designate statistical differences between treatment means based on Fishers's Protected LSD (P < 0.05).
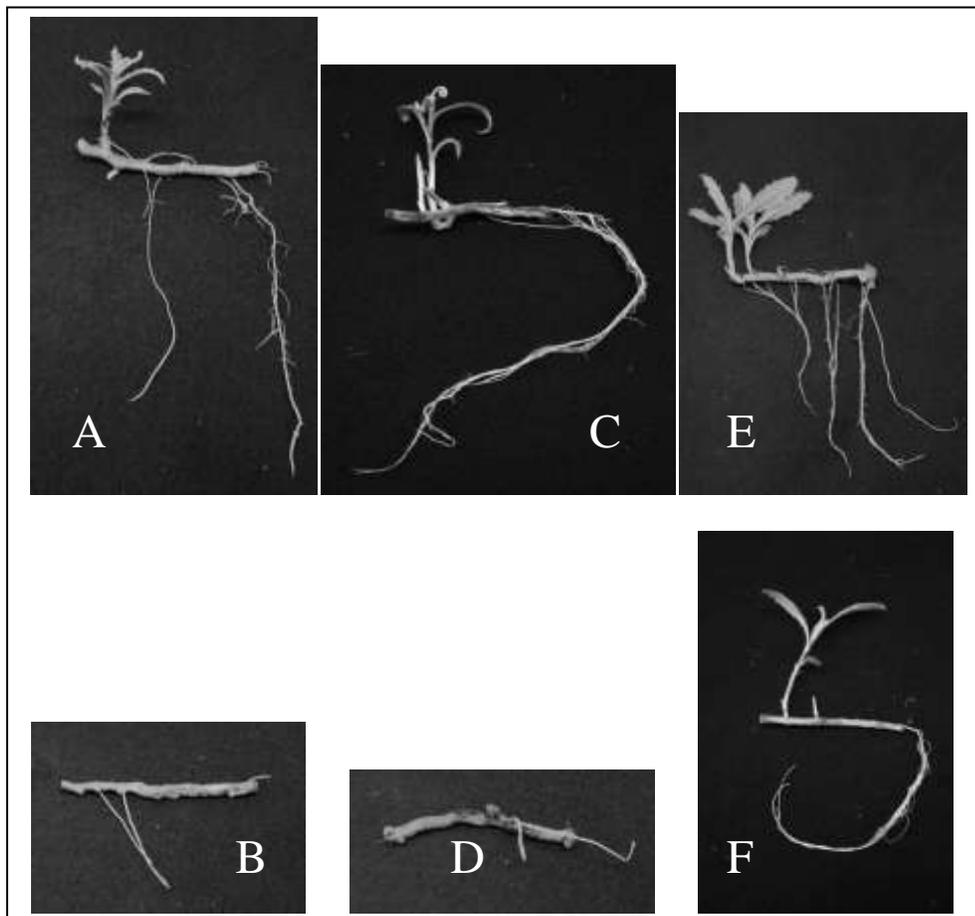
Figure 1.3. Photographs illustrating the visual differences in Canada thistle growth between surface and sub-surface herbicide applications. Panes A and B show the surface and sub-surface aminocyclopyrachlor applications, respectively, while panes C and D show the surface and sub-surface aminopyralid applications, respectively. Panes E and F show the surface and sub-surface untreated controls, respectively.

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CHAPTER TWO: AMINOCYCLOPYRACHLOR, AMINOPYRALID,

AND CLOPYRALID DISSIPATION IN SOIL UNDER FIELD

CONDITIONS AND ADSORPTION IN SIX NORTH AMERICAN SOILS

INTRODUCTION

Aminopyralid and clopyralid are pyridine carboxylic acid herbicides with a broad spectrum of weed control that includes species in the Asteraceae, Fabaceae, and Solanaceae families. Clopyralid has been the herbicide of choice for selective broadleaf weed control in cropland as well as range, pasture, natural areas, and rights-of-way, especially for Canada thistle (*Cirsium arvense* L.) (Carrithers et al. 2005). Clopyralid has soil residual activity providing substantial residual weed control with soil half-lives ranging from 12-70 d and an average soil half-life of 40 d (Senseman et al. 2007). Clopyralid is also known to be weakly adsorbed by soils and remains in the soil solution with an average K_{oc} of 6 mL g^{-1}, making vertical movement in the soil profile possible. Several studies have shown that that clopyralid is mobile in the soil profile (Bergstrom et al. 1991; Bovey and Richardson 1991; Bukun et al. 2010b; Elliot et al. 2000; Pik et al. 1977; Sakaliene et al. 2009; Smith and Aubin 1989). This has precluded its labeling for use in riparian areas.

Aminopyralid provides excellent control of similar weed species as clopyralid at lower application rates (Enloe et al. 2007). Aminopyralid is similar to clopyralid in its soil persistence with an average soil half-life of 34.5 d but a slightly more narrow range of soil half-lives of 25 to 35 d (Senseman et al. 2007). However, there is evidence that aminopyralid is less mobile in the soil. It has a slightly higher K_{oc} value than clopyralid

at 10.8 mL g^{-1}. Bukun et al. (2010b) reported that aminopyralid had a higher K_d (0.299

mL g^{-1}) compared with clopyralid (0.186 mL g^{-1}) and aminopyralid was also less mobile

($R_f = 0.82$) than clopyralid ($R_f = 0.91$). Fast et al. (2010) also found aminopyralid had

greater potential to bind to clay minerals than picloram. The low use rate and reduced

mobility has made aminopyralid a popular choice for land managers. These

characteristics have allowed the aminopyralid to be used in riparian areas due to its

favorable toxicological, ecotoxicological, and environmental fate profile (Jachetta et al.

2005).

Aminocyclopyrachlor is the first pyrimidine carboxylic acid herbicide with a

proposed use pattern in non-cropland and rangeland to control broadleaf weeds and

shrubs (Turner et al. 2009). Several species in numerous dicot families have been

controlled with aminocyclopyrachlor, including Asteraceae, Fabaceae, Chenopodiaceae,

Convulvulaceae, and Euphorbiaceae (Armel et al. 2009, Claus et al. 2008, Jenks 2010,

Turner et al. 2009). Aminocyclopyrachlor also has selectivity on some monocot species

and has great potential for use in ecosystem restoration work (Edwards 2008, Vassios et

al. 2009). Additionally there is evidence that aminocyclopyrachlor is even less mobile

than either clopyralid or aminopyralid with a K_{oc} of 28 mL g^{-1} (Finkelstein et al. 2008).

Finkelstein et al. (2008) also reported that aminocyclopyrachlor had a range of soil half-

lives from 72 to 128 d. However, there is very little additional information published

about aminocyclopyrachlor behavior in the soil.

The environment and certain edaphic factors can impact herbicide persistence in

the soil. Exposure to high amounts of sunlight can quickly degrade herbicides if left on

an exposed surface. Ultra-violet radiation can cause chemical phytolysis of several

auxinic herbicides, such as picloram, aminopyralid, and aminocyclopyrachlor if left on the soil surface for more than a few days (Finkelstein et al. 2008; Senseman et al. 2007). However, quinclorac and clopyralid appear to have little to no photodegradation (Senseman et al. 2007). The probability of photodegradation occurring increases with hotter and drier weather. Lack of precipitation will prevent the incorporation of the herbicide in to the soil where they are protected from degrading UV rays.

Microbial degradation is yet another source of herbicide dissipation in the environment. All auxinic herbicides are primarily broken down by soil microbes, but the rate at which this degradation occurs varies. The classic persistent auxinic herbicide, picloram, is susceptible to microbial degradation by aerobic microbes, but this process is slow, resulting in an average half-life of 90 days in the soil (Senseman et al. 2007). Quinclorac may also persist in the soil for over a year (Senseman et al. 2007) and injure susceptible crops like corn and sorghum. Precipitation level, C:N ratio, soil temperature, and history of herbicide applications can affect microbial degradation. High soil moisture, high C:N ratios, and warm soil temperatures will contribute to enhanced rates of herbicide degradation. There is also evidence to suggest that repeated applications of herbicides to soil can result in the build-up of microbe populations adapted to metabolizing specific herbicides. One example of enhanced degradation is atrazine in adapted agricultural fields with a 10-fold faster rate than non-adapted soils (Krutz et al., 2008).

Soil adsorption is another way herbicide uptake by plants can be limiting in the soil. In fact, soil adsorption may be one of the most important factors affecting herbicide fate in soil. Herbicide adsorption to soil particles can affect its degradation rate, plant

availability, and overall efficacy (Kah and Brown 2007). The relationship between

herbicide adsorption, soil organic matter (OM), and pH are often important parameters

used in pesticide fate models to predict herbicide behavior among different soils

(Farenhorst et al. 2003, 2008; Novak et al. 1997). However, adsorption is not a major

issue for most of the auxinic herbicides as they usually occur as the anionic free acids in

the soil due to the low pK_a value of their predominant ionizable carboxyl functional

group and their relatively low log K_{ow} values. Since most Western soils are neutral or

basic, soil adsorption could be considered negligible; however, adsorption becomes

important in acidic forest or prairie soils rich in organic matter.

Anion sorption has been found to vary with pH reaching maximum sorption

around the molecule's pK_a (Hinsington 1981). When the pH of a soil solution is above

the pK_a of an herbicide, the molecule will have a negative charge. In basic soils, anion

adsorption may be driven by the diffuse layer of Ca or Mg cations surrounding clay

particles according to the electric double layer theory first described by Gouy (1910) and

Chapman (1913). Sorption of anions to iron and aluminum oxides with high points of

zero charge (pzc) in acidic soils can also result in a net positive charge resulting in anion

adsorption (Sparks 2003).

For herbicides with soil residual activity, keeping the concentration of herbicide

high enough to be phytotoxic in the root zone long enough is key to acceptable control.

The relatively low log K_{ow} values of clopyralid, aminopyralid, and aminocyclopyrachlor

indicates that they will are hydrophilic. Aminocyclopyrachlor has a water solubility pH 7

and 20°C of 4.2 g L^{-1} making it the most water soluble of the herbicides included in this

strudy (Finkelstein et al. 2008). Aminopyralid follows at 2.48 g L^{-1} and clopyralid is 1.00

g L^{-1} (Senseman et al. 2007). These factors indicate that these three herbicides have a potential for leaching with water in the soil profile or being carried away with surface runoff.

Aminocyclopyrachlor has great potential to provide land managers with control of a wide spectrum of weed species spanning many different environments at attractive use-rates. Therefore, it will be important to better understand how it behaves the soil in terms of persistence in the environment, mobility in the soil profile, and plant availability. Since aminocyclopyrachlor may have similar potential use-patterns as clopyralid and aminopyralid, it will also be important to make comparisons to these widely used compounds. Thus, the objectives of the following studies were (1) to quantify and compare dissipation rates under field conditions for aminocyclopyrachlor, aminopyralid, and clopyralid and calculate their soil half-lives; (2) to determine and compare mobility of these herbicides under field conditions; and (3) to quantify and compare availability of the three herbicides by determining their distribution coefficients (K_ds).

MATERIALS AND METHODS

Herbicide Dissipation in Soil Under Field Conditions.

Herbicide Application. Two sites with similar soil properties were chosen for this experiment. One site was located at the Colorado State University Agricultural Research, Development, and Education Center (ARDEC) while the other was located at the Colorado State University Horticultural Farm (Hort Farm). The soil at ARDEC is a Fort Collins loam (fine-loamy, mixed, superactive, mesic aridic haplustalf) (1.5% OM, 44% sand, 41% silt, 15% clay) and soil at the Hort Farm is a Nunn clay loam (fine, smectitic, mesic aridic argiustolls) (2.5% OM, 35% sand, 34% silt, 31% clay). Both sites received

27 cm of precipitation during the study period and an additional 30 cm of irrigation. On

May 20, 2010, aminocyclopyrachlor (DuPont, Wilmington, DE 19898), aminopyralid

(Dow AgroSciences LLC, Indianapolis, IN 46268), and clopyralid (Dow AgroSciences

LLC, Indianapolis, IN 46268) were all applied at a rate of 1.12 kg ai ha^{-1} using a CO_2

backpack sprayer set to deliver 280 L ha^{-1} at 206 kPa to bare soil.

The individual plots measured 3 m by 9 m and each herbicide treatment was

replicated three times. To establish the initial spray application at day zero after

application (0 DAA) time point, three open 9.2 cm plastic petri dishes (Thermo Fisher

Scientific, Waltham, MA 02454) with 9.0 cm filter papers (Whatman International Ltd.,

England) placed inside were laid out evenly in each plot to capture the spray solution.

The lids were immediately place on the petri dishes after application, para-filmed, and

placed in coolers for transport to a -20 C freezer for storage until analysis.

Soil Sampling. Soil samples were taken from each site at predetermined intervals of 7,

14, 28, 56, 128, and 365 DAA to a depth of 30 cm using a zero-contamination soil

sampler (Clements Associates Inc., Newton, IA 50208) equipped with plastic sleeves

(Clements Associates Inc., Newton, IA 50208). Three soil cores were taken in each plot

and the locations of the samples were randomized within the plot areas by superimposing

a 30 cm^2 grid and assigning each sample location a set of coordinates. This ensured that

a sampling coordinate was not repeated over the sampling time points. The plastic

sleeves containing the soil cores were immediately capped and were placed in coolers for

transport to a -20 C freezer for storage until sample preparation and analysis.

0 DAA Sample Preparation. The filter papers were removed from the individual sealed petri dishes and were cut in to 1 cm^2 pieces. These pieces were placed in a 50 mL centrifuge tube (Thermo Fisher Scientific, Waltham, MA 02454) and 10 mL deionized water was added. The tubes were placed on a horizontal shaker for 2 h, then centrifuged at 2,000 rpm for 10 min to separate the paper from the liquid supernatant. A 100 µL sub-sample of the liquid supernatant was diluted with 10 mL of a solution consisting of 1% HPLC-grade acetonitirle, 99% HPLC-grade water, and 0.05% phosphoric acid. An aliquot was transferred to a sample vial for HPLC analysis.

Aminocyclopyrachlor Soil Sample Preparation. Three individual soil cores from each aminocyclopyrachlor plot were separated into three depths: 0-5, 5-15, and 15-30 cm. The soil from each depth was combined by depth to create one sample for each of the three depths from each plot. A 5 g sub-sample from each depth, plot, and site combination was weighed into a 50 mL plastic centrifuge tube (Thermo Fisher Scientific, Waltham, MA 02454) and 10 mL of deionized water was added. The soil solution was allowed to shake for 2 h and was then centrifuged at 4,700 rpm for 20 min to separate the soil and liquid fractions. An aliquot of the liquid supernatant was the placed in a 2 mL Eppendorf filter microfuge tube with a 0.45 µm nylon filter (Corning, Inc., Corning, NY 14831) and centrifuged at 15,000 rpm for 20 min. An aliquot of the filtered liquid was then placed in a 2 mL sample vial with limited volume inserts (Thermo Fisher Scientific, Waltham, MA 02454) for high performance liquid chromatography (HPLC) analysis. Another sub-sample of soil from each depth, plot, and site combination was oven dried at 110 C for 24

h to determine soil water content. Initial quality control (QC) samples had an average recovery of 95%.

Clopyralid Soil Sample Preparation. Preparation of the soil samples from plots sprayed with clopyralid was similar to that of aminocyclopyrachlor samples, but due to low clopyralid recoveries from spiked soil samples in method development, the pH of the extraction solution was manipulated. The 5 g sub-samples of soil were combined with 10 mL of a 0.05N ammonium hydroxide solution in a plastic 50 mL centrifuge tube. This basic extraction solution was prepared by adding 1.7 mL 14M ammonium hydroxide to 500 mL deionized water. This soil solution was allowed to shake for 2 h and was centrifuged at 4,700 rpm for 20 min. A 5 mL aliquot of the liquid supernatant was placed in a 15 mL glass centrifuge tube and 25 µL of ACS grade formic acid was added. The samples were vortexed for 5 sec to thoroughly mix the solution and an aliquot of this acidified solution was centrifuge filtered using the Eppendorf filter microfuge tubes. Like the aminocyclopyrachlor samples, a final aliquot of the filtered liquid was placed in a sample vial with a limited volume insert for HPLC analysis. A small soil sub-sample was also taken for final soil moisture adjustment. Average QC sample recovery was 97%.

Aminopyralid Soil Sample Preparation. Preparation of the samples from plots sprayed with aminopyralid was similar to the other two herbicides; however, due to interfering peaks, the HPLC method was not suitable and liquid chromatography/mass spectrometry (LC/MS) was used requiring slightly different methods. A 10 g sub-sample from each of

the depth, plot, and site combination was weighed into a plastic 50 mL centrifuge tube

and was combined with 20 mL of LC/MS-grade water and shaken overnight. An aliquot

of the soil solution was placed in a glass 20 mL test tube and was centrifuged at 5,000

rpm for 20 min. An aliquot of the liquid supernatant was then drawn into a plastic 10 mL

syringe with a Luer-Lok tip (Thermo Fisher Scientific, Waltham, MA 02454) and then

forced back out through an affixed 25 mm 0.45 μm PTFE syringe filter (Thermo Fisher

Scientific, Waltham, MA 02454) into a sample vial for LC/MS analysis. A small soil sub-

sample was also taken for final soil moisture adjustment. Average QC sample recovery

was 115%.

HPLC Analysis. For the 0 DAA samples extracted from the filter papers, HPLC analysis

was performed for all three compounds. The diluted extract samples from the filter

papers were injected onto a 250 x 4.6 mm Zorbax RX-C8 column (Agilent Technologies,

Inc., Santa Clara, CA 95051) with a particle size of 5 μm at a volume of 50 μL. Two

mobile phase solutions were used during the run. Mobile phase A was a 1% HPLC-grade

acetonitrile, 99% HPLC-grade water, and 0.05% phosphoric acid solution, while mobile

phase B consisted of a solution 30% HPLC-grade acetonitrile, 60% HPLC-grade water,

and 0.05% phosphoric acid. The mobile phase was run at a flow rate of 1.4 mL min^{-1} on

a gradient of 100% mobile phase A transitioning to 100% mobile phase B over the first

10 min, holding at 100% mobile phase B until 15 min, then reverting to 100% mobile

phase A to re-equilibrate through the end of the 20 min run. The detector wavelength

was set to 250 nm for the first 10.5 min, then shifted to 280 nm until 12.5 min, then it

shifted back to 250 nm until the end of the run. The retention times of the compounds

were approximately 6.5, 8.3, and 11.1 min after injection for aminocyclopyrachlor, aminopyralid, and clopyralid, respectively. The limit of quantitation for this method was 0.1 µg g^{-1}. A standard curve was included at the beginning and end of each run that spanned concentrations from 1 to 0.25 µg g^{-1}.

Only aminocyclopyrachlor and clopyralid soil samples could be analyzed by HPLC with some variations on the methods used for each individual compound to optimize sensitivity. The filtered aminocyclopyrachlor samples were injected onto a 150 x 2 mm phenyl-hexyl column (Phenomonex, Torrance, CA 90501) with a particle size of 3µm at a volume of 25 µL. Two mobile phase solutions were used during the run. Mobile phase A consisted of a 1% HPLC-grade methanol, 99% HPLC-grade water, and 0.05% phosphoric acid solution, while mobile phase B was a solution of 15% HPLC-grade methanol, 85% HPLC-grade water, and 0.05% phosphoric acid. For aminocyclopyrachlor, the mobile phases were run at a flow rate of 0.3 mL min^{-1} on a gradient of 100% mobile phase A transitioning to 100% mobile phase B over the first 10 min, holding at 100% mobile phase B until 15 min, then reverting to 100% mobile phase A to re-equilibrate until the end of the 25 min run. The detector wavelength was set to 250 nm. Aminocyclopyrachlor's retention time was approximately 9.7 min. The limit of detection (LOD) for this given method was 0.05 µg g^{-1}. A standard curve was included at the beginning and end of each run that spanned concentrations from 1 µg g^{-1} to 0.05 µg g^{-1}. Duplicate quality control samples of soil from each site spiked at 1.0, 0.5, and 0.1 µg g^{-1} were also included in each run to ensure consistent recovery levels.

The filtered clopyralid samples analyzed by HPLC were injected onto 250 x 4.6 mm C8 column with a particle size of 5 µm at a volume of 100 µL. The clopyralid HPLC

gradient also employed two mobile phases. Mobile phase A was a solution of 10%

HPLC-grade methanol, 90% HPLC-grade water, and 0.05% phosphoric acid, while

mobile phase B consisted of a 30% HPLC-grade methanol, 70% HPLC-grade methanol,

and 0.05% phosphoric acid solution. For clopyralid, the mobile phases were run at a flow

rate of 1.4 mL min^{-1} at a gradient similar to that which was used for

aminocyclopyrachlor. The detector wavelength was set to 280 nm. The approximate

retention time of clopyralid was 12 min after injection. The LOD for clopyralid was also

0.05 μg g^{-1}. Standard curves and quality control samples were included in each run for

clopyralid similar to the aminocyclopyrachlor analysis.

Aminopyralid LC/MS Analysis. The LC/MS instrument used was a qudrapole instrument

set to selectively monitor for positive ions with a molecular weight of 207 g mol^{-1}.

Filtered aminopyralid samples were injected onto a 150 x 4.6 mm C18 column (Waters

Corp., Milford, MA 01757) with a particle size of 3 μm at a volume of 10 μ L. Two

mobile phase solutions were used in the LC/MS analysis of aminopyralid. Mobile phase

A consisted of a 100% LC/MS-grade acetonitrile and 0.1% formic acid solution, while

mobile phase B was a solution of 100% LC/MS-grade water and 0.1% formic acid. The

mobile phase solutions were mixed in-line over time at different concentrations to create

a gradient. The run began with 20% mobile phase A and 80% mobile phase B and held

those concentrations until 8 min. From 8 to 9 min the gradient built so that by 9 min the

concentrations were 80% mobile phase A and 20% mobile phase B. These

concentrations held until 15 minutes, at which point they reverted back to 20% mobile

phase A and 80% mobile phase B. The column was allowed to re-equilibrate until the

end of the 24 min run. The flow rate of the mobile phase was 0.5 mL min^{-1} through the column. The approximate retention time of aminopyralid was 6.5 min. The LOD for aminopyralid with LC/MS analysis was 1 ng g^{-1}. A standard curve ranging from 0.3 ng g^{-1} to 1 μg g^{-1} and quality control samples spiked at 1 μg g^{-1} were run prior to the samples to establish a link between peak areas and actual soil concentrations as well as to ensure acceptable levels of recovery.

Centrifugation K_d Assay.

Soils. The soils used in this experiment were collected from a variety of locations throughout the United States and were chosen for their unique properties that span a range of physical and chemical soil characteristics (Table 2.1). The soils were air dried, sieved, and maintained in the dark at 4 C until use in the experiment. Soil textures and properties were determined by Harris Laboratory, Kansas City KS. Soil textural analysis was done using the hydrometer method, while soil pH was measured using a buffered 1:1 slurry method, and soil organic matter content was quantified using the loss-on-ignition method.

Sample Preparation and Analysis. The centrifugation assay developed by Walker and Jurado-Exposito (1998) that depends on treating soil at field capacity and centrifuging out the plant-available water is best suited for organic compounds with high water solubility (Kah and Brown 2007) like aminocyclopyrachlor, aminopyralid, and clopyralid and was, therefore, used in this experiment. The field capacity of each soil was determined using the pressure-plate technique (Klute 1986). The following methods are similar to those

used by Bukun et al. (2010b) with a few minor changes. 100 g of air dried soil was

weighed into a glass 500 mL jar. Enough water was added to each soil to bring the

moisture level up to 150% of its individual field capacity. It was necessary to wet the soil

beyond field capacity because the centrifuge speeds were not high enough to extract

enough water from certain soils, especially from those with already low water holding

capacities. In the water used to wet each soil, enough formulated aminocyclopyrachlor,

aminopyralid, or clopyralid was added to fortify the soil at a concentration of 1 $\mu g\ g^{-1}$ as

well as enough radiolabeled herbicide to treat the soil at 171.7 Bq g^{-1}. The wetted soils

were allowed to equilibrate for 24 h before they were gently mixed and 15 g of each soil

and herbicide combination was weighed into a stainless steel insert that had a filter

apparatus in the bottom (USDA-ARS, Fort Collins, CO 80521) (Fig. 2.1). The filter

apparatus consisted of a rubber O-ring, a perforated stainless steel disc, a nylon mesh

disc, and a millipore glass fiber filter (Whatman International Ltd., England). The insert

was placed in a 50 mL tube and centrifuged at 4,700 rpm for 1 hr. Following

centrifugation, the water that collected in the tube was transferred to a scintillation vial

and the volume of the plant available water was determined by weight. The amount of

radioactivity in the extracted water was determined by adding 10 mL scintillation cocktail

(Ultima Gold LLT [6013371], Perkin ElmerLife and Analytical Sciences, Inc., Waltham,

MA 02451) to the water collected from centrifugation and analyzing using liquid

scintillation spectroscopy (LSS) (Packard Trio-Carb [Model 2500 TR], Packard

Instrument Co., Meridien, CT 06450). A small sub-sample of each soil was oven dried at

110 C and soil moisture was determined to adjust the original sample weights by

weighing the sub-samples before and after drying. Each soil and herbicide combination was replicated three times and the study was repeated.

The concentration of herbicide adsorbed to each of the soils was calculated using the procedure of Kah and Brown (2007) with the following equation:

$$C_s = [C_i - (vC_e)]/S_{wt},\qquad\qquad [1]$$

where C_s is the concentration of the herbicide adsorbed to the soil (mg g^{-1}); C_i is the total amount of herbicide applied to the system (g); v is the volume of water centrifuged from the soil (mL); C_e is the concentration of herbicide in the centrifuged water (g mL^{-1}); and S_{wt} is the dry weight of the soil (g). Using the C_s value from the above equation, the soil adsorption coefficient K_d (mL g^{-1}) was then calculated using the following equation:

$$K_d = C_s/C_w,\qquad\qquad [2]$$

where C_w is equal to C_e from equation 1.

Data Analysis.

Data from all experiments were subjected to Levene's test for homogeneity of variance (SAS Institute, Cary, NC 27519) to determine if data from different sites or repeated experiments could be combined. First order exponential decay models were created in SigmaPlot version 10 (Systat Software, Inc., San Jose, CA 95440) for the three herbicides in the soil dissipation experiment and 95% confidence intervals were used to determine if there were significant differences between the models for the three herbicides. Soil half-lives for each herbicide were also calculated. The data for the soil adsorption (K_d) experiment were subjected to ANOVA (SAS Institute, Cary NC 27519). Treatment means were separated at the 5% significance level using the Fisher's Protected

Least Significant Difference (LSD) test (SAS Institute, Cary, NC 27519). Pearson's

correlation (SAS Institute, Cary, NC 27519) was also performed between soil organic

matter content (OM), pH, sand, silt, clay, and K_d values for each herbicide.

RESULTS AND DISCUSSION

Herbicide Dissipation in Soil Under Field Conditions.

Dissipation, for the purposes of this experiment, may be defined as the loss of

active herbicide from the sampled soil profile depth by any mechanism (i.e.: adsorption,

microbial degradation, or leaching). The objective of this experiment was not to

determine the mechanism of loss, but to quantify herbicide losses over time to better

understand the plant availability. The results of the Levene's test comparing dissipation

between sites were not significant (P > 0.05) so the data from both sites were combined

for each herbicide. The herbicide dissipation rates for the three compounds were

modeled using first order exponential decay functions ($r^2 = 0.80$ for each of the

herbicides). Due to overlapping 95% confidence intervals, there were no significant

differences between the dissipation rates for these herbicides (data not shown). Based on

the individual models for each herbicide, the half-lives were 32.5, 26.6, and 28.9 d for

aminocyclopyrachlor, clopyralid, and aminopyralid, respectively, with no appreciable

herbicide residue left for any of the three herbicides by 365 DAA (Fig. 2.2). Again,

because the models were not significantly different, the half-lives cannot be considered

significantly different.

The half-lives found in this experiment for aminopyralid and clopyralid agree

with published data. Aminopyralid has a published half-life range in soil of 25 to 35 d

(Senseman et al. 2007) with average half-life of 34.5 d for 8 North American soils according to Dow AgroSciences (2005), while clopyralid has a published half-life range in soil of 12-70 d (Senseman et al. 2007), with an average of 40 d (Wauchope et al. 1985). Aminocyclopyrachlor's half-life was substantially shorter than the published average (72 to 128 d) (Finkelstein et al. 2008). It is possible that the additional irrigation in this experiment favored a microenvironment that supported more rapid microbial degradation of aminocyclopyrachlor than the previous study.

It is interesting that all three herbicides dissipated at similar rates; however, observed weed control duration differed among the herbicides with aminocyclopyrachlor appearing to have the longest, followed by aminopyralid, and clopyralid having the shortest duration of weed control. Clopyralid weed control has been shown to fail before aminopyralid based on different levels of control one year after treatment in a study by Enloe et al. (2007) even at lower aminopyralid rates. Observations from the field also indicate that aminocyclopyrachlor provides better weed control at lower rates 14 MAT than aminopyralid (Lindenmayer et al. 2009). Given that all three herbicides dissipate at similar rates, but weed control seems to last longer for aminopyralid and aminocyclopyrachlor, one could suggest that aminopyralid and aminocyclopyrachlor are inherently more biologically active compounds, not that they necessarily persist in the environment longer than clopyralid. This may be evidenced by the fact that aminocyclopyrachlor and aminopyralid control Canada thistle longer than clopyralid at lower application rates (Enloe et al. 2007; Lindenmayer et al 2009). Additional research is needed to quantify biological responses to each herbicide across a range of concentrations to simulate soil residual activity.

Besides the total concentration of herbicide in the soil profile, movement in the soil profile is important. The vast majority of all three herbicides stayed in the top five cm of the profile until 28 DAA (Fig. 2.3). It should be noted that at 14 DAA about 18% of the total clopyralid had moved to the deepest half of the 30 cm sampled, with very little detected in the 5-15 cm depth. Due to the higher leaching potential associated with clopyralid, it is possible that some of the herbicide moved rapidly at first through the profile with an irrigation or rain event. By 28 DAA there was a substantial decrease in the total herbicide recovered from the soil for all compounds. In general, roughly 25% was found in the top 0-5 cm, 60% had moved into the next 5-15 cm, and only 15% had reached the 15-30 cm depth. By 56 DAA, a greater proportion of the total recovered herbicide for each of the three compounds had reached the deepest sampled depth. For all three herbicides, 25% of the recovered herbicide was found in both the 0-5 and 5-15 cm depths, with approximately 50% having moved to the 15-30 cm depth. By 128 DAA very little aminopyralid was recovered and no clopyralid was detected, while aminocyclopyrachlor had roughly equal distribution among the depths of the remaining total recovered herbicide. At the end of the experiment (365 DAA) there was no detectable aminopyralid or clopyralid, but there was a trace amount of aminocyclopyrachlor left, mostly found in the 15-30 cm depth. Leaching beyond 30 cm was not accounted for in this experiment and may have contributed to herbicide dissipation.

Centrifugation K_d Assay.

The traditional batch slurry technique for determining soil distribution coefficients is best suited to compounds that have a higher potential for soil binding. It

has been suggested that these studies should be conducted at a soil-to-solution ratio that achieves between 30 and 50% adsorption (OECD 1997). It is difficult to use the batch slurry technique for herbicides that do not have an affinity for soil binding as the lowest practical ratio is 1:1. Therefore, the centrifugation assay was used in this experiment. Based on the Levene's test for homogeneity of variance, there were no significant differences between repeated experiments with any of the herbicides (P > 0.05); therefore, data were combined across repeated experiments for each herbicide.

For aminocyclopyrachlor, the ANOVA for the centrifugation K_d assay revealed a significant soil effect (P < 0.0001) indicating differential adsorption between the soils used in the experiment. The LSD means separation test (Table 2.2) showed that the Drummer silty clay loam had the greatest potential for adsorbing aminocyclopyrachlor, followed by the Webster clay loam and the Imperial silty clay. The Spinks loamy sand had the least potential for soil adsorption, with the Gilead sandy loam and Fort Collins loam having statistically similar, but numerically intermediate soil adsorption coefficients to both the Imperial silty clay and Gilead sandy loam. The range of K_d values observed in this experiment are similar to values reported by Olivera et al. (2011) who studied aminocyclopyrachlor sorption and desorption in 14 Brazilian soils, even though they used the batch equilibrium method to determine the sorption coefficients. The Pearson's correlation test revealed that aminocyclopyrachlor soil adsorption across all of the soils (Table 2.3) was significantly and positively correlated with OM and silt. There was also a moderately significant positive correlation with clay. Sand was found to be significantly and negatively correlated to aminocyclopyrachlor soil adsorption.

Aminopyralid soil adsorption was significantly affected by soil type ($P < 0.0001$). A pattern of soil adsorption similar to aminocyclopyrachlor was also observed for aminopyralid for each of the soils included in the experiment, though the range of soil adsorption coefficients was narrower for aminopyralid than for aminocyclopyrachlor (Table 2.2). For aminopyralid, the greatest soil adsorption was found in the Webster clay loam, followed by the Drummer silty clay loam, Imperial silty clay, and Fort Collins loam. Aminopyralid adsorbed the least to the The Gilead sandy loam and Spinks loamy sand and was statistically similar. These values were similar to those found by Bukun et al. (2010b), with a few exceptions. The K_d observed for aminopyralid in the Imperial silty clay soil for this experiment was substantially greater than that found by Bukun et al. (2010b). Additionally, the K_d values for the Drummer silty clay loam and the Gilead sandy loam found in this experiment were somewhat lower than those observed by Bukun et al. (2010b). Based on the Pearson's correlation coefficients (Table 2.4), aminopyralid soil adsorption was significantly and positively correlated to OM, silt, and clay. This is similar to aminocyclopyrachlor, with the addition of a significant correlation to clay. Again, sand was found to be significantly and negatively correlated to soil adsorption of aminopyralid, while pH only had a less significant and negative correlation. In general, this agrees with the findings of Bukun et al (2010b) who also found a very significant positive correlation with OM, but only moderately significant positive correlations with silt and clay. Bukun et al. (2010b) also observed negative correlations with sand at a moderate significance level and pH at a weakly significant level.

Clopyralid, like the previous two herbicides demonstrated a significant soil effect from the ANOVA ($P < 0.0001$). Clopyralid had a soil adsorption pattern similar to that of aminopyralid (Table 2.2); however, clopyralid distribution coefficients were lower than those observed for aminocyclopyrachlor and aminopyralid. The Webster clay loam had the greatest clopyralid adsorption, followed by the Imperial silty clay, Drummer silty clay loam, and the Fort Collins loam. The Spinks loamy sand and Gilead sandy loam had the least observed clopyralid soil adsorption and were again statistically similar. Like aminopyralid, the clopyralid distribution coefficients found in this experiment tend to agree with those found by Bukun et al. (2010b) with a few exceptions. The clopyralid soil adsorption observed in this experiment for the Imperial silty clay was much greater than that observed by Bukun et al. (2010b). Additionally, the K_d values found in this experiment for the Webster clay loam were slightly greater and the Drummer silty clay loam were slightly lesser compared to those found by Bukun et al. (2010b). The Pearson's correlation coefficients for clopyralid soil adsorption across all the soils (Table 2.5) differed from the other two herbicides in that the most significant correlation was a positive one with clay and only moderately significant positive correlations with OM and silt. Interestingly, pH also had a moderately significant positive correlation with clopyralid soil adsorption. Similar to the other two herbicides, there was a significant negative correlation with sand. This is somewhat different than the results produced by the experiment conducted by Bukun et al. (2010b) who observed significant positive correlations with OM, silt, and clay, a significant negative correlation with sand, and a weakly significant negative correlation with pH.

In general, aminocyclopyrachlor, aminopyralid, and clopyralid had low K_d values relative to other herbicides. This can be attributed, in part, to the water soluble nature of the three herbicides. The published water solubility values are 4.20, 2.48, and 1.00 g L^{-1} for aminocyclopyrachlor, aminopyralid, and clopyralid, respectively. This is also reflected in the low log K_{ow} values for each of the herbicides. The log K_{ow} values are -2.48, -2.87, and -2.63 at pH 7 and 20 C for aminocyclopyrachlor, aminopyralid, and clopyralid, respectively (Dow AgroSciences 2005; Finkelstein et al. 2008; Senseman et al. 2007). These herbicides are also weak acids herbicides with carboxylic acid side-chains. As such, they each have a relatively low dissociation constant (pK$_a$). Low pK$_a$ values of 4.65, 2.56, and 2.30 for aminocyclopyrachlor, aminopyralid, and clopyralid, respectively, (Bukun et al. 2010a; Dow AgroSciences 2005; Senseman et al. 2007) indicate that the vast majority of each of the herbicide molecules will be in the dissociated and ionic form. Additionally, the negative charge on OM or clay colloid surfaces will repel the negatively ionized carboxyl herbicide molecules, further promoting its solubilility in the soil solution.

Herbicide water-solubility is a double-edged sword in that it simultaneously increases the plant availability of the herbicide through root uptake, which can lead to increased efficacy as well as increasing the herbicide leaching potential. Roots have no cuticle and water soluble herbicides can be taken up through mass flow; however, the water solubility of weak acid herbicides also poses a problem. Since the herbicides are part of the soil water solution, they are prone to dilution or leaching in the soil. Due to the highly water-soluble nature of all three of these herbicides and low distribution coefficients, both aminopyralid and clopyralid should theoretically have high relative

leaching potentials (Bukun et al. 2010b; Pik et al. 1977; Smith and Aubin 1989).

Compared to dicamba, mecoprop, and pendimethlin, clopyralid was found to be more mobile (Sakaliene et al. 2009). On the other hand, other studies have shown clopyralid is mobile, but generally stays in the upper 30 cm of the soil profile (Bergstrom et al. 1991; Bovey and Richardson 1991; Elliot et al. 2000), making it available for root absorption. Bukun et al. (2010b) also quantified the mobility of both clopyralid and aminopyralid using thin-layer chromatography (TLC). The average R_f values measured for aminopyralid (0.82) and clopyralid (0.91) indicate that clopyralid is slightly more mobile than aminopyralid, but varied with soil type.

Since aminocyclopyrachlor is so closely related in its chemical structure and properties to aminopyralid and clopyralid, it follows that aminocyclopyrachlor should also be prone to movement in the soil profile. Based on published K_{oc} values for each the herbicides and the results of this experiment, one may hypothesize that aminocyclopyrachlor would be less mobile than the other two herbicides. Aminocyclopyrachlor has the greatest K_{oc} (28 mL g^{-1}), followed by aminopyralid (10.8 mL g^{-1}), and clopyralid has the lowest K_{oc} (6 mL g^{-1}) (Finkelstein et al. 2008; Senseman et al. 2007) indicating a greater affinity for soil sorption. Combined with the results of the centrifugation K_d study, there is greater evidence that aminocyclopyrachlor would be less mobile than either aminopyralid or clopyralid. Across all of the soils included in the study, aminocyclopyrachlor had an average K_d of 0.503 mL g^{-1}, while aminopyralid and clopyralid had average K_d values of 0.378 and 0.236 mL g^{-1}, respectively. The results of the herbicide dissipation experiment also provide evidence that aminocyclopyrachlor and aminopyralid would be less mobile than clopyralid as the vast majority of both

aminocyclopyrachlor and aminopyralid stayed in the upper five cm of the soil profile for two weeks, while about 18% of the clopyralid had already moved to the lower 15 cm of the sampled soil profile. However, more research is needed to more adequately describe aminocyclopyrachlor mobility by sampling to greater depths in the soil profile or with more direct measurements like TLC.

The adsorption of these weak acid herbicides did not have a strong correlation to soil pH which is similar to observations by Bukun et al. (2010b). Under slightly acidic soil conditions when the pH is below the point of zero charge (pzc) of the adsorbent, but above the pK_a of the herbicide, kaolinite clays and iron hydroxides will be positively charged, providing a surface for adsorption. Ligand exchange can also occur where hydroxyl groups coordinated to Al^{3+} or Si^{4+} atoms at edges of octahedral and tetrahedral sheets, respectively, in clay minerals or a metal cation can be exchanged for an organic anion (Stumm 1987; Stumm 1992), such as an ionized carboxyl group on an auxinic herbicide molecule. Ligand exchange is also most common in soils rich in kaolinite clays or iron oxide minerals like gibbsite or goethite (Sparks 2003). These possible mechanisms may explain why the Webster clay loam had a consistently high K_d for all three of the herbicides, while the sandy soils like the Spinks loamy sand and the Gilead sandy loam had consistently low herbicide adsorption. This is also congruent with the fact that aminopyralid and clopyralid both had significant positive Pearson's correlations with clay. The moderately significant correlation of aminocyclopyrachlor adsorption to clay may be explained by its greater pK_a relative to the other two herbicides. However, the confounding correlations between OM, sand, silt, and clay make it difficult to isolate the individual effects of those soil properties.

The results of research presented here agrees with previous studies concerning aminopyralid and clopyralid (Bergstrom et al. 1991; Bovey and Richardson 1991; Bukun et al. 2010b; Elliot et al. 2000; Pik et al. 1977; Sakaliene et al. 2009; Smith and Aubin 1989) helping to describe the behavior of aminocyclopyrachlor in soil. These herbicides had similar dissipation curves when applied at similar rates; however shorter durations of weed control for clopyralid compared with aminopyralid and aminocyclopyrachlor have been observed (Enloe et al. 2007; Lindenmayer et al. 2009). Furthermore, Bukun et al. (2010a) demonstrated reduced foliar absorption and translocation of aminopyralid compared with clopyralid, which serves as additional evidence of the importance of soil residual activity for long-term weed control with aminopyralid and aminocyclopyrachlor. It can also be concluded that all three herbicides are quite plant-available, as evidenced by the low levels of soil adsorption observed in this and other experiments (Bukun et al. 2010b).

This is confirmed by the results of the centrifugation K_d assay that showed that clopyralid had the least potential for adsorption to soil, while aminopyralid and aminocyclopyrachlor had greater potential for soil adsorption, though still quite low compared to other herbicides. While the potential for mobility with these herbicides is high, previous research (Bergstrom et al. 1991; Bovey and Richardson 1991; Bukun et al. 2010b; Elliot et al. 2000) suggests that they may stay in the upper portion of the soil profile. It was also confirmed, based on the K_d values generated in this experiment and previous research (Bukun et al. 2010b), that clopyralid would have the most potential for leaching, followed by aminopyralid. Finally the results of these experiments would

suggest that aminocyclopyrachlor would have the least potential for leaching, making it

potentially a safer alternative for use in riparian areas or areas with high water tables.

Table 2.1. Physical and chemical soil properties

Location	Soil Series	Taxonomy	pH	OM[a]	Sand	Silt	Clay
					------------------ % ------------------		
Michigan	Spinks sandy loam	Sandy, mixed, mesic Lamellic Hapludalfs	5.9	1.2	81	11	8
North Carolina	Gilead sandy loam	Fine, kaolinitic, thermic Aquic Hapludalts	5.2	1.6	90	3	7
California	Imperial silty clay	Fine, smectitic, calcareous, hyperthermic Vertic Torrifluvents	8.0	2.0	32	23	45
Colorado	Fort Collins loam	Fine-loamy, mixed, superactive, mesic Aridic Haplustalfs	8.2	1.9	55	18	27
Illinois	Drummer clay loam	Fine-silty, mixed, superactive, mesic Typic Enoaquolls	5.4	5.9	16	48	36
Minnesota	Webster clay loam	Fine-loamy, mixed, superactive, mesic Typic Endoaquolls	6.9	7.9	36	30	34

[a]OM, soil organic matter

Table 2.2. Soil adsorption (K_d) of aminocyclopyrachlor, aminopyralid, and clopyralid. Means with different letters indicate significant differences within an individual herbicide.

Soil	Aminocyclopyrachlor	Aminopyralid	Clopyralid
	K_d		
	--- kg L^{-1} ---		
Spinks loamy sand	0.094 e	0.105 e	0.077 e
Gilead sandy loam	0.304 cd	0.126 e	0.071 e
Imperial silty clay	0.396 c	0.431 c	0.389 b
Fort Collins loam	0.178 de	0.243 d	0.179 d
Drummer silty clay loam	1.216 a	0.633 b	0.238 c
Webster clay loam	0.827 b	0.728 a	0.462 a
Average	0.503	0.378	0.236

Table 2.3. Pearson's correlation between aminocyclopyrachlor soil adsorption (K_d) and soil properties.

	OM	pH	Sand	Silt	Clay
K_d	0.852**	-0.306	-0.779*	0.898**	0.529
OM		-0.107	-0.667	0.748*	0.492
pH			-0.307	0.005	0.570
Sand				-0.932***	-0.930***
Silt					0.733*

* P < 0.10
** P < 0.05
*** P < 0.01

Table 2.4. Pearson's correlation between aminopyralid soil adsorption (K_d) and soil properties.

	OM	pH	Sand	Silt	Clay
K_d	0.926***	0.118	-0.880**	0.860**	0.778*
OM		-0.107	-0.667	0.748*	0.492
pH			-0.307	0.005	0.570
Sand				-0.932***	-0.930***
Silt					0.733*

* P < 0.10
** P < 0.05
*** P < 0.01

Table 2.5. Pearson's correlation between clopyralid soil adsorption (K_d) and soil properties.

	OM	pH	Sand	Silt	Clay
K_d	0.685	0.504	-0.757*	0.561	0.850**
OM		-0.107	-0.667	0.748*	0.492
pH			-0.307	0.005	0.570
Sand				-0.932***	-0.930***
Silt					0.733*

* P < 0.10
** P < 0.05
*** P < 0.01

Figure 2.1. Centrifuge filter apparatus used for centrifugation K_d assay.
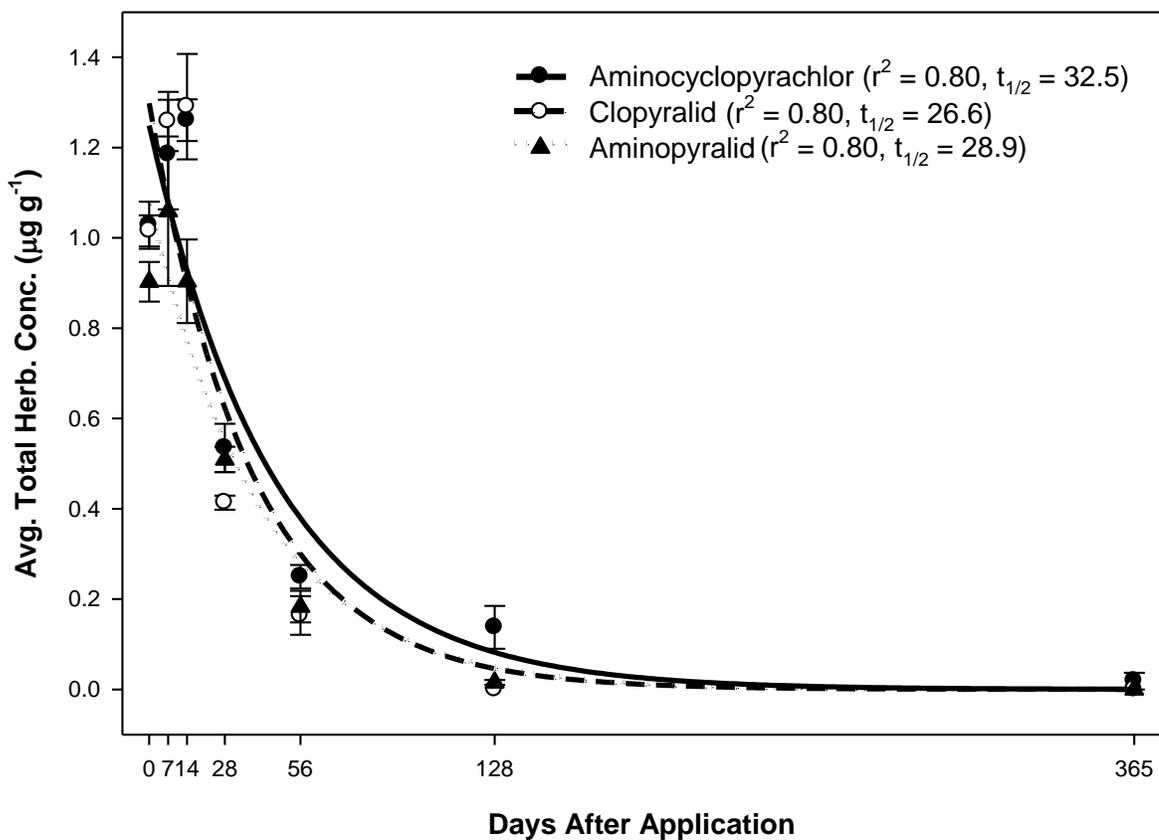
Figure 2.2. Herbicide dissipation rates under field conditions for aminocyclopyrachlor, clopyralid, and aminopyralid. Lines represent modeled dissipation rates based on complete raw data set, while symbols represent the average total herbicide concentration for the samples soil profile at each sampling time point.
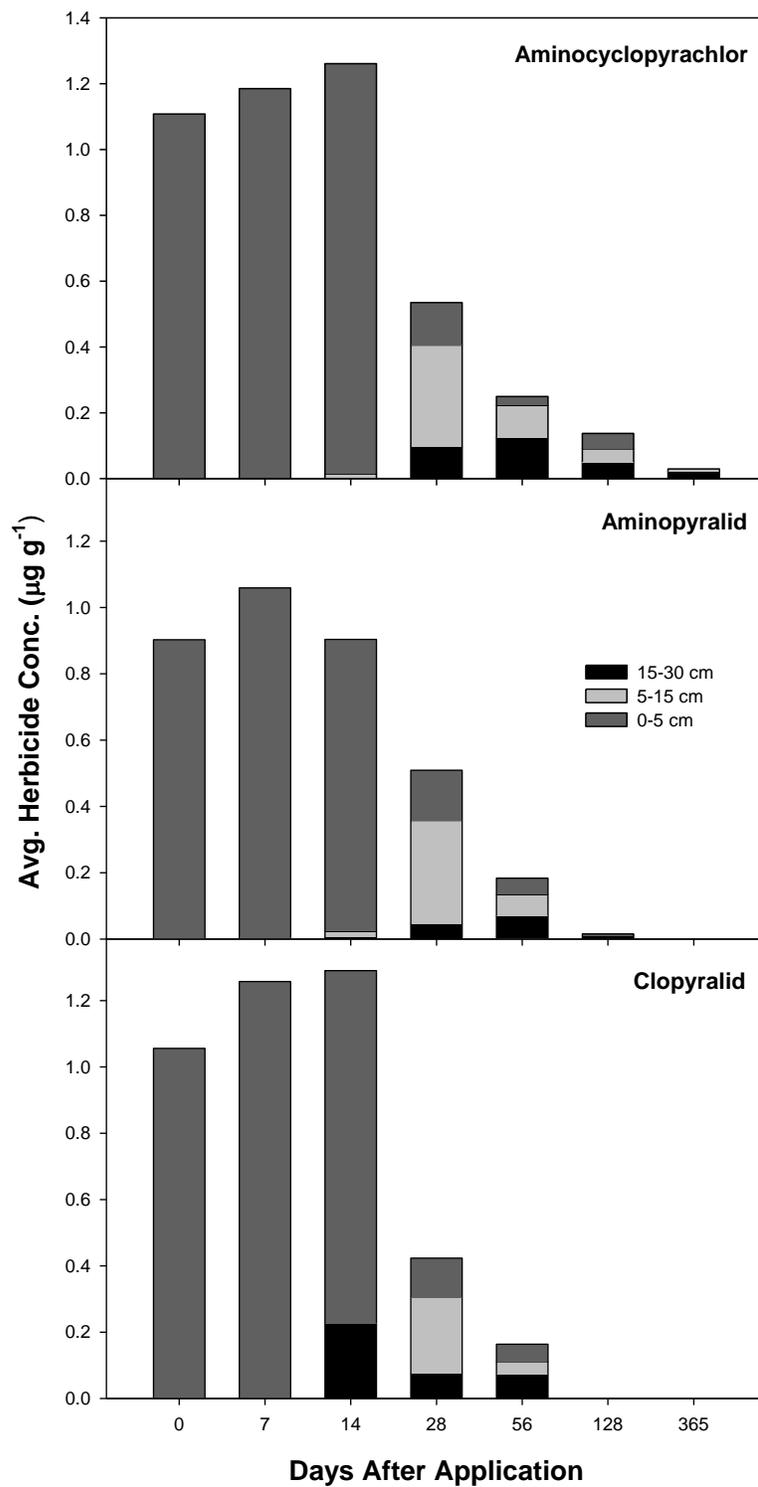
Figure 2.3. Average total herbicide recovery over time separated by sampling depth for aminocyclopyrachlor, aminopyralid, and clopyralid under field conditions.

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CHAPTER THREE: AMINOCYCLOPYRACHLOR ABSORPTION,

TRANSLOCATION AND METABOLISM IN FIELD BINDWEED

(*CONVOLULUS ARVENSIS*)

INTRODUCTION

Field bindweed (*Concolvulus* arevensis) is a twinning, herbaceous, perennial plant

native to Eurasia. It was likely introduced to North America from Europe as a

contaminant of crop seeds as early as 1739 (Coombs et al. 2004) and has become a

widespread and serious problem for much of the U.S. Primarily a weed of disturbed

lands, field bindweed has invaded cultivated areas and wastelands (Whitson et al. 2006)

and is found in every U.S. state except for Alaska (plants.usda.gov). In fact, field

bindweed is still considered a major pest in Europe and many agricultural areas of the

world (Maillet 1988; Weaver and Riley 1982) and has been ranked as the twelfth worst

weed in the world (Holm et al. 1977). In crops, field bindweed has been especially

problematic. The plants can quickly form dense mats that out-compete crops for below-

ground resources such as water and nutrients. Field bindweed vines climb crop plants,

shading them and causing lodging, especially in small-grains. The vines generally make

harvest difficult by clogging harvesting equipment. Field bindweed also provides habitat

for insects that damage adjacent crops (Tamaki et al. 1975) and can serve as an

alternative host for crop diseases (Feldman and Gracia 1977; Holm et al. 1977). These

problems combine to reduce crop yields by as much as 50-60% and result in economic

losses exceeding $377 million in 1998 (Coombs et al. 2004). Field bindweed is also a

major pest of non-cropland, such as range, pasture, and riparian areas, and poses a

specific threat to restoration efforts as it competes with native grasses and forbs. It quickly invades areas through its creeping root system, forming dense monocultures that decrease habitat biodiversity and hinder native species survival.

The biology of field bindweed makes it a challenging invasive species for land managers. Field bindweed has an extensive root system that can penetrate soil as deep as 7 m (Whitson et al. 2006) and produces very long rhizomes from which new, adventitious buds will arise as soon as three to four weeks after germination (Elmore and Cudney 2003). A single plant produced 197 vertical roots that totaled over 260 m in length only six months after germination. The same plant also produced 34 rhizomes totaling over 45 m of additional growth, from which, 141 new shoots were established in the same time period (Zollinger and Lym 2000). The roots also act as a carbohydrate storage organ that provides energy reserves for both above- and below-ground growth. Finally, a single plant may produce as many as 500 seeds (Coombs et al. 2004), which may remain viable for up to 50 years (Whitson et al. 2006).

A persistent weed, such as field bindweed, requires a multi-faceted management approach including a combination of mechanical, cultural, biological, and chemical practices. Intense and timely cultivation has been used to control newly emerged seedlings by destroying the plants and can have an impact on established stands by depleting root carbohydrate reserves and promoting the germination of dormant seeds (Zollinger and Lym 2000). Research has shown that carbohydrate root reserves are at the lowest for the season between April and May (Frazier, 1943) indicating that field bindweed management through cultivation may be most effective early in the season. Additional research has shown that cultivating at two or three week intervals throughout

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the growing season can eliminate 95% of established field bindweed stands (Timmons 1941; Timmons and Bruns 1951). However, intense tillage would not be practical or sustainable as it would not be cost effective, crops could not tolerate the disturbance, and it would put the soil at high risk for erosion.

Other research has indicated that intense early spring cultivation followed by densely planted crops such as forage sorghum (*Sorgum vulgare* Pers.), soybeans (*Glycine max* (L.) Merr.), or forage sudangrass (*Sorghum Sudanese* (Piper) Stapf.), followed by fall cultivation for a three year period was able to eliminate established field bindweed infestations (Bakke 1939; Stahler 1948; Timmons 1941). Alfalfa (*Medicago sativa* L.) or alfalfa-perennial grass mixes can out-compete field bindweed when combined with intense tillage (Bakke 1939; Franzke and Hume 1936; Stahler 1948; Timmons 1941).

Biological control efforts have focused on insect and fungal pathogens. A gall mite species (*Aceria malherbae* Nuzzaci) imported from Greece has had limited releases in some areas of the U.S (Boldt and Sobhian 1993). Additionally, over 600 fungi have been isolated from populations in Europe, with the most successful being from the *Stagnospora* genus (Pfirter et al. 1997). Combinations of cover crops and applications of fungal spores have also been considered (Pfirter et al. 1997).

Chemical control of field bindweed has remained a challenge (Derscheid et al. 1970; Westra et al. 1992; Wiese and Rea 1959) due to the weed's extraordinary regenerative ability and control usually requires multiple applications (Timmons 1949) of selective systemic herbicides, such as 2, 4-D, dicamba, picloram (Westra et al. 1992) imazapyr (Schoenhals et al 1990), and quinclorac (Enloe et al. 1999). The non-selective

herbicide glyphosate can also be used in appropriate land management systems (Westra et al. 1992). The systemic nature of the aforementioned herbicides is critical to their success as translocation to the root system of field bindweed is necessary to effect long-term control. Limited below-ground translocation has been identified as one potential reason for variable control with herbicides (Lauridson et al. 1983). Short-term control of above ground vegetation is easily achieved with herbicides, but long-term effect on the root system and its reproductive structures are minimal.

Chemical control has been complicated by the high degree of phenotypic polymorphism in field bindweed (Brown 1945; Darmency 1979; DeGennaro and Weller 1984b; Garcia-Baudin and Kiss 1973) resulting in erratic chemical control (Derscheid 1947; DeGennaro and Weller 1984a; Hamner and Tukey 1944; Weaver & Riley 1982; Wiese and Rea 1955; Woestermeyer 1950). The variability in phenotypes is reflected in reduced leaf area and increased cuticular thickness under arid conditions (Dall'Armellina & Zimdahl 1989), but were not highly correlated with differences in 2, 4-D susceptibility (Whitworth and Muzik 1967) and were thought to be due to differences in 2, 4-D binding within plant cells (Harvey and Muzik 1973). In addition, five phenotypically distinct biotypes of field bindweed have been described based on their growth, reproduction (DeGennaro and Weller 1984b) and variation in sensitivity to glyphosate (DeGennaro and Weller 1984a). It was initially thought that these differences were due to variability among the biotypes in herbicide absorption and translocation (D'Anieri et al 1990; Sandberg et al 1980), but new evidence suggests the tolerance to glyphosate is based on multiple mechanisms at the cellular level including increased 3-deoxy-D-*arabino*-heptosonate-7-phosphate synthase (DAHPS) activity and higher 5-*Enol*-

pyruvylshikimate-3-phosphate synthase (EPSPS) activity (Westwood and Weller 1997). With such variability in herbicide efficacy and plant susceptibility, a new solution is needed for field bindweed control.

Aminocyclopyrachlor is a new auxinic compound and is the first pyrimidine carboxylic acid herbicide with a proposed use pattern in non-cropland and rangeland to control broadleaf weeds and shrubs (Turner et al. 2009). Several species in numerous dicot families have shown sensitivity to aminocyclopyrachlor, including Asteraceae, Fabaceae, Chenopodiaceae, Convulvulaceae, and Euphorbiaceae (Armel et al. 2009; Claus et al. 2008; Jenks 2010; Turner et al. 2009). Aminocyclopyrachlor has selectivity on some monocot species and has great potential for use in ecosystem restoration work (Edwards 2008; Vassios et al. 2009). Effective control of perennial weeds is paramount in these situations. Translocation to roots and below-ground reproductive structures is the key to long-term control of perennial species, such as field bindweed. Aminocyclopyrachlor absorption, translocation, and metabolism has been studied in Canada thistle (*Cirsium arvense*) (Bukun et al. 2010), as well as prickly lettuce (*Latuca serriola* L.), rush skeletonweed (*Chondrilla juncea* L.), and yellow starthistle (*Centaurea solstialis* L.) (Bell et al. 2011). Observations from the field indicate that aminocyclopyrachlor is a very effective herbicide for field bindweed providing excellent control for up to 16 months after treatment with use-rates as low as 35 g ai ha^{-1}, which outperformed both quinclorac and picloram, industry standards for field bindweed control (Lindenmayer et al. 2009). Finally, aminocyclopyrachlor had greater field bindweed control at low rates than was observed for Canada thistle, suggesting biological activity is species dependent (Lindenmayer et al. 2009).

Aminocyclopyrachlor is a new active compound and, as such, there is still limited information available about its absorption, translocation and metabolism in a variety of susceptible target species. With field bindweed being a major pest in cropland as well as non-cropland and the with the weed appearing to be quite sensitive to aminocyclopyrachlor, more research is needed to understand how this compound behaves in field bindweed. Therefore, the objectives of this study were to (1) evaluate the absorption and translocation of aminocyclopyrachlor in field bindweed and (2) determine if any metabolites of aminocyclopyrachlor are formed in field bindweed and the rate at which they form.

MATERIALS AND METHODS

Plant Material.

Field bindweed seeds were scarified by soaking the seeds in 17.8 M sulfuric acid for 15 min and were immediately rinsed with tap water for 10 min. The seeds were then planted in flats of 3 cm x 3 cm plugs (American Clayworks, Denver, CO 80204) filled with an organic potting mix (Fafard C-1P Mix Conrad Fafard, Inc, Agawam, MA 01001). It should be noted that plants grown from seed appeared to be more robust than those grown from root segments from previous research (Dall'Armellina and Zimdahl 1989); thus, this method was chosen. Once the germinated seedlings had three to four true leaves, they were transplanted into sand-filled cones (Deepot cones, Stuewe and Sons, Inc., Corvallis, OR 97333) measuring 6.5 cm in dia and 25 cm in length. A slow-release, nitrogen fertilizer (Osmocote Flower & Vegetable Smart-Release Plant Food, Scotts Miracle-Gro Co., Marysville, OH 43041) was mixed with the sand at 1% v/v and weekly additions of a water-soluble complete nutrient (20-20-20) fertilizer (Miracle-Gro

fertilizer, Scotts Miracle-Gro Co., Marysville, OH 43041) were also made. Plants were

watered as needed and grown in a greenhouse for 6 wk at 26/22 C day/night temperature

with a 16 h photoperiod. Natural light was supplemented with 1,000 watt metal halide

lamps, providing a midday photosynthetic flux (PPF) of 700 μmol m^{-2} s^{-1}. Plants were

periodically pruned to remove axial shoots and buds to maintain a single shoot and

prevent flowering. Plants were also allowed to vine up 30cm wooden stakes in the cones

to facilitate upright growth. Plants for both experiments that had reached the 12-15 leaf

growth-stage were chosen for uniformity.

Aminocyclopyrachlor Absorption and Translocation.

The plants selected for the aminocyclopyrachlor absorption and translocation

experiment were treated according to the methods used by Bukun et al. (2010) with a few

minor adjustments. One leaf, located midway up the stem, was protected by a piece of

aluminum foil and the whole plant was oversprayed with aminocyclopyrachlor (DuPont,

Wilington, DE 19898) applied at a rate of 0.14 kg ae ha^{-1} with 1% v/v MSO in a single

nozzle, overhead track spray chamber (DeVries Manufacturing Corp., Hollandale, MN

56045) calibrated to deliver 187 L ha^{-1} at 206 kPa.

Following the foliar herbicide application, the protected leaves were treated with 20,

0.5 μL droplets of the spray solution with the addition of radiolabeled

aminocyclopyrachlor (DuPont, Wilmington, DE 19898). Thus, each plant received a 3.3

kBq dose of ^{14}C-aminocyclopyrachlor. The treated plants were then placed in a growth

chamber (Model 15, Conviron Controlled Environments Ltd., Winnipeg, MB Canada)

(16 h photoperiod, 22/18 C day/night, PPF of 500 μmol m^{-2} s^{-1}) and allowed to grow until their predetermined harvest interval.

Field bindweed plants were harvested at 0, 6, 12, 24, 48, 96, and 192 h after treatment (HAT) and were separated by plant part into the treated leaf, above-ground tissue, and below-ground tissue. To determine absorption, the treated leaves were shaken in 10 mL of a leaf-wash solution consisting of 90% water, 10% methanol and 0.25% non-ionic surfactant (NIS) for 15 min. The treated leaves were then removed from the leaf-wash solution and 10 mL of scintillation cocktail (Ultima Gold LLT, Perkin Elmer Life and Analytical Sciences, Inc., Waltham, MA 02454) was added and the leaf wash was analyzed using liquid scintillation spectroscopy (LSS) (Model 2500, Packard Tri-Carb, Packard Instrument Co., Meriden, CT 06450) . Aminocyclopyrachlor absorption was then calculated as the difference between radioactivity in the original treatment solution and the radioactivity in the leaf-wash solution. The treated leaf tissue was then triple rinsed in de-ionized water and were, along with all of the other plant parts, frozen in liquid nitrogen in individual 50 mL plastic centrifuge tubes (Thermo Fisher Scientific, Waltham, MA 02454) and stored at -50 C for later processing. Additionally, the sand potting medium was shaken with 250 mL de-ionized water for 30 min and a 10 mL sample was combined with 10 mL of scintillation cocktail and analyzed using LSS to determine root exudation.

To determine translocation of radioactivity, the individual frozen plant parts were ground in 10 mL of a 90% methanol and 10% water extraction solution using a tissue homogenizer (Power Gen 125, Thermo Fisher Scientific, Waltham, MA 02454) in the 50 mL plastic centrifuge tubes they were stored in. The extraction solution was centrifuged

at 4700 rpm for 20 min and the liquid was decanted into a glass 50 mL test tube. A 1 mL

sample of the extraction solution was removed and combined with 10 mL of trapping

cocktail and analyzed also using LSS to determine the soluble fraction of radioactivity in

each plant tissue. The remaining tissue pellet for each plant tissue was oven-dried for 48

h at 60 C and combusted in a biological oxidizer (0X-500, R.J. Harvey Instrument Co.,

Tappan, NJ 10983) to determine the bound fraction of radioactivity in each plant tissue.

Radioactivity was trapped with 10 mL of xylene ^{14}C trapping cocktail (OX-161, R.J.

Harvey Instrument Co., Tappan, NJ 10983) and analyzed using LSS. The experiment

was a randomized complete-block design with four replications and was repeated.

Aminocyclopyrachlor Metabolism.

The methods for determining aminocyclopyrachlor metabolism in field bindweed

are similar to those described above for the absorption and translocation experiment with

these additional steps. The remaining 9 mL of the extraction solution decanted after

centrifugation of the ground plant tissue were placed in a sample evaporator[11] where the

methanol was evaporated off, leaving 0.9 mL of water containing the soluble herbicide.

This liquid was transferred to a 2 mL, 40 μm eppendorf centrifuge filter and centrifuged

at 15,000 rpm for 30 min. The filtered extract was then transferred to a sample vial and

analyzed using high performance liquid chromatography (HPLC) using a phenyl-hexyl, 2

x 150 mm column (Phenomonex, Torrance, CA 90501) with an injection volume of 100

μL, the wavelength set at 250 nm, and a flow rate of 0.3 mL/min. Aminocyclopyrachlor

eluted at nine min using the following gradient: 1% methanol: 99% water: 0.05%

phosphoric acid solution to 15% methanol: 85% water: 0.05% phosphoric acid solution

over 10 min, holding for 5 min, then returning to the original solution for the remainder

of the 25 min run. Radioactivity was quantified using an inline radioactive detector (Beta-Ram, LabLogic Systems, Inc., Brandon, FL 35511). The experiment was a randomized complete-block design with four replicates and was repeated.

Data Analysis.

Data from all experiments were subjected to Levene's test for homogeneity of variance (SAS Institute, Cary, NC 27519) to ensure the data from repeat experiments could be pooled. Data for all of the time-courses were then analyzed using nonlinear regression and plotted in SigmaPlot version 10 (Systat Software, Inc., San Jose, CA 95440).

RESULTS AND DISCUSSION

Aminocyclopyrachlor Absorption and Translocation.

Aminocyclopyrachlor absorption in field bindweed was rapid and reached a maximum of 48.3% by 48 HAT (Fig. 3.1). The absorption of aminocyclopyrachlor in to leaf tissue is most similar to 2, 4-D (59%) (Agbakoba and Goodin 1970), but less than picloram (68%) (Agbakoba and Goodin 1970) and dicamba (64%) in field bindweed (Flint and Barrett 1989); however, Flint and Barrett (1989) did observe absorption of 2,4-D as high as 83% at 72 HAT in a separate experiment. Flint and Barrett (1989) also observed ^{14}C-glyphosate absorption of 32% in field bindweed at an application rate of 0.28 kg ha^{-1}. Aminocyclopyrachlor absorption was far greater than absorption of quinclorac by 36 HAT (7.3%) (Enloe et al.1999). Therefore, compared to several herbicides commonly used for field bindweed control, aminocyclopyrachlor appears to have average absorption.

Aminocyclopyrachlor exhibited similar but slightly lower absorption in bindweed compared to C. thistle (57%) by 24 HAT (Bukun et al. 2010) and rush skeletonweed (55%) (Bell et al. 2011). In prickly lettuce and yellow starthistle, aminocyclopyrachlor absorption was much less compared to bindweed with 10% and 5%, respectively (Bell et al. 2011). It is interesting that field bindweed had similar aminocyclopyrachlor absorption as the two other perennial species previously studied. It also interesting to note that the two annual species studied had very low aminocyclopyrachlor absorption.

There was a clear pattern of accumulation into and subsequent translocation out of the treated leaf with time (Fig.3.2). Aminocyclopyrachlor accumulation in field bindweed treated leaves reached a maximum of 25% of the applied radioactivity by 12 HAT; however accumulation diminished to 12% by 192 HAT due to translocation. Aminocyclopyrachlor translocation to above- and below-ground tissue increased with time and reached a maximum for both tissues by 192 HAT at 14% for both tissues. Total radioactivity recovery declined from 95% at 6 HAT to 84% by 192 HAT (data not shown). Losses could have been incurred through sample processing or loss as $^{14}CO_2$, but are within acceptable levels considering the number of processing steps during harvest and extraction, as well as the recovery efficiency during sample oxidation.

Aminocyclopyrachlor accumulation in the treated leaf was about twice that of quinclorac at 48 HAT, but ended the study at similar values (12% and 13% for aminocyclopyrachlor and 192 HAT and quinclorac at 168 HAT, respectively) (Enloe et al. 1999). At the end of the respective studies, aminocyclopyrachlor had much greater translocation to above-ground tissue than did quinclorac (14% at 192 HAT compared to 1.7% at 168 HAT) (Enloe et al. 1999). Similarly, aminocyclopyrachlor had much greater

translocation to below-ground tissue than did quinclorac (14% at 192 HAT compared to 1.6% at 168 HAT) (Enloe et al. 1999). Aminocyclopyrachlor differs in translocation patterns from quinclorac, as it seems to be more evenly distributed between the treated leaf, above- and below-ground tissues by 192 HAT. In contrast, Enloe et al. (1999) observed that the majority of the quinclorac remained in the treated leaf, even 168 HAT. Flint and Barrett (1989) found that, when applied at 0.28 kg ha^{-1}, only 21% of the glyphosate translocated out of the treated leaf by 72 HAT. Of the absorbed ^{14}C-glyphosate, the greatest amount of radioactivity also remained in the treated leaf. Since these data were based on a percentage of absorbed radioactivity in field bindweed, it is very difficult to make comparisons between aminocyclopyrachlor and glyphosate translocation. Agbakoba and Goodin (1970) saw almost equivalent accumulation of ^{14}C-2, 4-D in apical meristem and root tissue, but much higher accumulation of ^{14}C-picloram in the apical meristem compared to the roots in field bindweed. Radioactivity was reported as the specific activity for each plant part on a fresh weight basis, so again, direct comparisons with the aminocyclopyrachlor translocation data from this study are impossible. Overall, it would appear that aminocyclopyrachlor translocated throughout bindweed plants, especially to roots as well as or better than other commonly used herbicides.

Compared to Canada thistle, aminocyclopyrachlor translocation to above-ground tissue in bindweed was less with 14% compared to 18% 192 HAT (Bukun et al. 2010). Translocation to above-ground tissue in field bindweed was similar to yellow starthistle (9%) 72 HAT, but greater compared to rush skeletonweed and prickly lettuce (2% and 1%, respectively) (Bell et al. 2011). However, translocation to below-ground tissue was

greater in bindweed compared to Canada thistle at 14% and 6%, respectively 192 HAT (Bukun et al 2010). Similarly, aminocyclopyrachlor translocation to below-ground tissue was much greater in field bindweed compared to rush skeletonweed (2.5%), yellow starthistle (1.5%), and prickly lettuce (<1%) by 72 HAT. Compared to other species that have been treated with aminocyclopyrachlor, field bindweed seems to have the greatest translocation to root tissue, which undoubtedly contributed to the elevated level of control at use rates reported from the field.

Long-term field bindweed control with aminocyclopyrachlor appears to be related to the elevated levels of translocation to below-ground plant tissue compared to other herbicides and is greater when compared to other species treated with aminocyclopyrachlor. The duration of control observed with aminocyclopyrachlor in field bindweed could possibly also be due to soil residual activity and direct herbicide absorption by plant roots over time. Increased biological activity of aminocyclopyrachlor in field bindweed compared to other herbicides and other species may also be a factor in long-term perennial weed control. More research is needed to elucidate how this herbicide behaves in the soil and its activity relative to other non-cropland and rangeland herbicides.

Aminocyclopyrachlor Metabolism.

No soluble aminocyclopyrachlor metabolites were found in any field bindweed tissue at any time during the experiment. Bukun et al. (2010) observed rapid conversion of the methyl-ester to the free acid in Canada thistle (82% 6HAT). Bukun et al. (2010) postulate that, when applied as the methyl-ester, the ester group facilitates absorption

through the leaf cuticle and acts as a pro-herbicide. Once in the cuticle, carboxylesterases

metabolize the herbicide to the free-acid form (Gershater and Edwards 2007). This is

supported by Bell et al. (2011) who only observed conversion of the methyl-ester to the

free acid with a half-life of only 3.5 h, which occurred solely in the treated leaves of rush

skeletonweed. Bell et al. (2011) did not detect any subsequent metabolites of the free

acid in any plant tissue, indicating that the free acid is the mobile form of the herbicide.

Similarly, Enloe et al. (1999) saw very little metabolism of quinclorac in field bindweed

with <5% converted to metabolites 168 HAT.

However, the radioactivity in field bindweed was observed in the bound fraction

in all plant parts and the amount of radioactivity associated with the bound fraction

increased with time based on the total absorbed radioactivity (Fig. 3.3). Using 95%

confidence intervals, the amount of bound herbicide differed significantly between all

three plant parts by 192 HAT. The above-ground tissue had the greatest amount of

incorporated herbicide (4%), followed by the treated leaf (2%), with the below-ground

tissue having the least amount of incorporated herbicide (0.05%) as a percentage of

absorbed radioactivity.

Further study is needed, but the rate of incorporation may be due to different

concentrations of the enzyme responsible for the herbicide metabolism in the different

plant parts. While the exact mechanism of incorporation cannot be determined from the

current study, one possible reaction is the conjugation to a sugar catalyzed by either *O-* or

N-glucosyltransferases, forming a glucose ester at the carboxylic acid side-chain using

UDP-glucose as the sugar donor (Devine et al. 1993; Kreuz et al. 1996). Glucose

esterification is not a stable detoxification and can easily be converted back into the

active herbicide; however, glucosylated compounds have been shown to be sequestered in the cell vacuole (Davidonis et al. 1982; Devine et al. 1993; Shmidt & Sandermann 1982). Since no soluble metabolites were observed, it is not likely that this is the specific mechanism responsible for the metabolism of aminocyclopyrachlor.

The chlorine atom on the aminocyclopyrachlor molecule also makes for an excellent leaving group that can be replaced with glutathione via glutathione-*S*-transferases (GSTs) in a nucleophilic displacement reaction. This is well documented as the mechanism of herbicidal safening for thiocarbamate and chloroacetamide herbicides (Breaux et al. 1987; Fuerst and Gronwald 1986; Gronwald et al. 1987; Komives et al. 1985; Lay & Cassida 1976). Like glucose conjugation, glutathione conjugates are sequestered in the valcuole (Kreuz et al. 1996; Martinoia et al. 1993). Since no soluble metabolites were found, glutathione conjugation may not be the most likely mechanism of aminocyclopyrachlor metabolism either.

Another possible reaction is the conjugation to an amino acid at the carboxylic acid side-chain, usually aspartate or glutamate, forming a carboxylic acid amide (Devine et al. 1993). These bonds are also easily broken, but there is evidence that amide conjugates are excreted into the cell wall space (Davidonis et al. 1982), where it may eventually be incorporated into the protein portions of lignin. Lignin binding is a common metabolic mechanism of auxinic herbicides (Devine et al. 1993, Reinke and Bandurski, 1987). Conjugation to an amino acid is perhaps more likely than conjugation to a sugar as Lewer & Owen (1987) have shown that susceptible species, like lambsquarters, predominantly form aspartate conjugates and tolerant species, like wheat, predominantly form glycoside esters. It follows that field bindweed, being a susceptible

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species would more likely conjugate with amino acids. Herbicides containing heterocyclic rings, like aminocyclopyrachlor, are particularly susceptible to this eventual fate. Also, since no soluble metabolites were found, amino acid conjugation and subsequent lignin incorporation may be the most likely mechanism of aminocyclopyrachlor metabolism.

Even though amino acid conjugation has been well documented for over 50 years (Andrea and Good, 1955), the biosynthetic pathways remain elusive (Staswick et al. 2005). Bacterial IAA-Lys synthetase has been identified by Roberto et al. (1990) but not much has been revealed about the nature of the IAA-amido conjugating counterparts in plants. The most recent advancement was the characterization of synthases that produce IAA-glucosyl esters by Szerszen et al. (1994) and the hydrolases that release IAA from those bonds (Jakubowska et al. 1993).

Regardless of the exact mechanism of aminocyclopyrachlor metabolism in field bindweed, the relative amounts of the compound that were incorporated into the plant tissue were not substantial. It would appear that the herbicide is translocated throughout the plant to sink tissues as the intact molecule. Once at its destination, it is slowly incorporated into the plant tissue itself, but the incorporation itself may be rapid since no soluble metabolites were detected at any time point during the experiment. Our research agrees with previous studies using other species and indicates that aminocyclopyrachlor translocated as the free acid and is relatively stable in susceptible plants. There is no information available in the literature to shed light on aminocyclopyrachlor absorption, translocation, or metabolism in more tolerant species. Such information might help

explain why aminocyclopyrachlor displays such biological activity on susceptible

species, such as field bindweed.

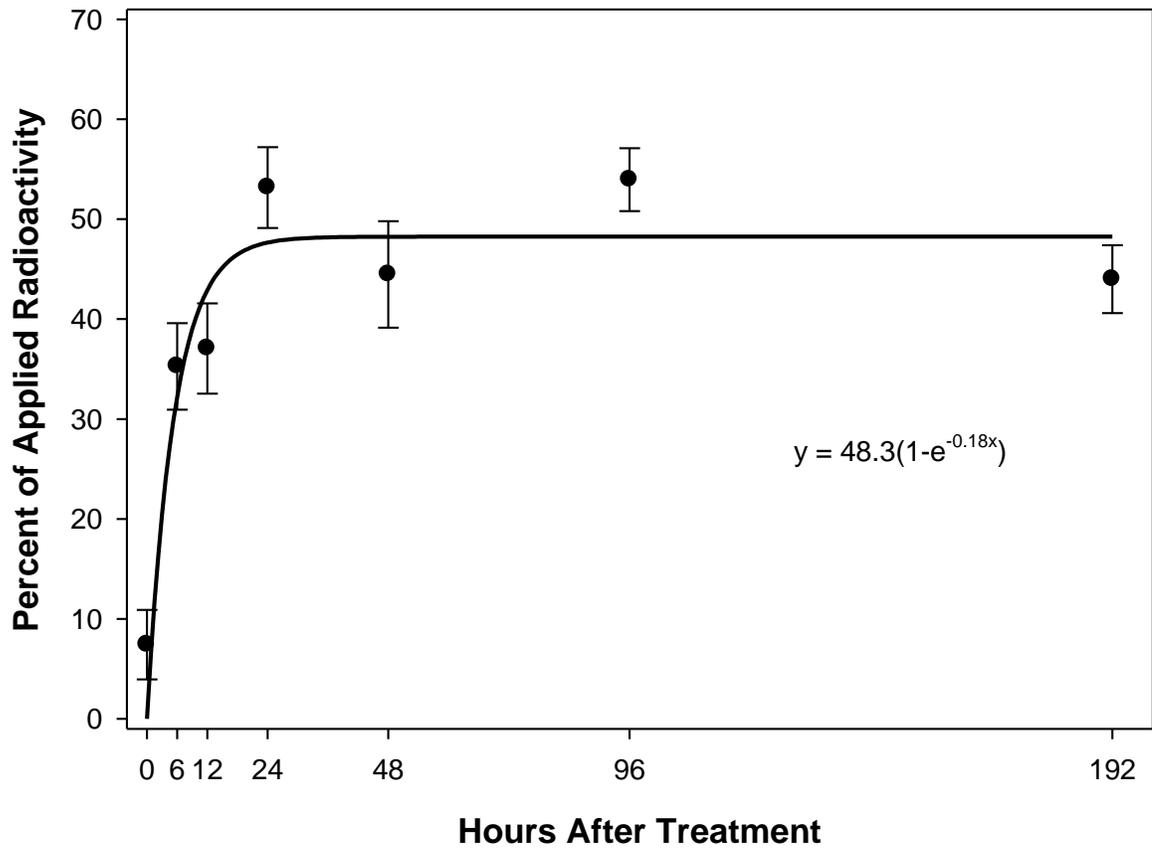Figure 3.1. ^{14}C-Aminocyclopyrachlor absorption into field bindweed leaf tissue over time as a percentage of applied radioactivity.
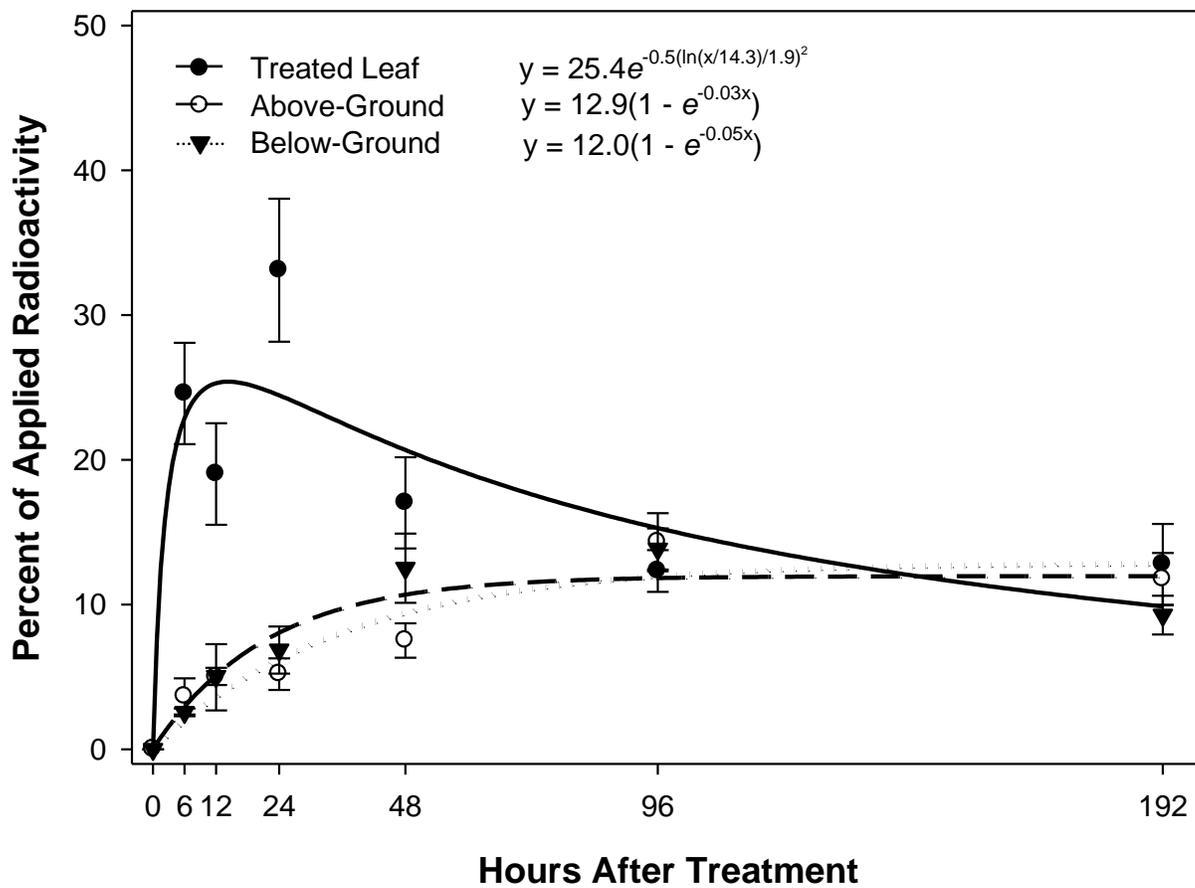
Figure 3.2. ^{14}C-Aminocyclopyrachlor translocation in field bindweed over time as a percentage of applied radioactivity.
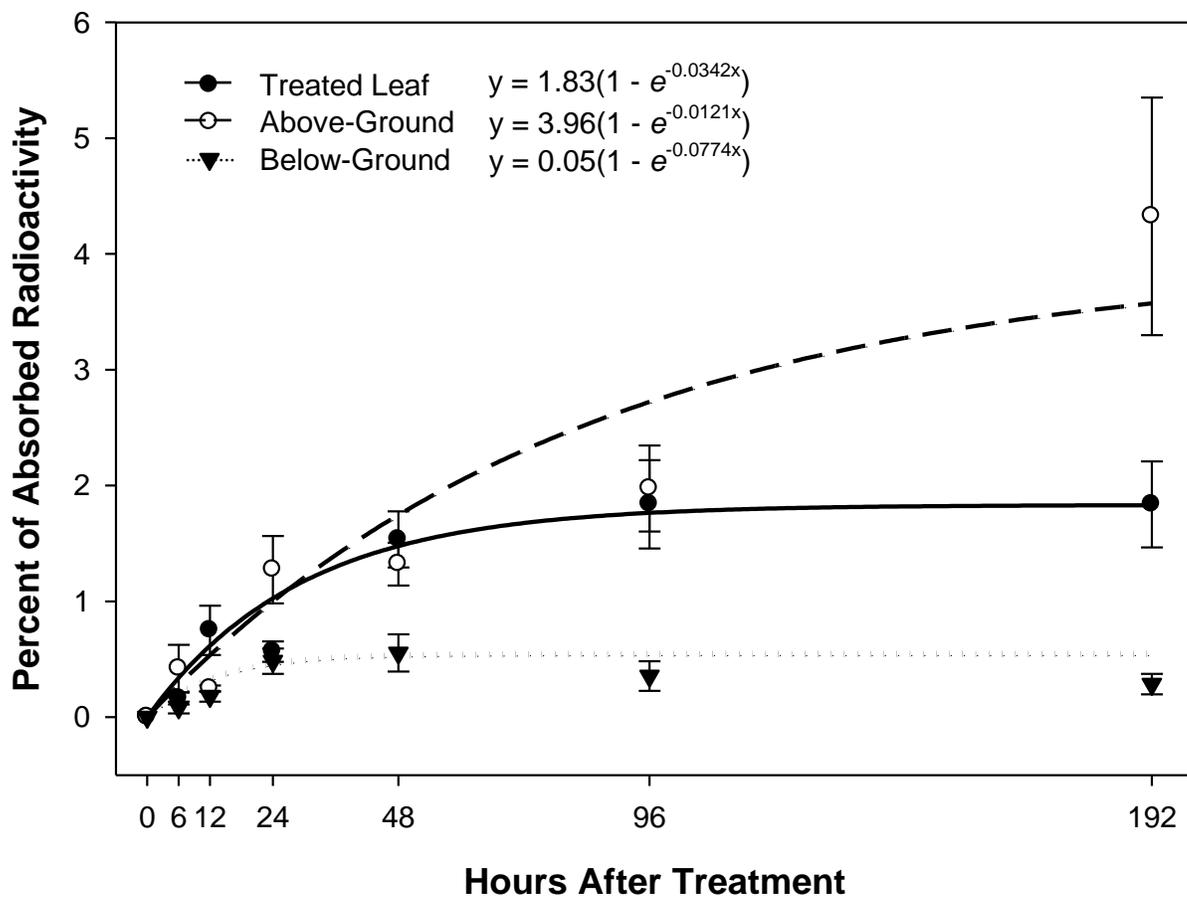
Figure 3.3. ^{14}C-Aminocyclopyrachlor incorporation into the insoluble fraction over time as a percentage of absorbed radioactivity.

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