To my wife Sandra in appreciation for her love, support, help, and encouragement
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Aminopyralid is a synthetic auxin herbicide of the pyridinecarboxylic acid family that was introduced in 2005; thus, the amount of information available on this herbicide is limited. Greenhouse experiments were conducted to determine the absorption, translocation, and metabolism of $^{14}$C-aminopyralid in the southern forage bahiagrass ($Paspalum notatum$ Fluegge). Absorption of $^{14}$C-aminopyralid was 99% at 4 DAT with 89% of the $^{14}$C recovered remaining in the treated leaves. Shoots of plants contained 9% of recovered $^{14}$C, and less than 1.0% of recovered $^{14}$C was translocated to the roots of bahiagrass plants. The portion of recovered $^{14}$C that remained in the form of aminopyralid was 94% or greater. Due to the environmental persistence of this herbicide, field experiments were conducted to determine the sensitivity of bell pepper, $Capsicum annuum$ L.; eggplant, $Solanum melongena$ L.; muskmelon, $Cucumis melo$ L.; tomato, $Lycopersicon esculentum$ Mill.; watermelon, $Citrullus lanatus$ (Thunb.) Matsum. & Nakai to aminopyralid soil residues. Aminopyralid was applied at six rates from 0.0014 kg ae ha$^{-1}$ to 0.0448 kg ae ha$^{-1}$, and vegetable crops were planted in the treated areas. At an aminopyralid soil concentration of 0.2 µg kg$^{-1}$, the limit of quantitation (LOQ) for aminopyralid in this research, crop injury ratings 6 wk after planting were 48 (bell pepper), 67 (eggplant), 71 (tomato), 3 (muskmelon), and 3% (watermelon), and fruit yield losses (relative to the untreated...
control) at 0.2 µg kg⁻¹ aminopyralid were 61, 64, 95, 8, and 14% in those respective crops.

Laboratory experiments were conducted to determine the sorption of aminopyralid to soil and clay minerals. Freundlich distribution coefficients ($K_f$) for aminopyralid ranged from 0.35 in a Cecil sandy loam to 0.96 in an Arredondo fine sand. It was concluded that soil sorption of aminopyralid was greater than that of picloram and that the potential for off-target movement of aminopyralid is less than that of picloram. Mineralization of picloram and aminopyralid in Sharkey clay was 15.2 and 23.7%, respectively, and mineralization of those respective herbicides in Arredondo fine sand was 5.4 and 9.2%.
ABSORPTION, TRANSLOCATION, AND METABOLISM OF AMINOPYRALID AND PICLORAM IN BAHIAGRASS

Introduction

Aminopyralid is a pyridinecarboxylic acid herbicide that was registered in 2005 for annual and perennial broadleaf weed control in rangeland, permanent grass pastures, and non-cropland areas at rates of 0.05 to 0.12 kg ae ha\(^{-1}\) (Anonymous 2008; USOPPEPTS 2005). It provides foliar and soil residual weed control, is moderately sorbed \(K_f = 0.35–0.96\), and is a relatively persistent herbicide in the soil (half-life = 34.5 d) (Fast et al. 2010a; Senseman 2007b). While the residual activity of aminopyralid is desirable from a weed management standpoint, aminopyralid carryover can injure broadleaf crops that are planted on a site where aminopyralid was previously applied. This issue is of extreme importance in Florida where aminopyralid is commonly used for weed control in bahiagrass \(Paspalum notatum\) Fluegge) pastures. Vegetable crops are often grown in rotation with bahiagrass pasture (Hopkins and Elmstrom 1984), and several of Florida’s most economically important vegetable crops, including bell pepper \(Capsicum annuum\) L., eggplant, \(Solanum melongena\) L., and tomato \(Lycopersicon esculentum\) Mill., are sensitive to aminopyralid at soil concentrations of 1 µg kg\(^{-1}\) or less (Fast et al. 2010b).

Tropical soda apple \(Solanum viarum\) Dunal.) is one of the most common and troublesome pasture weeds in Florida (Webster 2008) due to prolific seed production, high seed germination rates (70 to 90%), and limited herbicide options (Bryson and Byrd 2007; Mullahey and Cornell 1994). Plants typically produce 150 to 400 fruits per plant with 400 to 500 seeds per fruit with rapid germination and seedling establishment (Bryson and Byrd 2007). The herbicide triclopyr is capable of providing 90% control of emerged tropical soda apple, but control is ephemeral as seedlings quickly re-infest the treated area in the months following herbicide application (Ferrell
et al. 2006; Mullahey et al. 1993). Conversely, aminopyralid provides excellent control of emerged plants and residual control of seedlings for up to one year after treatment (Ferrell et al. 2006). Most herbicides that are used for soil residual control are applied directly to bare soil; however, several of these types of herbicides are intercepted by cover-crop foliage and mulch residues in no-till production systems and herbicide efficacy is reduced (Chokor et al. 2008; Ghadiri et al. 1984; Schmitz et al. 2001). Likewise, aminopyralid is commonly applied to dense and vigorously growing vegetation (Ferrell et al. 2006), and it is likely that this vegetation intercepts the vast majority of the herbicide, preventing direct soil sorption. The fact that aminopyralid has soil residual activity even when the herbicide is not applied directly to the soil suggests that some mechanism may be present to allow the herbicide to move from vegetation to the soil.

Picloram and clopyralid are two herbicides that are nearly structurally identical to aminopyralid. The metabolism of picloram in pea (Pisum sativum L.), barley (Hordeum vulgare L.), soybean [Glycine max (L.) Merr.], Canada thistle [Cirsium arvense (L.) Scop.], horsenettle (Solanum carolinense L.), and leafy spurge (Euphorbia esula L.) is negligible (Gorrell et al. 1988; Lym and Moxness 1989; Scott and Morris 1970; Sharma and Vanden Born 1973). Additionally, clopyralid is not readily metabolized in hemp dogbane (Apocynum cannabinum L.) and yellow starthistle (Centaurea solstitialis L.) (Orfanedes et al. 1993; Valenzuela-Valenzuela et al. 2001). In many plants, these herbicides are conjugated in the parent form and sequestered in the cell vacuole. Thus, the herbicide remains in the active form within the plant tissues. The lack of metabolism of these herbicides is problematic, particularly when treated plants are used for mulch in sites where sensitive crop species will be grown. Because picloram and clopyralid are not typically metabolized, mulch that is composed of plants that were treated with these
herbicides may contain enough herbicide to injure susceptible broadleaf plants as it decomposes and the herbicide is released (Anonymous 2009a, 2009b). When spearmint (Mentha cardiaca Baker) was treated with clopyralid and the spearmint residue was incorporated into soil, potato (Solanum tuberosum L.) grown in the treated soil sustained injury (Boydston 1994). Compost that contained 50 µg kg⁻¹ clopyralid was applied on a site at 112 metric tons ha⁻¹, and tomato plants that were grown on the site sustained injury (Blewett et al. 2005). It has also been reported that clopyralid in mulch can injure susceptible plants at concentrations as low as 10 µg kg⁻¹ (Rynk 2002). Clopyralid can remain in plant residue at concentrations high enough to injure sensitive plants for a long period of time; moreover, grass clippings that had an initial clopyralid concentration of 200 µg kg⁻¹ showed a concentration of 20 µg kg⁻¹ 98 weeks after treatment (Miltner et al. 2003). Problems with clopyralid in mulch causing crop injury became so prevalent in California, Oregon, and Washington that restrictions were placed on clopyralid use in those states (Brank 2003; ODA 2003; WSDA 2003). Due to the chemical similarity of aminopyralid, picloram, and clopyralid, it is questioned whether or not the behavior of aminopyralid in plants is similar to that of clopyralid and picloram. The aminopyralid product label states that aminopyralid-treated plant residues should not be used in mulch that will be applied where susceptible broadleaf plants will be grown (Anonymous 2008). It has also been reported that aminopyralid was not metabolized in Canada thistle (Bukun et al. 2009). This information suggests that aminopyralid, like picloram and clopyralid, is sequestered in plants and released in the active form as the plant tissue decomposes. However, no published data are available on aminopyralid absorption, translocation, and metabolism in bahiagrass or the release of aminopyralid from treated plant residue. It is important to have a clear understanding of the anthropomorphic behavior of aminopyralid in plants and release into the soil to prevent
unwanted crop injury from occurring as a result of aminopyralid carryover. Therefore, the objective of this research was to quantify aminopyralid absorption, translocation, and metabolism in bahiagrass and compare to the known herbicide picloram.

**Materials and Methods**

Bahiagrass rhizomes were collected from established pasture in North Central Florida, cut into 8 cm segments, and placed in 12 cm diameter pots containing potting soil media. Pots were placed in a greenhouse and plants were treated with aminopyralid (0.12 kg ae ha\(^{-1}\)) or picloram (0.56 kg ae ha\(^{-1}\)) after reaching a height of 25 cm (approximately 4 weeks). Herbicides were applied with a track sprayer calibrated to deliver 140 L ha\(^{-1}\) with an 8002E nozzle, and each spray solution contained 0.25% v/v non-ionic surfactant. Immediately after application, plants were spotted with 6.7 kBq of ring-labeled \(^{14}\)C-aminopyralid (specific activity = \(1.01 \times 10^9\) kBq mol\(^{-1}\), radiochemical purity = 98%) or \(^{14}\)C-picloram (specific activity = \(9.10 \times 10^8\) kBq mol\(^{-1}\), radiochemical purity = 99%) in a total volume of 10 µL. Spotting consisted of four 2.5-µL droplets of \(^{14}\)C herbicide solution placed on the adaxial surface of four random leaves of each plant.

**Absorption and Translocation**

Plants were harvested 1 and 4 days after treatment (DAT). Treated leaves were excised and washed with 5 mL of deionized water (five 1-mL aliquots). The leaf rinse solutions were combined for each plant and 2 mL of the combined leaf wash was added to 15 mL of scintillation cocktail\(^1\). Soil was washed from roots, and plants were placed in a plant press and oven dried at 40 C for 48 hr. Oven-dried plants were then sectioned into treated leaves, shoot, and roots, and sections were combusted in a biological oxidizer\(^2\). Evolved \(^{14}\)CO\(_2\) was trapped in 20 mL of scintillation cocktail\(^3\), and radioactivity in leaf washes and oxidations was quantified using liquid scintillation spectrometry\(^4\). Mean \(^{14}\)C recovery was determined by summing the \(^{14}\)C present in
the leaf washes and plant segments. Average $^{14}$C recovery was 97% of applied for aminopyralid and 85% of applied for picloram. The experimental design was a completely randomized design with three replications, and the experiment was repeated.

**Metabolism**

Plants were grown and treated with $^{14}$C-herbicide as described above and harvested for metabolism analysis at 1 and 4 DAT. Leaf washes were performed, and plants were sectioned into roots and shoots, immediately frozen in liquid nitrogen, and placed in -10°C storage until laboratory analysis was performed. Herbicide metabolism was determined using thin layer chromatography (TLC) as described by Lym and Moxness (1989). Tissue from treated leaves (0.2 g) was ground with a tissue homogenizer in 2 mL of 80:20 (v/v) ethanol/water. The suspension was then centrifuged at 5000 × g for 8 min, and the supernatant decanted. An additional 1 mL of 80:20 (v/v) ethanol/water was added, and the pellet was rehomogenized. The suspension was again centrifuged for 8 min at 5000 × g and the supernatant was added to the test tube. The supernatant was evaporated to a volume of 200 µL in a nitrogen evaporator at 50°C. A two-way TLC analysis was then performed using 20- by 20-cm silica gel plates. The 200 µL of plant extract was placed on the plates using a micropipette, and the plates were then developed using 80:40:10:5 (v/v/v/v) benzene/acetone/acetic acid/methanol. Plates were then air dried, rotated 90 degrees, and developed in 65:25:4 (v/v/v) chloroform/methanol/water. Plates were air dried and scanned using a radiochromatogram scanner. For comparison, separate plates were also developed using this procedure that contained 10 µL of stock radiolabeled solution. The experimental design was a completely randomized design with three replications, and the experiment was repeated.

A bioassay experiment was conducted with aminopyralid to provide additional metabolism data. Aminopyralid (0.12 kg ae ha$^{-1}$) was applied to Arredondo fine sand (Loamy, siliceous,
semiactive, hyperthermic Groassarenic Paleudults) in 8 cm square pots and was also applied to
field-grown bahiagrass. Aboveground bahiagrass biomass was harvested 0 DAT (immediately
after application when herbicide solution had dried on the leaf) and 2 DAT (after 2.5-cm of
simulated rainfall had been applied to the treated area to wash unabsorbed herbicide from
leaves). Bahiagrass was dried at 40 C for 48 hr and ground to pass a 2-mm sieve. A 1.2 g
sample of the bahiagrass was incorporated into 590 g of Arredondo fine sand in 8-cm square
pots. Water was added to bring soil to 80% field capacity, and three tomato seeds were planted
in the pots three weeks later. Tomato plants were thinned to one plant per pot one week after
emergence. Aboveground tomato plant biomass was harvested 4 weeks after planting and
percentage fresh weight reduction relative to the untreated control was calculated. The
experimental design was a completely randomized design with three replications and the
experiment was repeated.

**Results and Discussion**

**Absorption and Translocation**

\(^{14}\)C-aminopyralid was readily absorbed by bahiagrass, with approximately 93.0 and 98.7%
absorbed at 1 and 4 DAT, respectively (Table 1-1). Of the \(^{14}\)C recovered, 82.1% (1 DAT) and
88.7% (4 DAT) remained in the treated leaves, 10.3% (1 DAT) and 9.5% (4 DAT) was
translocated to the shoot, and less than 1.0% (1 and 4 DAT) was translocated to the roots.
Absorption of \(^{14}\)C-picloram was less than that of \(^{14}\)C-aminopyralid. Moreover, \(^{14}\)C-picloram
absorption was 63.6% at 1 DAT and 92.1% at 4 DAT. The distribution of \(^{14}\)C among plant
segments was similar for plants treated with either aminopyralid or picloram. Of the \(^{14}\)C
recovered from plants treated with \(^{14}\)C-picloram, 56.0% (1 DAT) and 80.8% (4 DAT) remained
in the treated leaves, 7.0% (1 DAT) and 10.6% (4 DAT) was translocated to the shoot, and less
than 1.0% (1 and 4 DAT) was translocated to the roots. Other researchers have likewise shown
limited translocation from foliar applications on Canada thistle and woolly loco (*Astragalus mollisimus* Torr.) (Bukun et al. 2009; Sterling and Jochem 1995).

**Metabolism**

Metabolism of both aminopyralid and picloram was negligible in bahiagrass (Table 1-2). Approximately 94.0 and 96.2% of $^{14}$C was found to be in the parent form of $^{14}$C-aminopyralid at 1 and 4 DAT, respectively. Picloram was found to be unmetabolized, with 99.0 and 96.6% of $^{14}$C remaining in the form of $^{14}$C-picloram 1 and 4 DAT, respectively. Plants were not harvested beyond 4 DAT because metabolism of other synthetic auxin herbicides typically occurs within 4 DAT; moreover, metabolism of triclopyr in rice (*Oryza sativa* L.), 2,4-D common milkweed (*Asclepias syriaca* L.) and honeyvine milkweed (*Cynanchum laeve* (Michx.) Pers.), and picloram in broom snakeweed (*Gutierrezia sarothrae* (Pursh) Britt. & Rusby) occurred within 4 DAT (Braverman 1995; Coble et al. 1970; Gibbs and Sterling 2004; Wyrill and Burnside 1976).

In the bioassay experiment, a 93% reduction in tomato fresh weight was observed when plants were grown in soil treated with 0.12 kg ae ha$^{-1}$ aminopyralid (Table 1-3). When aminopyralid-treated bahiagrass leaves were harvested 0 and 2 DAT and mixed with soil, growth reductions in seedling tomato were 85 and 67%, respectively. Clopyralid can also injure susceptible plant species when clopyralid-treated plants are used for mulch (Anonymous 2009b; Blewett et al. 2005; Boydston 1994). These data indicate that aminopyralid is not metabolized in bahiagrass and that it can be released into the soil and affect susceptible plant species as the bahiagrass decomposes.

Based on the results of this research, it was concluded that aminopyralid and picloram translocation from foliar applications in bahiagrass is minimal and that these herbicides are not readily metabolized in bahiagrass. It was also concluded that aminopyralid can be sequestered in bahiagrass and released into the soil as the plant tissue decomposes. This suggests that
bahiagrass contributes to the soil residual activity of aminopyralid by absorbing the herbicide and slowly releasing it into the soil as the plant tissue decomposes.

Sources of Materials


2 Biological Oxidizer, Model OX500, R. J. Harvey Instrument Corporation, Hillsdale, NJ 07642.

3 Carbon-14 Cocktail, R. J. Harvey Instrument Corporation, Hillsdale, NJ 07642.


5 Dremel Model 300 Tissue Homogenizer, Dremel Corporation, Racine, WI.

6 Zymark TurboVap LV Evaporator, SOTAX Corporation, Hopkinton, MA 01748.

7 Whatman K6F Silica Gel 60 Å Thin Layer Chromatography Plates, Whatman Incorporated, Clifton, NJ 07013.

Table 1-1. Absorption and distribution of $^{14}$C-aminopyralid and $^{14}$C-picloram in bahiagrass 1 and 4 d after treatment (DAT).

<table>
<thead>
<tr>
<th>Herbicide</th>
<th>% of recovered $^{14}$C (SE)</th>
<th>1 DAT</th>
<th>4 DAT</th>
<th>1 DAT</th>
<th>4 DAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treated leaf</td>
<td></td>
<td>82.1 (3.2)</td>
<td>88.7 (1.5)</td>
<td>56.0 (2.1)</td>
<td>80.8 (2.4)</td>
</tr>
<tr>
<td>Shoot</td>
<td></td>
<td>10.3 (2.0)</td>
<td>9.5 (1.7)</td>
<td>7.0 (2.3)</td>
<td>10.6 (3.5)</td>
</tr>
<tr>
<td>Root</td>
<td></td>
<td>0.7 (0.1)</td>
<td>0.5 (0.1)</td>
<td>0.6 (0.1)</td>
<td>0.7 (0.1)</td>
</tr>
<tr>
<td>Unabsorbed</td>
<td></td>
<td>6.9 (1.5)</td>
<td>1.3 (0.2)</td>
<td>36.4 (5.1)</td>
<td>7.9 (1.0)</td>
</tr>
</tbody>
</table>

Table 1-2. Percentage of recovered $^{14}$C remaining in the form of the parent herbicide ($^{14}$C-aminopyralid or $^{14}$C-picloram) in bahiagrass 1 and 4 d after treatment (DAT).

<table>
<thead>
<tr>
<th>Herbicide</th>
<th>Harvest timing</th>
<th>% of recovered (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{14}$C-aminopyralid</td>
<td>Standard$^1$</td>
<td>99.6 (0.2)</td>
</tr>
<tr>
<td>$^{14}$C-aminopyralid</td>
<td>1 DAT</td>
<td>94.0 (1.5)</td>
</tr>
<tr>
<td>$^{14}$C-aminopyralid</td>
<td>4 DAT</td>
<td>96.2 (1.2)</td>
</tr>
<tr>
<td>$^{14}$C-picloram</td>
<td>Standard</td>
<td>99.3 (0.3)</td>
</tr>
<tr>
<td>$^{14}$C-picloram</td>
<td>1 DAT</td>
<td>99.0 (0.5)</td>
</tr>
<tr>
<td>$^{14}$C-picloram</td>
<td>4 DAT</td>
<td>96.6 (1.6)</td>
</tr>
</tbody>
</table>

$^1$Standard = 333 Bq of $^{14}$C herbicide applied directly to TLC plate

Table 1-3. Percentage reduction in tomato fresh weight from plants grown in soil that received 0.12 kg ae ha$^{-1}$ aminopyralid or soil that contained 1.2 g of bahiagrass that had been treated with 0.12 kg ae ha$^{-1}$ aminopyralid 0 or 2 d before harvest.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tomato plant fresh weight reduction % relative to untreated control (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil</td>
<td>93 (3)</td>
</tr>
<tr>
<td>Bahiagrass harvested 0 DAT</td>
<td>85 (9)</td>
</tr>
<tr>
<td>Bahiagrass harvested 2 DAT</td>
<td>67 (3)</td>
</tr>
</tbody>
</table>
CHAPTER 2
AMINOPYRALID SOIL RESIDUES AFFECT VEGETABLE CROPS

Introduction

Florida is the second largest producer of fresh market vegetables in the US, with nearly 2 million metric tons valued at 1.4 billion dollars produced in 2009 (USDA–NASS 2010). The warm, humid climate and sandy soils in Florida are a favorable environment for soilborne pests, including damping-off (*Rhizoctonia solani* Kühn and *Pythium* spp.), Fusarium wilt (*Fusarium oxysporum* Schltdl.), Phytophthora root rot (*Phytophthora* spp.), Verticillium wilt (*Verticillium dahliae* Kleb.), white mold (*Sclerotinia sclerotiorum* (Lib.) de Bary], and sting (*Belonolaimus longicaudatus* Rau) and root-knot (*Meloidogyne* spp.) nematodes (Koike et al. 2003; Noling 2007; Sumner et al. 1999). These major soilborne pests of vegetable crops in Florida have been managed effectively since the 1960’s with the use of methyl bromide as the primary method of soilborne pest control (Chellemi 2002). The production and import of methyl bromide in the US was banned by the EPA in 2005 (USEPA 2008a) as a result of its negative effects on the stratospheric ozone layer. Methyl bromide is currently being phased out with new production only possible for quarantine and preshipment uses and those crops that qualify for a critical use exemption (CUE) as decided by the Parties of the Montreal Protocol (UNEP 2009). This management tool will no longer be available when CUEs are no longer granted and the existing supplies of methyl bromide are depleted. The reduction in the availability of methyl bromide for soilborne pest control has forced producers to rely more heavily on other control methods. An effective and inexpensive way to control soilborne pests is to use crop rotations in which vegetable crops are rotated with crops that do not serve as host plants for these soilborne pests (Chellemi 2002; Momol et al. 2007; Noling 2007).
Bahiagrass (*Paspalum notatum* Flueggé) is the most commonly planted warm-season grass in Florida, and it occupies an estimated 1 million ha in the state (Chambliss et al. 1999). It tolerates a wide range of environmental conditions and fertility regimes while requiring less intensive management than many other forage grasses (Ball et al. 2002;). Since bahiagrass pasture is abundant in Florida and bahiagrass is not a suitable host plant for numerous soilborne pests, vegetable crops are commonly grown in rotation with bahiagrass pasture (Hopkins and Elmstrom 1984). Research has demonstrated that damping-off, fusarium wilt, yellow nutsedge (*Cyperus esculentus* L.), and root galls from root-knot nematode decreased and crop yields increased when cucumber (*Cucumis sativus* L.), snap bean (*Phaseolus vulgaris* L.), and watermelon were planted after three years of bahiagrass (Hopkins and Elmstrom 1984; Sumner et al. 1999). In addition to controlling pests, crop rotations with bahiagrass increase crop yields by improving the water holding capacity, nitrogen content, organic matter content, and soil tilth (Beaty and Tan 1972; Breneman et al. 1995; Burton 1954; Hopkins and Elmstrom 1984). It should be noted that some weed species that grow in rotational crops can serve as alternative hosts for soilborne pests (Rich et al. 2009). Tropical soda apple (*Solanum viarum* Dunal), for example, is a common and troublesome weed of pastures in Florida (Mullahey and Cornell 1994), and it can serve as a host plant for root-knot nematode (Church and Rosskopf 2005). Therefore, weed control is imperative in bahiagrass pastures that are employed in a vegetable crop rotation.

Aminopyralid, a synthetic auxin herbicide registered in 2005, is labeled for annual and perennial broadleaf weed control in rangeland, permanent grass pastures, and non-cropland areas at rates of 0.05 to 0.12 kg ae ha\(^{-1}\) (Anonymous 2008; USOPPEPTS 2005). Because it is relatively persistent in the soil (half-life = 34.5 days), aminopyralid provides both foliar and soil
residual weed control; moreover, it provides excellent control of tropical soda apple for up to one year after application (Senseman 2007b; Ferrell et al. 2006). Because of its high efficacy and residual activity on several common and troublesome pasture weed species, aminopyralid is used for weed control in pastures throughout Florida. However, an aminopyralid product label states that pastures treated with aminopyralid are not to be rotated to any crop within one year after treatment. Further label restrictions state that a broadleaf crop should not be planted in a treated area until an adequately sensitive field bioassay reveals that the crop will not be adversely affected (Anonymous 2008). While the residual activity of aminopyralid is desirable from a weed control standpoint, injury in subsequent broadleaf crops arising from aminopyralid carryover is a potential negative consequence. As a result of the heavy use of aminopyralid for pasture weed control and the fact that vegetable crops are commonly planted in rotation with bahiagrass pasture, there is a need for information on the effects of aminopyralid on vegetable crops. The objective of this research was to quantify the sensitivity of bell pepper (*Capsicum annuum* L.), eggplant (*Solanum melongena* L.) tomato (*Lycopersicon esculentum* Mill.), muskmelon (*Cucumis melo* L.), and watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai.] to soil residues of aminopyralid.

**Materials and Methods**

Field experiments were conducted on a Lakeland fine sand (Thermic, coated Typic Quartzipsamments) at the University of Florida North Florida Research and Education Center near Live Oak, FL and on an Arredondo fine sand (Loamy, siliceous, semiactive, hyperthermic Groassarenic Paleudults) at the University of Florida Plant Science Research and Education Unit near Citra, FL. Bell pepper, eggplant, and tomato were seeded on 7 March 2008 in a greenhouse in Gainesville, FL and were transplanted in the field on 18 April 2008 (Live Oak) and 23 April 2008 (Citra). Muskmelon and watermelon were direct-seeded in the field when the other species
were transplanted. Plants were grown on polyethylene-mulched beds in plots that were five beds wide (one bed per crop species) by 4.6 m long. Beds at Live Oak were 94 cm wide with 1.5 m row spacing for bell pepper, eggplant, and tomato and 1.8 m row spacing for muskmelon and watermelon. Beds at Citra were 76 cm wide with 1.8 m row spacing for bell pepper, eggplant, and tomato and 2.4 m row spacing for muskmelon and watermelon. Eggplant, tomato, muskmelon, and watermelon were planted in a single row in the center of the beds, while beds for bell pepper contained two rows of plants per bed. In-row plant spacings were 30 cm (bell pepper), 45 cm (eggplant and tomato), 60 cm (muskmelon), and 91 cm (watermelon).

Aminopyralid was applied to bare soil in plots three weeks before planting at rates of 0.0014, 0.0028, 0.0056, 0.0112, 0.0224, and 0.0448 kg ae ha⁻¹. The soil was tilled with a disk, beds were formed using a PTO-driven power bedder, methyl bromide was applied, and polyethylene mulch was set in place the day following aminopyralid application.

A soil sample was collected from the center of the beds in each plot to a depth of 15 cm on the day that vegetables were transplanted. Samples were transported to the laboratory on ice and ultimately stored at -10°C. Aminopyralid soil concentration was then quantified via high performance liquid chromatography with tandem mass spectrometry (HPLC/MS/MS) at a commercial laboratory¹ (limit of quantitation was 0.2 µg kg⁻¹). At 6 weeks after planting (WAP) crop injury was visually estimated on a scale of 0 to 100 (0 = no injury, 100 = plant death), plant height or vine length of three random plants within each plot was measured, and open blooms on the three random plants in each plot were counted. For simplicity, “vine length” will be referred to as “plant height” herein. Marketable fruit was harvested four times at both locations (19 June, 26 June, 3 July, and 10 July 2008), with the culls being discarded.
The experimental design was a randomized complete block, and treatments were replicated four times. Plant height, bloom number, and yield data were converted to percentage reduction relative to the untreated control. No location interactions were detected; therefore, data were pooled across experiment sites. Crop injury, plant height and bloom number reduction, and crop yield loss were regressed as a function of aminopyralid soil concentration. The regression model, parameters, and $R^2$ values for the dependent variables of each crop are provided in Table 2-1.

**Results and Discussion**

Aminopyralid caused severe crop injury and plant height reduction in bell pepper, eggplant, and tomato (Figure 2-1). Plant height was reduced 30 to 40% as aminopyralid soil concentration increased from 0 to 1 µg kg$^{-1}$, with crop injury increasing by approximately twice the rate of plant height reduction. Conversely, little additional increase (<20%) in plant response was documented as aminopyralid concentration increased from 1 µg kg$^{-1}$ up to the maximum of 14.8 µg kg$^{-1}$. Maximum values of crop injury were 90 (bell pepper), 90 (eggplant), and 89% (tomato), and maximum plant height reductions were 69, 61, and 54%, respectively. These results were not surprising because synthetic auxin herbicides such as picloram and clopyralid are typically highly efficacious on species in the Solanaceae family, which includes bell pepper, eggplant, and tomato (O’Sullivan et al. 1999; Rocha and Yamashita 2009).

In addition to affecting the vegetative growth of bell pepper, eggplant, and tomato, aminopyralid also affected the reproductive capacity of those plants. Response curves of bloom number reduction and fruit yield loss for bell pepper, eggplant, and tomato were similar to those of crop injury and plant height reduction. Interestingly, there was a dramatic initial reduction between 0 and 1 µg kg$^{-1}$ aminopyralid with very little further reduction (≤10%) at concentrations above 1 µg kg$^{-1}$. At the maximum concentration tested (14 µg kg$^{-1}$), aminopyralid caused bloom
number reductions of 100, 92, and 100% and yield losses of 100, 95, and 100% in bell pepper, eggplant, and tomato, respectively.

The effects of aminopyralid on muskmelon and watermelon were less severe than those observed on bell pepper, eggplant, and tomato. As aminopyralid soil concentration increased, muskmelon and watermelon crop injury, reductions in plant height and bloom number, and fruit yield loss increased in a curvilinear manner (Figure 2-2). This contrasts the more drastic bi-phasic increase that occurred in bell pepper, eggplant, and tomato. Crop injury, plant height reduction, bloom number reduction, and fruit yield loss were 51, 49, 41, and 68%, respectively, in muskmelon, and values of those respective variables were 42, 28, 73, and 67% in watermelon.

The limit of quantitation (LOQ) for aminopyralid in soil was 0.2 µg kg⁻¹ in this research. The values of crop injury, plant height reduction, bloom number reduction, and fruit yield loss that were predicted to occur in those crops evaluated at this concentration are provided in Table 2-2. Crop injury values for bell pepper, eggplant, tomato, muskmelon, and watermelon were 48, 67, 71, 3, and 3%, respectively, and yield losses for those respective crops were 61, 64, 95, 8, and 14% at 0.2 µg kg⁻¹ aminopyralid. This demonstrates that aminopyralid can cause substantial injury and yield loss in bell pepper, eggplant, and tomato at soil concentrations below the LOQ; therefore, an alternative method of detection (e.g., a field bioassay) must be used to determine if the aminopyralid soil concentration is sufficient to cause unacceptable crop injury and yield loss. Pearson’s correlation coefficients were calculated to determine the strength of the correlation between fruit yield loss and crop injury, plant height reduction, and bloom number reduction (Table 2-3). With the exception of muskmelon, correlation coefficients for crop injury were ≥0.91 and P-values were <0.0001 for all crops. Correlation coefficients for plant height reduction and bloom number reduction were ≥0.80 and P-values were ≤0.0006 in all crops.
except watermelon and tomato. The strong correlation between fruit yield loss and crop injury, plant height reduction, and bloom number reduction suggests that aminopyralid soil concentration and the resulting fruit yield loss can be reliably predicted using a field bioassay. Although the aminopyralid soil concentration can be predicted using a field bioassay, it should be noted that these parameters were collected 6 WAP; therefore, a substantial amount of time is required to use this method.

The regression model and parameters from Table 2-1 were used to predict the soil concentrations of aminopyralid that would result in 10% crop injury (the maximum acceptable level of injury in vegetable crops) in the five crops evaluated (Table 2-4). The estimated time (mo) required for aminopyralid (when applied at the maximum labeled rate of 0.12 kg ae ha⁻¹) to reach those soil concentrations were then calculated using aminopyralid’s reported half-life of 34.5 d (Senseman 2007b) (Table 2-4). Our calculations suggest that bell pepper, eggplant, and tomato should not be planted within two yr after aminopyralid application, and muskmelon and watermelon should not be planted within one yr after aminopyralid application to avoid crop injury and yield loss from aminopyralid carryover. These estimates are not meant to serve as a replacement for a field bioassay, but to provide a general guideline as to when it is appropriate to conduct a field bioassay. Only through this accurate confirmation can a decision be made concerning when crops can be planted without sustaining unacceptable injury and yield loss from aminopyralid carryover. The rate of herbicide dissipation is influenced by a complex of dynamic factors (e.g., environmental conditions and soil chemical and physical properties) and is therefore highly variable (Nash 1988). Additionally, when clopyralid is applied in grass crops, the herbicide can be sequestered in the crop residue and then be re-released into the soil in a biologically active form as the crop residue decomposes (Branham and Lickfeldt 1997; Brinton
et al. 2006). Based on aminopyralid label statements regarding plant residues that contain aminopyralid, it appears that aminopyralid behaves in a similar manner to clopyralid and picloram (Anonymous 2008). This introduces an additional factor that influences the rate of aminopyralid dissipation because environmental conditions and management practices affect the rate and timing at which the crop residue decomposes and aminopyralid is re-released into the soil. Due to the extreme sensitivity of the crops included in this research to low aminopyralid soil concentrations (less than 1 µg kg⁻¹) and the unpredictability of the rate of aminopyralid dissipation in the soil, it is imperative that a field bioassay be conducted before planting a broadleaf crop on a site where aminopyralid has been applied. The data that we utilized was collected 6 WAP, which is unpractical for most producers; therefore, additional research needs to be conducted to determine if the bioassay period could be reduced to 1 or 2 WAP.

Sources of Materials

¹Carbon Dynamics Institute, LLC, 2835 Via Verde Drive, Springfield, IL 62703.
Table 2-1. Regression parameters and $R^2$ values for graphs in Figures 2-1 and 2-2. The hyperbolic regression model $Y = AX / (B + X)$ was used, where $Y =$ dependent variable, $X =$ independent variable, $A =$ vertical asymptote of the dependent variable, and $B =$ equation constant.

<table>
<thead>
<tr>
<th>Crop</th>
<th>Dependent variable</th>
<th>$A$</th>
<th>$B$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bell pepper</td>
<td>Crop injury</td>
<td>91.108</td>
<td>0.178</td>
<td>0.95</td>
</tr>
<tr>
<td>Bell pepper</td>
<td>Plant height reduction</td>
<td>71.325</td>
<td>0.593</td>
<td>0.94</td>
</tr>
<tr>
<td>Bell pepper</td>
<td>Bloom number reduction</td>
<td>102.487</td>
<td>0.073</td>
<td>0.98</td>
</tr>
<tr>
<td>Bell pepper</td>
<td>Fruit yield loss</td>
<td>103.011</td>
<td>0.136</td>
<td>0.97</td>
</tr>
<tr>
<td>Eggplant</td>
<td>Crop injury</td>
<td>89.913</td>
<td>0.069</td>
<td>0.90</td>
</tr>
<tr>
<td>Eggplant</td>
<td>Plant height reduction</td>
<td>62.796</td>
<td>0.343</td>
<td>0.57</td>
</tr>
<tr>
<td>Eggplant</td>
<td>Bloom number reduction</td>
<td>91.919</td>
<td>0.082</td>
<td>0.90</td>
</tr>
<tr>
<td>Eggplant</td>
<td>Fruit yield loss</td>
<td>94.476</td>
<td>0.095</td>
<td>0.96</td>
</tr>
<tr>
<td>Tomato</td>
<td>Crop injury</td>
<td>89.163</td>
<td>0.052</td>
<td>0.92</td>
</tr>
<tr>
<td>Tomato</td>
<td>Plant height reduction</td>
<td>54.498</td>
<td>0.273</td>
<td>0.78</td>
</tr>
<tr>
<td>Tomato</td>
<td>Bloom number reduction</td>
<td>99.585</td>
<td>0.076</td>
<td>0.91</td>
</tr>
<tr>
<td>Tomato</td>
<td>Fruit yield loss</td>
<td>98.878</td>
<td>0.008</td>
<td>0.96</td>
</tr>
<tr>
<td>Muskmelon</td>
<td>Crop injury</td>
<td>63.436</td>
<td>3.608</td>
<td>0.58</td>
</tr>
<tr>
<td>Muskmelon</td>
<td>Plant height reduction</td>
<td>56.568</td>
<td>2.262</td>
<td>0.77</td>
</tr>
<tr>
<td>Muskmelon</td>
<td>Bloom number reduction</td>
<td>41.509</td>
<td>0.124</td>
<td>0.44</td>
</tr>
<tr>
<td>Muskmelon</td>
<td>Fruit yield loss</td>
<td>75.679</td>
<td>1.764</td>
<td>0.73</td>
</tr>
<tr>
<td>Watermelon</td>
<td>Crop injury</td>
<td>52.742</td>
<td>3.467</td>
<td>0.70</td>
</tr>
<tr>
<td>Watermelon</td>
<td>Plant height reduction</td>
<td>30.654</td>
<td>1.464</td>
<td>0.55</td>
</tr>
<tr>
<td>Watermelon</td>
<td>Bloom number reduction</td>
<td>92.089</td>
<td>3.875</td>
<td>0.66</td>
</tr>
<tr>
<td>Watermelon</td>
<td>Fruit yield loss</td>
<td>70.791</td>
<td>0.781</td>
<td>0.85</td>
</tr>
</tbody>
</table>

Table 2-2. Values of crop injury, plant height reduction, bloom number reduction, and fruit yield loss that are predicted to occur at an aminopyralid soil concentration of 0.2 µg kg$^{-1}$ (the limit of quantitation for aminopyralid in this research) using the regression model and parameters from Table 2-1.

<table>
<thead>
<tr>
<th>Crop</th>
<th>Dependent variable</th>
<th>%</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Bell pepper</td>
<td>Crop injury</td>
<td>48</td>
<td>18</td>
<td>75</td>
</tr>
<tr>
<td>Eggplant</td>
<td>Plant height reduction</td>
<td>67</td>
<td>23</td>
<td>65</td>
</tr>
<tr>
<td>Tomato</td>
<td>Bloom number reduction</td>
<td>71</td>
<td>23</td>
<td>72</td>
</tr>
<tr>
<td>Muskmelon</td>
<td>Fruit yield loss</td>
<td>3</td>
<td>5</td>
<td>26</td>
</tr>
<tr>
<td>Watermelon</td>
<td>Fruit yield loss</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>
### Table 2-3. Pearson’s correlation coefficients for the correlation between fruit yield loss and crop injury, plant height reduction, and bloom number reduction.

<table>
<thead>
<tr>
<th>Crop</th>
<th>Dependent variable</th>
<th>Pearson’s correlation coefficient</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bell pepper</td>
<td>Crop injury</td>
<td>0.97</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Bell pepper</td>
<td>Plant height reduction</td>
<td>0.89</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Bell pepper</td>
<td>Bloom number reduction</td>
<td>0.96</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Eggplant</td>
<td>Crop injury</td>
<td>0.97</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Eggplant</td>
<td>Plant height reduction</td>
<td>0.81</td>
<td>0.0005</td>
</tr>
<tr>
<td>Eggplant</td>
<td>Bloom number reduction</td>
<td>0.98</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Tomato</td>
<td>Crop injury</td>
<td>0.94</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Tomato</td>
<td>Plant height reduction</td>
<td>0.74</td>
<td>0.0025</td>
</tr>
<tr>
<td>Tomato</td>
<td>Bloom number reduction</td>
<td>0.93</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Muskmelon</td>
<td>Crop injury</td>
<td>0.83</td>
<td>0.0002</td>
</tr>
<tr>
<td>Muskmelon</td>
<td>Plant height reduction</td>
<td>0.92</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Muskmelon</td>
<td>Bloom number reduction</td>
<td>0.80</td>
<td>0.0006</td>
</tr>
<tr>
<td>Watermelon</td>
<td>Crop injury</td>
<td>0.91</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Watermelon</td>
<td>Plant height reduction</td>
<td>0.71</td>
<td>0.0048</td>
</tr>
<tr>
<td>Watermelon</td>
<td>Bloom number reduction</td>
<td>0.83</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

### Table 2-4. Aminopyralid soil concentrations predicted to cause 10% crop injury.

<table>
<thead>
<tr>
<th>Crop</th>
<th>Aminopyralid soil concentration predicted to cause 10% crop injury&lt;sup&gt;a&lt;/sup&gt; µg kg&lt;sup&gt;-1&lt;/sup&gt; (SE)</th>
<th>Time required to reach aminopyralid soil concentration predicted to cause 10% crop injury&lt;sup&gt;b&lt;/sup&gt; mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bell pepper</td>
<td>0.022 (0.040)</td>
<td>13</td>
</tr>
<tr>
<td>Eggplant</td>
<td>0.009 (0.003)</td>
<td>15</td>
</tr>
<tr>
<td>Tomato</td>
<td>0.007 (0.002)</td>
<td>19</td>
</tr>
<tr>
<td>Muskmelon</td>
<td>0.675 (0.205)</td>
<td>7</td>
</tr>
<tr>
<td>Watermelon</td>
<td>0.811 (0.447)</td>
<td>7</td>
</tr>
</tbody>
</table>

<sup>a</sup>Concentrations were calculated using the regression model and parameters in Table 2-1.

<sup>b</sup>Times were calculated using a half-life of 34.5 d (Senseman 2007b) and assuming that aminopyralid was applied at the maximum labeled rate (0.12 kg ae ha<sup>-1</sup>), which would achieve a soil concentration of 50 µg kg<sup>-1</sup>.

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Figure 2-1. Crop injury, plant height reduction, bloom number reduction, and fruit yield loss (all expressed as a percentage of the untreated control) of bell pepper, eggplant, and tomato as a function of aminopyralid soil concentration. Crop injury, plant height reduction, and bloom number reduction data were collected 6 WAP.
Figure 2-2. Crop injury, plant height reduction, bloom number reduction, and fruit yield loss (all expressed as a percentage of the untreated control) of muskmelon and watermelon as a function of aminopyralid soil concentration. Crop injury, plant height reduction, and bloom number reduction data were collected 6 WAP.
CHAPTER 3
PICLORAM AND AMINOPYRALID SORPTION TO SOIL AND CLAY MINERALS

Introduction

Picloram and aminopyralid are auxin-type herbicides of the pyridinecarboxylic acid family. Picloram (Figure 3-1) was introduced in 1963 for the control of broadleaf weeds, including several deep-rooted perennial herbaceous species and woody brush species (Gantz and Laning 1963; Hamaker et al. 1963) and is currently labeled for use in pasture and rangeland, wheat, oats, barley, and non-crop areas at application rates of 0.07 to 1.12 kg ae ha\(^{-1}\) (Anonymous 2009a). Picloram is a moderately sorbed \(K_d = 0.5\) weak acid herbicide (\(pK_a = 2.3\)) with a water solubility of 430 mg L\(^{-1}\) (Senseman 2007a). Aminopyralid (Figure 3-1), which is structurally similar to picloram, was first registered in 2005 (USOPPEPTS 2005) and is labeled for use in range and pastureland and non-crop areas at application rates of 0.05 to 0.12 kg ae ha\(^{-1}\) for the control of several annual and perennial herbaceous broadleaf weed species (Anonymous 2008). Aminopyralid is a weak acid herbicide (\(pK_a = 2.56\)) that has a water solubility of 2480 mg L\(^{-1}\) and is moderately sorbed \(K_d = 0.72\) (Senseman 2007b).

Picloram is relatively mobile in soil, and picloram sorption to soil and clay minerals has been the focus of numerous experiments over the past 40 years. Light-textured soils with low organic matter (OM) content and high application rates cause picloram soil mobility to increase (Baur et al. 1972; Herr et al. 1966; Scifres et al. 1969), and the potassium salt formulation of picloram is more mobile than the triisopropanolamine salt formulation (Hunter and Stobbe 1972). In addition to the experiments that have been conducted on picloram soil mobility, numerous experiments have been conducted where picloram sorption was measured and reported in the form of the soil sorption constant \(K_d\) or the Freundlich distribution coefficient \(K_f\) (Arnold and Farmer 1979; Biggar and Cheung 1973; Farmer and Aochi 1974; Grover 1971).
Among those experiments, the lowest reported $K_f$ of picloram was 0.07 on an Ephrata sandy loam (60% sand, 32% silt, 8% clay, 0.94% OM, pH = 7.14) (Farmer and Aochi 1974), and the highest reported $K_f$ was 1.74 on a Palouse silt (12% sand, 61% silt, 27% clay, 3% OM, pH = 5.9) (Biggar and Cheung 1973). Additionally, picloram sorption is positively correlated to OM content (Farmer and Aochi 1974; Grover 1971; Hamaker et al. 1966) and negatively correlated to soil pH (Arnold and Farmer 1979; Biggar and Cheung 1973). Picloram’s weak soil sorption, correlation between sorption and soil pH, and the resulting high degree of soil mobility can be attributed to its weak acid nature. More than half of the molecules of a weak acid compound are in the undissociated-nonionic form at pH values that are below the $pK_a$ of the compound, and the number of molecules in the dissociated-anionic form increases as pH increases (Wauchope et al. 2002; Nicholls and Evans 1991). Because the pH of field soils is typically far above the $pK_a$ of picloram (2.30) and aminopyralid (2.56) (Senseman 2007a, 2007b), most of those molecules are in the dissociated-anionic form when present in field soil. The dissociation of weak acid herbicide molecules in soil affects sorption because dissociated-anionic molecules are repelled by the net negative charge of soil surfaces (Green and Karickhoff 1990). Weak acid herbicides are often strongly sorbed to clay minerals because the pH at the surface of many clay minerals can be 3 to 4 pH units lower than that of the soil solution (Bailey et al. 1968; Noyan et al. 2006); therefore, molecules at clay surfaces can convert to the undissociated-nonionic form and be adsorbed to the clay surfaces via coulombic interactions.

Due to its weak soil sorption and the resulting high degree of mobility in the soil, picloram can potentially move off-target to ground and surface water in and adjacent to treated areas (Lym and Messersmith 1988; Smith et al. 1988; Wood and Anthony 1997). Despite the environmental concerns caused by off-target movement to water, picloram continues to be heavily used because
of the high degree of efficacy and residual control that it provides on numerous broadleaf weed species. Aminopyralid, like picloram, is also highly efficacious on broadleaf weeds and provides residual weed control; however, aminopyralid has much lower use rates and a shorter half-life than picloram (Senseman 2007a, 2007b), which suggests that the potential for negative environmental impacts is much lower with aminopyralid than with picloram. In addition to its lower use rates and shorter soil half-life, the $K_d$ of aminopyralid (0.72) is greater than that of picloram (0.50) (Senseman 2007a, 2007b), which suggests that aminopyralid soil sorption is greater than that of picloram. No $K_f$ values or additional published data on aminopyralid sorption relative to soil texture and OM content were found in the literature. The objective of this research was to characterize picloram and aminopyralid sorption to five soils and three clay minerals and use that information to determine if the potential for off-target movement of aminopyralid in the soil is less than that of picloram.

**Materials and Methods**

**Sorption Kinetics**

Sorption kinetics of the potassium salt formulation of picloram and aminopyralid were determined on an Arredondo fine sand (see Table 3-1 for soil characteristics) using the batch equilibrium method (Weber 1986). Soil was placed in 50 mL plastic centrifuge tubes (2 g tube$^{-1}$) and 10 mL of 0.01 M calcium chloride solution that contained either picloram or aminopyralid at concentrations of 0.1 and 1.0 g ae L$^{-1}$ were added to each tube. Herbicide solutions contained mixtures of either picloram plus $^{14}$C-picloram or aminopyralid plus $^{14}$C-aminopyralid (supplied by Dow AgroSciences, Indianapolis, IN). The specific activity and radiochemical purity of picloram were $9.10 \times 10^8$ kBq mol$^{-1}$ and >99%, respectively, and the specific activity and radiochemical purity of aminopyralid were $1.01 \times 10^9$ kBq mol$^{-1}$ and 98%, respectively. Centrifuge tubes were placed on a wrist action shaker, removed after 1, 6, 12, 24, or 48 h, and
centrifuged at 3600 × g for 4 min. A 2 mL aliquot of the supernatant from each centrifuge tube was added to 15 mL of liquid scintillation cocktail, and the amount of radioactivity was quantified via liquid scintillation spectrometry (LSS). Herbicide not in solution was considered sorbed; hence, the amount of herbicide sorbed was calculated by subtracting the supernatant concentration from the concentration of herbicide initially added. Test concentrations did not exceed herbicide solubility, and a preliminary quality assurance experiment revealed that herbicide sorption to centrifuge tubes was negligible. Based on the relatively long half-lives of these compounds (Senseman 2007a; 2007b) and the report of Biggar et al. (1978) that no degradation of picloram occurred during a sorption experiment, we assumed that picloram and aminopyralid degradation would not occur in our experiments.

**Sorption to Soil and Clay Minerals**

Sorption of aminopyralid and picloram to five soils and three clay minerals was conducted in the same manner as the sorption kinetics experiments (see Table 3-1 for soil characteristics). Centrifuge tubes contained 2 g of soil or 0.15 g of clay, and herbicide concentrations added to the tubes were 0.01, 0.05, 0.1, and 1.0 g ae L⁻¹. Tubes were placed on a wrist action shaker for 24 h, and all other procedures were identical to those used in the sorption kinetics experiment.

Distribution of herbicide between the sorbed and solution phases was calculated using the Freundlich equation (Equation 3-1):

\[
x/m = K_f C^{1/n}
\]

Where: \(x/m\) is the amount of herbicide adsorbed per weight of dry soil (mg kg⁻¹), \(C\) is the concentration of the herbicide in soil solution at equilibrium (mg L⁻¹), and \(K_f\) and \(n\) are empirical constants.
**Molecular Modeling**

Molecular modeling software (ArgusLab 2004) was used to generate electrostatic potential-mapped electron density surface models of undissociated-nonionic and dissociated-anionic picloram and aminopyralid molecules. The software generated three-dimensional models of the herbicide molecules in their most logical configurations based on electron density, and a surface layer was then added to each model that displayed electronegativity colorimetrically. As others have done (Ferrell et al. 2005; Grey et al. 2000), we assumed that electronegative regions of the molecules would be repelled by negatively charged surfaces, while positively charged regions would contribute to the adsorption of the molecules to negatively charged surfaces via coulombic forces.

**Statistical Analyses**

Kinetics and sorption experiments were conducted twice using a completely randomized design, and each experiment contained three replications. Kinetics data were subjected to analysis of variance (SAS 2007), which revealed that the two-way interaction between experiment and herbicide concentration, the two-way interaction between experiment and time, and the three-way interaction between experiment, herbicide concentration, and time were not significant \( P > 0.05 \) for picloram and aminopyralid; therefore, sorption data were pooled across experiments and across herbicide concentrations within each herbicide. Percentage herbicide sorption was plotted against time and a hyperbolic regression model (Equation 3-2) was fit to the data (SigmaPlot 2006):

\[
Y = \frac{aX}{b + X}
\]  
(Eq 3-2)

Where: \( Y = \) percentage of herbicide sorbed (dependent variable), \( a = \) vertical asymptote (percentage herbicide sorption as time approaches infinity), \( X = \) time (independent variable), and \( b = \) the initial increase in \( Y \) per unit increase in \( X \). Sorption kinetics of the two herbicides were
compared using the 95% confidence intervals of the regression model parameters.

**Results and Discussion**

**Sorption Kinetics**

Picloram and aminopyralid sorption occurred in a short, initial phase of rapid sorption followed by a long phase during which sorption increased very little (Figure 3-2). The vertical asymptotes (maximum theoretical percentages of herbicide sorption) of picloram and aminopyralid were 10.3 and 15.2%, respectively, which indicates that aminopyralid sorption to an Arredondo fine sand is greater than that of picloram. Moreover, 46% (picloram) and 59% (aminopyralid) of the maximum theoretical sorption had occurred within 1 h, and more than 80% of the maximum theoretical sorption had occurred for both herbicides within 6 h. These results are consistent with the report of Hamaker et al. (1966) that picloram equilibrates rapidly in low OM soils.

**Sorption to Soil and Clay Minerals**

When herbicide sorption is described using the Freundlich equation, the empirical constant $n$ is an indicator of the linearity of the sorption isotherm; moreover, herbicide sorption is linearly proportional to the equilibrium solution concentration when $n = 1$. When herbicide sorption is a linear function of the equilibrium solution concentration, it is appropriate to use the soil sorption constant ($K_d$) to describe herbicide sorption and make comparisons between treatments; however, it is more appropriate to use $K_f$ when herbicide sorption is nonlinear (Seybold and Mersie 1996). Because our values of $n$ ranged from 0.62 to 1.24, we used $K_f$ to describe picloram and aminopyralid sorption in the soils and clay minerals included in this research.

The $K_f$ values of picloram and aminopyralid were less than 1 in all five soils included in this research (Table 3-2). The lowest $K_f$ values for picloram (0.12) and aminopyralid (0.35) were
observed in the Cecil sandy loam, and the highest $K_f$ values for picloram (0.81) and aminopyralid (0.96) were observed in the Arredondo fine sand. The low $K_f$ values ($< 1$) that we obtained for picloram are similar to those reported by others in several soils (Arnold and Farmer 1979; Biggar and Cheung 1973; Farmer and Aochi 1974). $K_f$ values of aminopyralid were greater than those of picloram within each soil, which suggests that soil sorption of aminopyralid is greater than that of picloram.

Herbicide sorption varied significantly among the three clay minerals included in this research (Table 3-2). $K_f$ values for picloram were 0.25 (kaolinite), 1.17 (bentonite), and 1016.40 (montmorillonite), and $K_f$ values for aminopyralid were 5.63 (kaolinite), 2.29 (bentonite), and 608.90 (montmorillonite). Kaolinite is a nonexpanding 1:1 clay (surface area = 29 m$^2$ g$^{-1}$), bentonite is a 2:1 expanding clay (surface area = 171 m$^2$ g$^{-1}$), and montmorillonite is a 2:1 expanding clay (surface area = 323 m$^2$ g$^{-1}$) (Fushiwaki and Urano 2001). Differences in surface area and expansion between kaolinite and montmorillonite explain why sorption was several times greater in montmorillonite than kaolinite. The differences in herbicide sorption among the three clay minerals included in this research suggest that clay minerals can significantly influence the amount of herbicide sorption that occurs in soil; moreover, the importance of clay surfaces to picloram soil sorption has been previously reported (Biggar and Cheung 1973).

A correlation analysis of our data revealed that sorption was not significantly correlated to soil texture, OM content, pH, or cation exchange capacity (CEC) (Table 3-3), which conflicts with previous reports that picloram sorption was positively correlated to OM content (Biggar and Cheung 1973; Farmer and Aochi 1974; Grover 1971) and negatively correlated to pH (Arnold and Farmer 1979; Biggar and Cheung 1973). In addition to the correlation analysis between sorption and OM, $K_{OM}$ values were also calculated for picloram and aminopyralid in the five
soils by dividing $K_f$ by percentage OM and multiplying by 100. If the contribution of OM to herbicide sorption was similar in the five soils, $K_{OM}$ should not vary substantially among soils. However, $K_{OM}$ values of the five soils included in this research were highly variable (data not shown), indicating that the contribution of OM to herbicide sorption differs among soils. The lack of correlation between herbicide sorption and soil texture, OM content, pH, and CEC indicates that additional factors or an interaction between factors influenced picloram and aminopyralid sorption. OM is not the only sorbent of herbicides in soil (Wauchope et al. 2002), and sorbents such as hydrated metal oxides and the mineral fraction of soil can contribute to picloram sorption (Hamaker et al. 1966; Cox et al. 1998). Clay minerals provide a suitable surface for weak acid herbicide sorption because their surface pH is often lower than that of the soil solution. The lower pH on the clay surface allows dissociated-anionic picloram molecules to enter the undissociated-nonionic form, which can be adsorbed to the clay surfaces by coulombic forces (Biggar and Cheung 1973; Bailey et al. 1968; Noyan et al. 2006).

It has been reported that picloram sorption was positively correlated to OM content, that picloram sorption was negatively correlated to pH, and that picloram movement was greater in light textured soils with low OM content compared to heavier soils with more OM (Arnold and Farmer 1979; Baur et al. 1972; Herr et al. 1966; Scifres et al. 1969; Biggar and Cheung 1973; Farmer and Aochi 1974; Hamaker et al. 1966). The Cecil sandy loam soil had 15% less sand, 0.47% more OM, and a pH that was 1.4 units below that of the Arredondo fine sand, which would lead one to predict that less picloram and aminopyralid sorption would occur in the Arredondo fine sand than in the Cecil sandy loam. However, $K_f$ values of picloram and aminopyralid were higher in the Arredondo fine sand than in the Cecil sandy loam. The fact that the Cecil sandy loam is a kaolinitic soil could provide an explanation for this phenomenon.
Sorption of picloram and aminopyralid to kaolinite was the lowest of the three clay minerals included in this research. Even though the Cecil sandy loam contained a greater percentage of clay, picloram and aminopyralid sorption may have been greater on the clay minerals that were present in the Arredondo fine sand. Given the differences we observed in sorption to the different clay minerals, the effect of differing clay minerals in different soils could outweigh the effect of soil texture, OM content, and pH. Results similar to ours have been reported with other weak acid herbicides; moreover, sorption of imazapyr, imazethapyr, and sulfometuron was lowest in the soils with the highest clay content (Gan et al. 1994; Wehtje et al. 1987). It should also be noted that OM can obstruct clay mineral surfaces and thus make them less available for herbicide sorption (Cox et al. 1998; Hance 1969). Another possible explanation for our results is that additional factors such as herbicide sorption to hydrated metal oxides may have contributed to picloram and aminopyralid sorption.

**Molecular Modeling**

Herbicide molecules that contain highly electronegative regions are repelled by the net negative charge of soil surfaces. In the undissociated-nonionic form, most regions of picloram and aminopyralid molecules are positively charged; however, small regions of high electronegativity exist on the nitrogen atom of the pyridine ring and on the oxygen atoms of the carboxyl group (Figure 3-3). All regions of picloram and aminopyralid molecules are highly electronegative when present in the dissociated-anionic form, except for the hydrogen atoms on the amino group. Because the pH of typical field soils is greater than the \( pK_a \) values of those herbicides, the majority of picloram and aminopyralid molecules in soil solution are in the dissociated-anionic form; this helps explain why these herbicides are weakly sorbed to soil. Decreasing pH can cause picloram and aminopyralid molecules to change from the dissociated-anionic to undissociated-nonionic form, which explains why the low surface pH of clay minerals...
can increase the soil sorption of these herbicides.

Picloram and aminopyralid are weakly sorbed to soils as a result of their weak acid nature, and the potential for these herbicides to move off-target is greater than that of many other herbicides. \( K_f \) values of aminopyralid and picloram were less than 1.00 in this research, and reported \( K_f \) values of atrazine and sulfentrazone were 11.08 and 3.35, respectively (DeSutter et al. 2003; Reddy and Locke 1998). Although there is potential for both picloram and aminopyralid to move off-target in the soil, \( K_f \) values of aminopyralid were significantly greater than those of picloram for the five soils included in this research. This indicates that there is less potential for off-target movement of aminopyralid in the soil compared to that of picloram. Sorption to clay minerals varied by an order of magnitude among the three clay minerals included in this research, which suggests that the type of clay mineral present in soils can affect picloram and aminopyralid sorption. Picloram and aminopyralid sorption to the five soils included in this research was not significantly correlated to soil texture, OM content, pH, or CEC; therefore, it was concluded that additional factors such as the type of clay minerals present in the soil or herbicide sorption to hydrated metal oxides have a significant influence on picloram and aminopyralid sorption to soil. Models of picloram and aminopyralid molecules with a colorimetric display of electronegativity indicate that herbicide molecules in the dissociated-anionic form are unlikely to be adsorbed because they would be repelled by the net negative charge of soil surfaces; moreover, most molecules are in the dissociated-anionic form at the pH values of typical field soils. The maximum labeled application rate of aminopyralid is less than that of picloram (Anonymous 2008; Anonymous 2009a), the average half-life of aminopyralid (34.5 d) is less than that of picloram (90 d) (Senseman 2007a, 2007b), and aminopyralid soil sorption was greater than that of picloram. The low \( K_f \) values of picloram and aminopyralid
indicate that there is potential for off-target movement of these compounds in the soil.
Additionally, the higher $K_f$ values of aminopyralid compared to those of picloram indicate that
the potential for aminopyralid to move off-target is slightly less than that of picloram.
Table 3-1. State of origin, taxonomy, and characteristics of the five soils included in this research.

<table>
<thead>
<tr>
<th>State</th>
<th>Series</th>
<th>Taxonomy</th>
<th>pH</th>
<th>CEC (cmol kg(^{-1}))</th>
<th>OM (% Sand)</th>
<th>Silt (%)</th>
<th>Clay (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Florida</td>
<td>Arredondo fine sand</td>
<td>Loamy, siliceous, semiactive, hyperthermic Grossarenic Paleudults</td>
<td>6.6</td>
<td>3.94</td>
<td>0.84</td>
<td>96.4</td>
<td>1.0</td>
</tr>
<tr>
<td>Georgia</td>
<td>Cecil sandy loam</td>
<td>Fine, kaolinitic, thermic Typic Kanhapludults</td>
<td>5.2</td>
<td>3.35</td>
<td>1.31</td>
<td>81.4</td>
<td>1.0</td>
</tr>
<tr>
<td>Kentucky</td>
<td>Maury silt loam</td>
<td>Fine, mixed, semiactive, mesic Typic Paleudalfs</td>
<td>5.6</td>
<td>27.90</td>
<td>2.31</td>
<td>33.4</td>
<td>19.6</td>
</tr>
<tr>
<td>Mississippi</td>
<td>Bosket fine sandy loam</td>
<td>Fine-loamy, mixed, active, thermic Mollic Hapludalfs</td>
<td>7.0</td>
<td>20.11</td>
<td>2.31</td>
<td>37.4</td>
<td>15.6</td>
</tr>
<tr>
<td>Mississippi</td>
<td>Sharkey clay</td>
<td>Very-fine, smectitic, thermic Chromic Epiaquerts</td>
<td>6.0</td>
<td>11.16</td>
<td>1.11</td>
<td>24.2</td>
<td>23.7</td>
</tr>
</tbody>
</table>

Table 3-2. Freundlich equation parameters (\(K_f\) and \(n\)) for picloram and aminopyralid in the five soils and three clay minerals included in this research. All \(R^2\) values were 0.99, and numbers in parentheses are 95% confidence intervals.

<table>
<thead>
<tr>
<th>Soil/clay</th>
<th>Picloram</th>
<th>Aminopyralid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(K_f)</td>
<td>(n)</td>
</tr>
<tr>
<td>Arredondo fine sand</td>
<td>0.81 (0.78–0.84)</td>
<td>0.88 (0.77–0.99)</td>
</tr>
<tr>
<td>Cecil sandy loam</td>
<td>0.12 (0.11–0.13)</td>
<td>0.97 (0.88–1.06)</td>
</tr>
<tr>
<td>Maury silt loam</td>
<td>0.36 (0.35–0.37)</td>
<td>1.21 (1.10–1.32)</td>
</tr>
<tr>
<td>Bosket fine sandy loam</td>
<td>0.40 (0.39–0.41)</td>
<td>1.02 (0.92–1.12)</td>
</tr>
<tr>
<td>Sharkey clay</td>
<td>0.52 (0.50–0.54)</td>
<td>1.24 (1.09–1.39)</td>
</tr>
<tr>
<td>Bentonite</td>
<td>1.17 (1.05–1.28)</td>
<td>1.08 (0.74–1.42)</td>
</tr>
<tr>
<td>Kaolinite</td>
<td>0.25 (0.23–0.27)</td>
<td>0.62 (0.08–1.16)</td>
</tr>
<tr>
<td>Montmorillonite</td>
<td>1016.40 (979.70–1053.10)</td>
<td>1.12 (1.10–1.14)</td>
</tr>
</tbody>
</table>
Table 3-3. Pearson’s correlation coefficients between picloram and aminopyralid sorption ($K_f$) and sand, silt, clay, pH, OM, and CEC of the five soils included in this research.

<table>
<thead>
<tr>
<th>Soil property</th>
<th>Picloram $K_f$</th>
<th>Aminopyralid $K_f$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sand</td>
<td>0.21 ($P = 0.73$)</td>
<td>-0.19 ($P = 0.76$)</td>
</tr>
<tr>
<td>Silt</td>
<td>-0.32 ($P = 0.59$)</td>
<td>0.18 ($P = 0.77$)</td>
</tr>
<tr>
<td>Clay</td>
<td>-0.001 ($P = 0.99$)</td>
<td>0.13 ($P = 0.84$)</td>
</tr>
<tr>
<td>OM</td>
<td>-0.45 ($P = 0.45$)</td>
<td>0.04 ($P = 0.95$)</td>
</tr>
<tr>
<td>pH</td>
<td>0.64 ($P = 0.25$)</td>
<td>0.41 ($P = 0.50$)</td>
</tr>
<tr>
<td>CEC</td>
<td>-0.18 ($P = 0.78$)</td>
<td>0.41 ($P = 0.49$)</td>
</tr>
</tbody>
</table>
Figure 3-1. Chemical structures of picloram and aminopyralid.

Figure 3-2. Picloram and aminopyralid sorption as a function of time.
Figure 3-3. Molecular models of picloram in the undissociated-nonionic (A) and dissociated-
anionic (B) forms and aminopyralid in the undissociated-nonionic (C), and
dissociated-anionic (D) forms. Ball-and-stick models directly below the surface-
mapped molecules indicate the configuration of the atoms within the surface-mapped
models.
CHAPTER 4
PICLORAM AND AMINOPYRALID MINERALIZATION IN SOIL

Introduction

Herbicide dissipation occurs when herbicide residues are removed from an environmental compartment (e.g., soil) by microbial or chemical degradation or transferred to another environmental compartment. The microbial fraction of herbicide degradation is referred to as “mineralization” (Stephenson et al. 2006). Degradation is an important component of herbicide dissipation, and mineralization makes a substantial contribution to degradation because of the abundant and diverse population of microorganisms that is typically found in soils (Walker 1987). The rate at which an herbicide degrades is influenced by numerous factors, including the molecular structure of the herbicide, the physical and chemical properties of the soil, and environmental conditions; therefore, the rate of herbicide degradation can be highly variable over time and space (Nash 1988; Walker 1987). Herbicide sorption to soil colloids is one factor that can affect herbicide mineralization; moreover, herbicides that are weakly sorbed can be mineralized more quickly than herbicides that are strongly sorbed because they are more readily available to soil microorganisms. (Wauchope et al. 2002).

Picloram is a synthetic auxin herbicide of the pyridine carboxylic acid family that was introduced in the 1960’s and provides foliar and soil residual control of many annual and perennial broadleaf weeds, including several woody brush species (Gantz and Laning 1963; Hamaker et al. 1963). Picloram has relatively high use rates (0.07 to 1.12 kg ae ha$^{-1}$), is persistent in the soil (half-life = 90 d), and is weakly sorbed to soil ($K_f = 0.12–0.17$) (Anonymous 2009a; Fast et al. 2010a; Senseman 2007a). Although these facts raise concern about the potential negative impacts of this herbicide on the environment, picloram is still commonly used because of unmatched efficacy and residual activity on common and troublesome weed species.
Aminopyralid, another pyridine carboxylic acid herbicide, was introduced in 2005 and provides foliar and soil residual control of numerous annual and perennial broadleaf weed species in rangeland, permanent grass pastures, and non-cropland areas at rates of 0.05 to 0.12 kg ae ha\(^{-1}\) (Anonymous 2008). Aminopyralid is a weakly sorbed herbicide (\(K_f = 0.35–0.96\)) with a soil half-life of 34.5 days (Senseman 2007b; Fast et al. 2010a). Aminopyralid has lower use rates, greater soil sorption, and a shorter soil half-life than picloram; therefore, it appears that aminopyralid would have less negative environmental impacts compared to picloram.

The persistence of aminopyralid and picloram in the soil can provide the benefit of residual weed control; however, the persistence of these herbicides also increases the probability of off-target movement and environmental contamination. The reported half-lives of picloram and aminopyralid are 90 and 34.5 d, respectively (Senseman 2007a; 2007b), which implies that aminopyralid is less persistent than picloram; however, it should be noted that those reported half-life values were averaged across several field experiments that were conducted in a wide range of soils and environmental conditions. In order to compare the persistence of aminopyralid and picloram, experiments should be conducted in the laboratory where environmental conditions are controlled and the same soil type is used for both herbicides. The objective of this research was to determine if aminopyralid mineralization is greater than that of picloram in two soils.

**Materials and Methods**

Mineralization of picloram and aminopyralid was quantified in Arredondo fine sand from Florida and Sharkey clay from Mississippi (see Table 4-1 for soil characteristics) using EPA testing guidelines (USEPA 2008b) and the methods described by Pramer and Bartha (1965). Soils were collected from field sites with no history of picloram or aminopyralid application, air dried, and passed through a 2 mm sieve. Fifty g of dry soil was then added to biometer flasks and
deionized water was added to bring soil to 40% field capacity. Flasks were then incubated in darkness for 1 wk at 25°C to allow soil microbial populations to establish. After the 1 wk incubation period, solutions of picloram plus $^{14}$C-picloram or aminopyralid plus $^{14}$C-aminopyralid were distributed to the soil surface to achieve a herbicide concentration in the soil of 1 µg g$^{-1}$. The specific activity and radiochemical purity of picloram were $9.10 \times 10^8$ kBq mol$^{-1}$ and >99%, respectively, and the specific activity and radiochemical purity of aminopyralid were $1.01 \times 10^9$ kBq mol$^{-1}$ and 98%, respectively. Flasks were sealed and 10 mL of 1 N KOH trapping solution was added to the sidearm of the biometer flasks to capture evolved $^{14}$CO$_2$. Flasks were placed in dark incubation, and KOH trapping solution was removed at sampling times of 1, 2, 4, 8, 16, 32, 48, and 64 DAT. At each sampling time, the biometer flasks were rinsed with 5 mL of trapping solution, 10 mL of fresh trapping solution was added, and 10 mL of fresh air was injected into the flasks to maintain an aerobic environment. Radioactivity in the trapping solution from each sampling time was quantified by adding a 2 mL aliquot of the trapping solution to 15 mL of scintillation cocktail and determining radioactivity via liquid scintillation counting. The experiment contained three replications and was conducted twice. Data were subjected to analysis of variance, which revealed that the effect of soil type on mineralization was significant. Mean $^{14}$CO$_2$ evolution was plotted against time for each soil type and each herbicide, and the two compartment biexponential regression model was fit to the data (Equation 4-1).

$$Y = a(1 - e^{-bX}) + c(1 - e^{-dX})$$ (4-1)

The regression parameters for the data sets are provided in Table 4-2.

**Results and Discussion**

Mineralization of $^{14}$C-aminopyralid was greater than that of picloram within each soil type, and mineralization of both herbicides was greater in the Sharkey clay than it was in Arredondo.
fine sand (Figure 4-1). At 64 DAT, mineralization of aminopyralid, as indicated by $^{14}$CO$_2$ evolution, was 23.7 and 15.2% in Sharkey clay and Arredondo fine sand, respectively, and picloram mineralization was 9.2 and 5.4% in those respective soils. The greater mineralization of both herbicides in the Sharkey clay compared to the Arredondo fine sand could be a result of differences in the microbial populations in the soils. Also, picloram and aminopyralid sorption was slightly greater in Arredondo fine sand than it was in Sharkey clay, which indicates that the herbicide would be less available to soil microbes for mineralization in Arredondo fine sand. The only structural difference between picloram and aminopyralid is that picloram has a chlorine atom in the number 5 position of the pyridine ring that is not present in aminopyralid. This small change in chemical structure may contribute to the increased mineralization of aminopyralid compared to picloram within each soil. Mineralization of picloram and aminopyralid was greater than that of flumioxazin (2.0–2.2%) and sulfentrazone (1.7–2.1%); moreover, soil sorption of aminopyralid and picloram appears to be less than that of flumioxazin and sulfentrazone (Fast et al. 2010a; Ferrell et al. 2003; Reddy and Locke 1998). This indicates that the weak sorption of picloram and aminopyralid contributes to their mineralization by making them more readily available to soil microbes. Research will be conducted to determine picloram and aminopyralid degradation (including mineralization and chemical breakdown) in these soils to provide further information on their persistence in the soil.
Table 4-1. State of origin, taxonomy, and characteristics of the five soils included in this research.

<table>
<thead>
<tr>
<th>State</th>
<th>Series</th>
<th>Taxonomy</th>
<th>pH</th>
<th>CEC cmol kg⁻¹</th>
<th>OM %</th>
<th>Sand %</th>
<th>Silt %</th>
<th>Clay %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Florida</td>
<td>Arredondo fine sand</td>
<td>Loamy, siliceous, semiactive, hyperthermic Grossarenic Paleudults</td>
<td>6.6</td>
<td>3.94</td>
<td>0.84</td>
<td>96.4</td>
<td>1.0</td>
<td>2.6</td>
</tr>
<tr>
<td>Mississippi</td>
<td>Sharkey clay</td>
<td>Very-fine, smectitic, thermic Chromic Epiaquerts</td>
<td>6.0</td>
<td>11.16</td>
<td>1.11</td>
<td>24.2</td>
<td>23.7</td>
<td>52.1</td>
</tr>
</tbody>
</table>

Table 4-2. Regression parameters for the graph in Figure 4-1. The model \( Y = a(1 - e^{-bX}) + c(1 - e^{-dX}) \) (biexponential two compartment model) was fit to the data. \( R^2 \) values for both soils and both herbicides were 0.99.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Herbicide</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sharkey clay</td>
<td>Aminopyralid</td>
<td>10.126</td>
<td>1.493</td>
<td>43.932</td>
<td>0.006</td>
</tr>
<tr>
<td>Arredondo fine sand</td>
<td>Aminopyralid</td>
<td>9.062</td>
<td>2.272</td>
<td>7.410</td>
<td>0.027</td>
</tr>
<tr>
<td>Sharkey clay</td>
<td>Picloram</td>
<td>2.895</td>
<td>1.350</td>
<td>119.728</td>
<td>0.001</td>
</tr>
<tr>
<td>Arredondo fine sand</td>
<td>Picloram</td>
<td>2.263</td>
<td>1.784</td>
<td>5.958</td>
<td>0.012</td>
</tr>
</tbody>
</table>
Figure 4-1. $^{14}$CO$_2$ evolution from $^{14}$C-picloram and $^{14}$C-aminopyralid as a function of time in Sharkey clay and Arredondo fine sand. Data points are means of six replications with standard error bars.
APPENDIX
PICLORAM AND AMINOPYRALID DEGRADATION EXPERIMENT

An additional experiment was conducted on the degradation of picloram and aminopyralid in soil. Herbicide extracted from soil samples is being quantified, and this research will be incorporated into Chapter 4 when the results of the research become available. Picloram and aminopyralid dissipation were evaluated in Arredondo fine sand from Florida and Sharkey clay from Mississippi (See Table 1 for soil characteristics) in 50-mL polypropylene centrifuge tubes. Dry soil (15 g) was added to centrifuge tubes, deionized water was added to bring soil to the appropriate moisture content (40 or 70% field capacity), and tubes were sealed and incubated in darkness at the appropriate temperature (10 or 20 C) for 1 wk to allow the soil microbial population to become established. After the 1 wk incubation period, soil was fortified with picloram or aminopyralid to achieve an initial herbicide concentration of 1 µg g⁻¹. Tubes were then sealed and incubated for 1, 2, 4, 8, 16, 32, 48, and 64 DAT in darkness, and they were placed in freezer storage (-10 C) at the end of the incubation period until herbicide extraction occurred. All tubes were opened once per week during incubation to ensure that aerobic conditions were maintained in the tubes.

Aminopyralid was extracted from soil by adding 25 mL 90:10 (v/v) acetonitrile/1N HCl to the centrifuge tubes. The suspension was agitated on a wrist action shaker for 1 h and centrifuged at 3600 × g for 6 min; supernatant was then transferred to 50-mL beakers. The extraction procedure was repeated using 15 mL of the 90:10 (v/v) acetonitrile/1N HCl solution, and the tubes were agitated for 30 min. The supernatants from the two extractions were combined and 10 mL of the supernatant was transferred to 15-mL polypropylene centrifuge tubes and evaporated to dryness at 50°C in a nitrogen evaporator. Samples were brought to 3 mL with 1N HCl, and 2 of the 3 mL was concentrated on a Strata X SPE column that was
conditioned with 1 mL each of methanol and distilled water. The column was washed with 0.75 mL 95:5 (v/v) water/methanol, dried under negative pressure for 15 min, and eluted twice with 0.5 mL acetonitrile per elution. Samples were then evaporated to dryness at 50°C in a nitrogen evaporator; 0.2 mL 22:2:1 (v/v) acetonitrile/pyridine/butanol, 0.1 mL butyl chloroformate, and 0.7 mL 50:50:0.1 (v/v/v) methanol/water/acetic acid were then added to bring samples to a final volume of 1 mL. Samples were then transferred to HPLC vials and analyzed with high performance liquid chromatography/mass spectrometry.

Picloram was extracted from soil in the same manner as aminopyralid; however, extraction columns were conditioned with 1 mL methanol followed by 1 mL of 1N HCl. Additionally, columns were eluted twice with 0.5 mL methanol for picloram extraction. Unlike the aminopyralid extraction, no reconstitution was required with picloram; the extract was added to HPLC vials for analysis immediately after column elution.
LIST OF REFERENCES


BIOGRAPHICAL SKETCH

Brandon James Fast was born in Fairview, Oklahoma in 1979. After graduating from Fairview High School in 1999, he attended Oklahoma State University in Stillwater, Oklahoma and obtained a bachelor of science degree in plant and soil sciences in 2003 and a master of science degree in plant and soil sciences in 2007. Upon completion of his master of science degree, Brandon attended the University of Florida and obtained a doctor of philosophy degree in agronomy in summer 2010. Upon completion of his doctor of philosophy degree, Brandon began employment with Dow AgroSciences in Indianapolis, Indiana.