ABSTRACT

LEWIS, DUSTIN FRANKLIN. Environmental Fate and Biological Impact of Aminocyclopyrachlor in Turfgrass and Neighboring Systems. (Under the direction of Drs. Fred H. Yelverton and Robert J. Richardson).

Aminocyclopyrachlor is a newly developed synthetic auxin herbicide belonging to the pyridine carboxylic acid family. Aminocyclopyrachlor has a chemical structure and mode of action similar to phenoxyacetic and pyridine carboxylic acid herbicides and has the potential to be widely used in turfgrass systems. Aminocyclopyrachlor has a favorable environmental profile with low toxicity; minimal volatility potential; and broad spectrum weed control at low application rates compared to available phenoxy and pyridine alternatives. Aminocyclopyrachlor was registered in 2010 by DuPont Crop Protection under the trade name Imprelis® for broadleaf weed control in commercial and residential fine turf. However, many questions remain unanswered regarding the environmental fate and biological impact of aminocyclopyrachlor in turfgrass and neighboring systems.

Research was conducted to determine the effect of ambient moisture on aminocyclopyrachlor efficacy. Results indicated the presence of dew on the turfgrass canopy at the time of herbicide application increased aminocyclopyrachlor efficacy and no losses due to photodegradation were observed.

Research was conducted to determine the utility of recycling synthetic auxin treated turfgrass clippings for additional weed control in utility turfgrass settings. Results indicated bioavailable herbicide residues in turfgrass clippings collected from a previously treated aminocyclopyrachlor turfgrass stand could provide significant weed control.
Research was conducted to determine the bioavailability of synthetic auxin residues from turfgrass clippings in aquatic and riparian plants. Results indicated herbicide residues were available in aqueous environments and could injure aquatic/riparian plant species.

Research was conducted to characterize the absorption, translocation, and metabolism of aminocyclopyrachlor in tall fescue. Results indicated rapid aminocyclopyrachlor absorption but translocation was limited to the aboveground foliage. No aminocyclopyrachlor metabolism was detected during the course of the study.

Research was conducted to determine the effect of simulated aminocyclopyrachlor drift on flue-cured tobacco. Results indicated tobacco was sensitive to all tested rates and application timings of aminocyclopyrachlor.
Environmental Fate and Biological Impact of Aminocyclopyrachlor in Turfgrass and Neighboring Systems

by

Dustin F. Lewis

A dissertation submitted to the Graduate Faculty of North Carolina State University in partial fulfillment of the requirements for the degree of Doctor of Philosophy

Crop Science

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DEDICATION

I would like to dedicate this dissertation in remembrance of my late grandfathers, Samuel F. Lewis and Billy T. Hutson. Sam Lewis was raised in Meigs County, TN and held numerous employments throughout his life while feverously maintaining our family farm in McMinn County, TN. Sam was an instrumental leader in our community, as he believed in always putting others’ needs above his own. Sam taught me to appreciate the beauty of the natural world and always lent a smile and helpful hand to any friend, neighbor, or stranger in need. Bill Hutson was raised in Anderson County, TN and fought for his country in the European theatre of World War II. Upon completion of his service, Bill graduated from the University of Tennessee with his bachelors and masters degrees which led him to a career in academia where he had the longest recorded tenure of any professor in the history of Tennessee Wesleyan College, Athens, TN. Bill instilled in me the value of an education and the passion to always follow your dreams. Both are two of the greatest men I have ever met, and I am honored to be part of their bloodline.
BIOGRAPHY

Dustin F. Lewis was born in the Claxton community of McMinn County in rural East Tennessee. He was raised on a small beef cattle farm by his parents, Douglas F. and Kimberly H. Lewis, along with his sister, Sarah Lewis Philpott. Being the only son, Dustin was taught by his father to develop self-motivation, a strong work ethic, and a tireless enthusiasm for working with his hands. Besides working on the family farm, Dustin spent his summers on the grounds crew at Cleveland Country Club where he fell in love with golf course management. He graduated with honors and an FFA State Degree from McMinn Central High School in 2003. He then attended the University of Tennessee to pursue a degree in Plant Sciences with a concentration of Turfgrass Management. During his bachelors, Dustin completed a competitive internship at Medinah Country Club where he prepared the grounds for the 2006 PGA Championship. Returning to the University of Tennessee, he graduated Cum Laude in 2007 and began his masters degree at the University of Tennessee focusing his studies in Turfgrass Weed Science under the direction of Drs. J. Scott McElroy and John C. Sorochan. While pursuing his masters, he was given charge of a United States Golf Association grant and administered research trials at Little Course in Franklin, TN; Honors Course in Ooltewah, TN; New Life Turf in Norway, SC; and Atlanta Athletic Club in Duluth, GA. Aside from his research, Dustin competed and placed in numerous graduate competitions at professional society meetings. Upon completion of his masters degree in 2009, Dustin began pursuit of his doctorate degree in Weed Science with minors in Ecology and Plant Biology at North Carolina State University under the direction of Drs. Fred H. Yelverton and Robert J. Richardson. The focus of his dissertation was on the
environmental fate and biological impact of aminocyclopyrachlor. Dustin has continued to excel in all his research and professional endeavors while at North Carolina State University. A few of his accolades include preparing the grounds for the 2010 and 2011 Masters Tournament at Augusta National Golf Club, serving as president of the Southern Weed Science Society Graduate Student Association, and leading his NCSU Graduate Weed Science Team to victory in the Northeastern Weed Science Region of the 2011 Weed Olympics. Upon graduation, Dustin has accepted a Field Biologist position with BASF Chemical Company in Champaign, IL where he will assist in the development of new agronomic products.
ACKNOWLEDGMENTS

There are many people who have been instrumental throughout my life who deserve acknowledgement. First, I would like thank God for the love and blessings He continues to place in my life. Without faith in His guidance, nothing would be possible. Secondly, I want to thank my wife and best friend Mary E. Lewis for her enduring support and unconditional love. Equally, I also want thank my family which built the foundation that I have and will continue to base my life upon. Their love and encouragement is cherished far beyond words can describe. I would also like to acknowledge Les Marlow, Superintendent of Cleveland Country Club, and Dr. John Sorochan, University of Tennessee, who taught me the beauties of turfgrass management.

Professionally and personally, I would like to acknowledge my North Carolina State University graduate committee members, Drs. Fred H. Yelverton, Robert J. Richardson, Harry J. Strek, Thomas R. Wentworth, and Wesley J. Everman for their leadership, direction, guidance, and friendship. I would also like to acknowledge Mathew D. Jeffries, Travis Gannon, and Leon Warren who comprise ‘Team Turfweeds’, as well as Steve Hoyle, Rory Roten, Sarah True Meadow, Justin Nawrocki, and Trevor Israel who embody ‘Team Aquatics/Noncropland’. These individuals have been critical to my success as a student, and their friendship is truly invaluable. Last but not least, I would like to thank the remaining Crop Science Department faculty, staff, and graduate students at the North Carolina State University. I will carry the memories I have shared with everyone at NCSU for the remainder of my life. God Bless and GO PACK!
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Aminocyclopyrachlor (AMCP) is a newly developed synthetic auxin herbicide for broadleaf weed control in turfgrass systems. AMCP has been observed to undergo rapid photodecomposition in shallow water when exposed to sunlight. However, most herbicide applications on golf courses occur during the morning when dew is still present on the turfgrass canopy. These conditions could result in efficacy loss if photolysis occurs while AMCP is suspended in dew droplets. Research was conducted to determine the effect of ambient moisture on AMCP efficacy. AMCP (79 and 105 g ae ha\(^{-1}\)), aminopyralid (280 g ha\(^{-1}\)), and two AMCP granular formulations (84 g ha\(^{-1}\)) were applied to dew covered (WET) and dew excluded (DRY) ‘Tifway’ bermudagrass plots. Herbicide treatments applied to WET plots had greater visual bermudagrass injury than respective treatments applied to DRY plots.


Keywords: Dew, photolysis
plots at 7 and 21 days after treatment (DAT), with the exception of aminopyralid at 21 DAT. NDVI turfgrass quality complemented visual ratings, indicating greater turfgrass quality reductions when applied to WET vs DRY plots. These results indicate AMCP applications made to dew-covered turfgrass can increase herbicidal efficacy and no significant losses due to photodegradation were observed.
Synthetic auxin herbicides are commonly used in turfgrass settings to control broadleaf weeds. The phenoxyacetic and pyridine carboxylic acids mimic the natural plant hormone indole-3-yl-acetic acid but are not metabolized rapidly in susceptible dicotyledonous plants (Grossmann 2009). Synthetic auxin compounds are translocated systemically through the phloem and/or xylem to meristematic plant regions where injury can be characterized by a loss in apical dominance, leaf cupping, epinastic curvature, and unregulated plant growth (Cobb and Reade 2010; Grossmann 2009). For synthetic auxin herbicides to be efficacious, the compound must first contact the leaf surface, absorb through the plant cuticle, translocate to the intended target site, and affect a specific biochemical process (Ross and Lembi 1999). While the aforementioned process seems easily achievable through typical herbicide application methods, numerous physiological, biochemical, and environmental barriers can inhibit herbicide absorption into the targeted plant causing reduce efficacy (Cobb and Reade 2010; Hess and Falk 1990; Monaco et al. 2002).

Terrestrial plant leaf surfaces are covered with a protective cuticular layer composed of hydrophobic/lipophilic trichomes, waxes, cutin, and/or pectin that protects plants from desiccation and pathogen invasion but poses a major barrier to herbicide uptake (Cobb and Reade 2010; Currier and Dybing 1959; Hess and Falk 1990; Ross and Lembi 1999; Taiz and Zeiger 2006). Using scanning electron microscopic techniques, Hess and Falk (1990) noted diverse epidermal morphology among various plant species that greatly affected herbicide distribution on the leaf surface. The authors observed less MCPA distribution on bermudagrass (*Cynodon dactylon* L. Pers.) compared to sugar beet (*Beta vulgaris* L), which was attributed to the thick epicuticular wax layer in bermudagrass. Adjuvants are often tank-
mixed with herbicides to assist in cuticle penetration and increase foliar absorption (Cobb and Reade 2010; McCarty et al. 2010).

Physicochemical properties such as chemical structure, octanol-water partition coefficient (Log $K_{ow}$), vapor pressure (VP), and water solubility ($K_s$) greatly influence herbicide foliar absorption. Synthetic auxin herbicides typically have a high $K_s$ and low log $K_{ow}$, indicating they do not bind strongly to lipophilic cuticular constituents on the leaf surface (Senseman 2007). However, absorption is also dependent on compound stability within the environment, as herbicide losses from volatility and/or photolysis can reduce retention time on the leaf surface. The vapor pressure for pyridine compounds are higher than those of phenoxy acids, making them more stable on the leaf surface due to decreased volatility potential (Senseman 2007). Changes to a synthetic auxin formulation or the addition of adjuvants can reduce volatility risks (Cobb and Reade 2010; McCarty et al. 2010).

Environmental conditions prior to, during, and following herbicide application can also impact synthetic auxin herbicide efficacy. Barrier and Loomis (1957) reported increasing temperatures from 15 to 30 C enhanced 2,4-D foliar absorption on soybean (*Glycine max* L.). Periods of high relative humidity can increase herbicide efficacy due to prolonged spray droplet drying time on the leaf surface (Richardson 1977). Pallas (1960) reported greater 2,4-D absorption and translocation in kidney bean (*Phaseolus vulgaris* L.) under 70 to 74% humidity than between 34 to 48% humidity. Cuticular thickness may increase under low humidity conditions or extended dry periods, reducing herbicide penetrability (Currier and Dybing 1959). Furthermore, low humidity conditions can increase
spray droplet drying time and create unabsorbable synthetic auxin crystalline deposits on the leaf surface (Hess and Falk 1990). Due to the high $K_s$ of synthetic auxin herbicides, dew formation following an application can resuspend crystalline deposits making them available for plant uptake (Monaco et al. 2002; Senseman 2007).

Aminocyclopyrachlor (AMCP) is a synthetic auxin herbicide belonging to the newly developed pyrimidine carboxylic acid herbicide family (Claus et al. 2008). AMCP has a similar mode of action and chemical structure to phenoxy and pyridine compounds (Senseman 2007). AMCP has a favorable environmental profile with low toxicology, minimal volatility potential, and broad spectrum weed control at low application rates (Claus et al. 2008; Finkelstein et al. 2008; Senseman 2007; Strachan et al. 2010). These characteristics make AMCP applicable for broadleaf weed control in turfgrass systems (Claus et al. 2008; Curtis et al. 2009; Flessner et al. 2011a; Gannon et al. 2009). AMCP received registration under the trade name Imprelis® in 2010 for use in commercial and residential fine turf (Anonymous 2010). AMCP has a fairly persistent half-life ($T_{1/2}$) in turfgrass systems, ranging from 37 to 103 d; however, AMCP undergoes rapid photodegradation in shallow water when exposed to sunlight ($T_{1/2} = 1.2$ d) (Claus et al. 2008; USEPA 2010). A field study conducted by DuPont Crop Protection indicated AMCP underwent rapid field photolysis following a morning application to a dew-covered centipedegrass [$Eremochloa ophiuroides$ (Munro.) Hack.] stand (unpublished data). Most herbicide applications on golf courses are made during the early morning to avoid patron exposure and utilize the dew pattern created by sprayer tires for guiding proper application coverage. Due to current application practices utilized by golf course managers, potential
photolysis could result in efficacy loss if AMCP applications are made to a dew-covered turfgrass stand. Based on this premise, research was conducted to determine the effect of ambient moisture on AMCP efficacy.

**Materials and Methods**

Field trials were initiated October 1, 2009 and October 1, 2011 at the North Carolina State University Turf Field Laboratory in Raleigh, NC to mature ‘Tifway’ bermudagrass [C. dactylon (L.) Pers. X C. transvaalensis Burtt-Davy] (Table 1). ‘Tifway’ bermudagrass was selected as an indicator species because it displays an intermediate tolerance to AMCP (Flessner et al. 2011b). Experimental design was a 6 x 4 factorial treatment arrangement (six herbicide treatments by two ambient moisture levels) in a randomized complete block with four replications and two experimental runs.

Herbicide treatments included: foliar applied AMCP\(^1\) (79 and 105 g ae ha\(^{-1}\)) and aminopyralid\(^2\) (280 g ha\(^{-1}\)); two granular AMCP\(^3\) formulations (coarse and fine prill size at 84.1 g ha\(^{-1}\)); and a nontreated check. Aminopyralid was applied for comparative purposes, as it has a similar chemical structure to AMCP (Senseman 2007). Foliar herbicide applications were made with a CO\(_2\) pressurized spray boom\(^4\) equipped four TeeJet 8002 XR flat fan nozzles\(^5\) on 24 cm spacings calibrated to deliver 304 L ha\(^{-1}\) at 224 kPa. Granular herbicide applications were made using shaker jars\(^6\).

Ambient moisture levels included plots (2.25 m\(^2\); 1.5 m x 1.5 m) covered overnight to prevent dew formation (DRY) and plots left uncovered so natural dew could form (WET). Tent structures on the DRY plots were constructed using PVC piping (1.25 cm diameter) to create a 1.5 m x 1.5 m x 1 m frame covered with a 7.3 m\(^2\) plastic tarp\(^7\). To allow air
movement for guttation reduction, a 15 cm gap was left between the bottom of the tarp and turfgrass canopy. The tent structures were placed over DRY plots the evening prior to experiment initiation and removed the following morning before herbicide applications. A 1.5 m x 1.5 m buffer was included between replications to not disturb dew formation in WET plots while removing tent structures (Figure 1).

Data collection included visual bermudagrass injury rated weekly on a 0 to 100% scale (0% = no visible turfgrass injury; 100% = complete turfgrass death). In addition, normalized difference vegetation index (NDVI) was collected 21 days after treatment (DAT) using a Field Scout TCM 500 NDVI Turf Color Meter. Four reflectance readings were recorded within each plot and NDVI was calculated. NDVI values were converted to turfgrass quality ratings (1 to 9 scale: 1 = poorest quality; 7 = acceptable quality; and 9 = highest quality) using the equation: \[ (\text{NDVI} \times 6.6) + 2.26 \] (Kieffer 2009). Research has indicated NDVI can accurately and non-subjectively evaluate turfgrass quality in relation to herbicide injury (Bell et al. 2000).

ANOVA was conducted using mixed model methodology (SAS 2004). Herbicide treatment and ambient moisture levels were considered fixed variables in the model and evaluated to determine if there was an interactive effect. Experimental run was considered a random variable, allowing for the comparison of treatment means and interactions over multiple environments (Carmer et al. 1989; Lewis et al. 2010). Experimental run, replication, and interactions between these effects were considered random effects in the model. Means were separated using Fisher’s Protected LSD (P ≤ 0.05).
Results and Discussion

ANOVA determined herbicide treatment and ambient moisture level main effects were significant (P < 0.05) for visual injury and NDVI turfgrass quality at 7 and 21 DAT (Tables 2 and 3). A significant interaction (P < 0.01) was also evident between the main effects on visual injury and NDVI turfgrass quality at the aforementioned rating dates (Table 4). Visual injury and NDVI data collection ceased 21 DAT, as bermudagrass was entering dormancy (October 21, 2009 and 2011); however, no treatments delayed spring greenup the following season (data not shown).

Herbicide Treatment Main Effect

In general, all herbicide treatments caused significant bermudagrass injury and reduced NDVI turfgrass quality compared to the nontreated at all rating dates (Table 2). At 7 DAT, bermudagrass injury was greatest (23%) from the highest foliar applied AMCP rate. Foliar applications of the low AMCP rate and aminopyralid injured bermudagrass 19 and 18%, respectively. Injury was less for the coarse and fine AMCP granular formulations (13 and 4%, respectively). Similar trends in bermudagrass visual injury were apparent 21 DAT, where the highest rate of foliar applied AMCP resulted in 38% injury. The low AMCP rate and aminopyralid injured bermudagrass 29 and 28%, respectively. The fine AMCP granular formulation caused greater injury than the coarse AMCP granular formulation (23 vs. 19%, respectively). NDVI turfgrass quality showed similar patterns to visual injury assessments 21 DAT. Lowest turfgrass quality was recorded from the high AMCP rate and aminopyralid (5.9 and 5.9, respectively), followed by the low AMCP rate (6.2). The fine AMCP granular
formulation reduced turfgrass quality greater than the coarse AMCP granular formulation (6.6 vs. 7.0, respectively).

**Ambient Moisture Level Main Effect**

At 7 and 21 DAT, WET plots had greater bermudagrass injury than DRY plots (Table 3). Bermudagrass injury was 15 and 25% at 7 and 21 DAT, respectively, in WET plots, whereas in DRY plots injury was 10 and 21% at the same respective rating dates. NDVI turfgrass quality taken 21 DAT demonstrated WET plots had reduced quality compared to DRY plots (6.3 vs. 6.6, respectively).

**Herbicide Treatment and Ambient Moisture Level Interaction**

Regarding the interaction between main effects, greater herbicidal activity was demonstrated in WET plots than DRY plots at 7 and 21 DAT (Table 4). When comparing within a herbicide treatment, bermudagrass injury was greater from treatments applied to WET plots compared to DRY plots 7 and 21 DAT, with the exception of aminopyralid at 21 DAT. NDVI turfgrass quality showed a similar response, as quality was more reduced from the high AMCP rate, aminopyralid, and fine AMCP granular applied to WET plots compared to DRY plot quality. While not statistically different, a trend in reduced turfgrass quality in WET versus DRY plots was noted from the low AMCP rate and coarse AMCP granular formulation. Excluding the low AMCP rate applied to DRY plots, all herbicide treatments applied to WET or DRY plots had reduced turfgrass quality (≤ 6.9) compared to the WET and DRY nontreated (7.3 and 7.2, respectively).

**Pearson's Correlation Coefficients**

Pearson's correlation coefficients were analyzed between bermudagrass visual injury and NDVI turfgrass quality taken 21 DAT (Table 5). Visual injury and NDVI turfgrass
quality demonstrated a strong negative relationship \( (r = -0.88; P < 0.001) \), indicating an increase in visual injury corresponded to a decrease in NDVI turfgrass quality. This result is similar to past research indicating strong correlations between visual ratings and non-subjective rating assessments, supporting the utility of visual evaluations to assess herbicide injury within the scientific community (Lewis et al. 2010; Yelverton et al. 2009).

**Research Implications**

Results from this study were contrary to the original hypothesis that AMCP applied to dew-covered turfgrass would experience efficacy loss due to photolysis. In fact the exact opposite was observed, as herbicidal efficacy increased when applied to WET plots. Kogan and Zuñiga (2001) reported dew did not affect glyphosate efficacy when applied at low and medium application volumes (150 and 300 L ha\(^{-1}\)). The presence of dew on turfgrass leaves can result in cuticle hydration and increase herbicide coverage, leading to greater herbicide absorption (Caseley 1989; Johnstone 1973). Dew may also reduce crystalline herbicide deposits from forming on the leaf surface (Hess and Falk 1990). Due to the high water solubility of AMCP and aminopyralid, it is speculated coverage increased as the foliar applied herbicides became suspended in dew droplets on the turfgrass leaves. Furthermore, applications to WET plots likely allowed for a longer retention time on the leaf surface than applications made to DRY plots, which may have undergone formation of crystalline deposits as spray droplets evaporated. Granular herbicide formulations are intended for root absorption; however, the presence of dew at application can suspend the herbicide on the turfgrass leaves leading to foliar uptake. While AMCP photodegradation in shallow water bodies has been reported as 1.2 d, the dew was only present on the leaf surface in this study.
for approximately 4 hr after application. Based on the reported observations, suspension
time in dew had minimal effects on AMCP photodegradation and did not reduce herbicidal
efficacy. Turfgrass managers need to be aware AMCP efficacy can be increased when
applied in the presence of ambient moisture on the plant leaf, which equates to better weed
control. Conversely, intermediately tolerant turfgrass species can incur greater injury and
reduced quality when AMCP is applied to dew-covered turf.
Source of Materials

1 Imprelis® herbicide, E. I. DuPont de Nemours, Wilmington, DE 19805-1523.

2 Milestone® herbicide, Dow AgroSciences, Indianapolis, IN 46268.

3 Aminocyclopyrachlor granular herbicide, E. I. DuPont de Nemours, Wilmington, DE 19805-1523.

4 CO₂-pressurized sprayer, Spraying Systems Co., Wheaton, IL 60189-7900.

5 Teejet nozzles, TeeJet Technologies, Springfield, IL 62703.

6 Ball® 16 oz canning jar, Jarden Home Brands, Daleville, IN 47334.

7 Wel-Bilt® woven poly tarp, Northern Tools + Equipment, Raleigh, NC 27606.

8 Field Scout™ TCM 500 Turf Color Meter, Spectrum™ Technologies, Inc. Plainfield, IL 60585.

Literature Cited


Table 1: Environmental conditions at the Lake Wheeler Turf Field Laboratory, Raleigh, NC surrounding initiation of experimental runs.

<table>
<thead>
<tr>
<th>Time (EST)</th>
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<td>21.9</td>
<td>11.2</td>
</tr>
</tbody>
</table>

\(^a\) Abbreviations: EST, eastern standard time; Temp., temperature; PAR, photosynthetically active radiation.

\(^b\) Air temperature (°C) from a two meter height.

\(^c\) Calculated dew point from a two meter height.

\(^d\) Percent relative humidity from a two meter height.

\(^e\) Soil temperature (°C) from 0.1 meter depth.

\(^f\) Photosynthetically active radiation (µmol sec\(^{-1}\) m\(^{-2}\)) from a two meter height.

\(^*\) Denotes time of herbicide applications.
Table 2: Main effect of herbicide treatment on ‘Tifway’ bermudagrass visual injury and NDVI turfgrass quality 7 and 21 DAT, respectivelyab.

<table>
<thead>
<tr>
<th>Herbicide Treatment</th>
<th>Rate (g ae ha⁻¹)</th>
<th>Visual Injury⁹</th>
<th>Turf Quality¹⁰</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7 DAT</td>
<td>21 DAT</td>
<td>21 DAT</td>
</tr>
<tr>
<td>AMCP</td>
<td>79 B</td>
<td>29 B</td>
<td>6.2 D</td>
</tr>
<tr>
<td>AMCP</td>
<td>105 A</td>
<td>38 A</td>
<td>5.9 E</td>
</tr>
<tr>
<td>Aminopyralid</td>
<td>280 B</td>
<td>28 B</td>
<td>5.9 E</td>
</tr>
<tr>
<td>AMCP Fine</td>
<td>84 D</td>
<td>23 C</td>
<td>6.6 C</td>
</tr>
<tr>
<td>AMCP Coarse</td>
<td>84 C</td>
<td>19 D</td>
<td>7.0 B</td>
</tr>
<tr>
<td>Nontreated</td>
<td>--- E</td>
<td>0 E</td>
<td>7.3 A</td>
</tr>
</tbody>
</table>

⁹ Abbreviations: NDVI, normalized difference vegetation index; DAT, days after treatment; AMCP, aminocyclopyrachlor; AMCP Fine, aminocyclopyrachlor fine granular; AMCP Coarse, aminocyclopyrachlor coarse granular; g ae ha⁻¹, grams acid equivalent per hectare.

¹⁰ Pooled analysis over ambient moisture level main effect and two experimental runs.

¹ Evaluated visually on a 0 to 100% scale (0% = no injury; 100% = complete plant death).

¹² Evaluated using TCM 500 Turf Color Meter radiometer on a 1 to 9 scale (1 = poorest quality; 7 = acceptable quality; and 9 = highest quality).

¹⁵ Means within columns with same letter (A-E) are not significantly different according to Fisher’s Protected LSD (P < 0.01).
Table 3: Main effect of ambient moisture level on ‘Tifway’ bermudagrass visual injury and NDVI turfgrass quality 7 and 21 DAT, respectivelya,b.

<table>
<thead>
<tr>
<th>Ambient Moisture</th>
<th>Visual Injuryc</th>
<th>Turf Qualityd</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7 DAT</td>
<td>21 DAT</td>
<td>21 DAT</td>
</tr>
<tr>
<td>DRY</td>
<td>10 B</td>
<td>21 B</td>
<td>6.6 A</td>
</tr>
<tr>
<td>WET</td>
<td>15 A</td>
<td>25 A</td>
<td>6.3 B</td>
</tr>
</tbody>
</table>

a Abbreviations: NDVI, normalized difference vegetation index; DAT, days after treatment; DRY, treatments applied in the absence of dew; WET, treatments applied in the presence of dew.

b Pooled analysis over herbicide treatment main effect and two experimental runs.

c Evaluated visually on a 0 to 100% scale (0% = no injury; 100% = complete plant death).

d Evaluated using TCM 500 Turf Color Meter radiometer on a 1 to 9 scale (1 = poorest quality; 7 = acceptable quality; and 9 = highest quality).

e Means within columns with same letter (A-B) are not significantly different according to Fisher's Protected LSD (P < 0.01).
Table 4: Interaction of herbicide treatment and ambient moisture level main effects on ‘Tifway’ bermudagrass visual injury and NDVI turfgrass quality 7 and 21 DAT, respectivelyab.

<table>
<thead>
<tr>
<th>Herbicide Treatment</th>
<th>Rate (g ae ha⁻¹)</th>
<th>Ambient Moisture</th>
<th>Visual Injuryc</th>
<th>Turf Qualityd</th>
<th>7 DAT</th>
<th>21 DAT</th>
<th>21 DAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMCP 79</td>
<td>DRY</td>
<td>17 CD³</td>
<td>27 D</td>
<td>7.1 AB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>WET</td>
<td>21 B</td>
<td>32 BC</td>
<td>6.9 BC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMCP 105</td>
<td>DRY</td>
<td>19 BC</td>
<td>33 B</td>
<td>6.8 C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>WET</td>
<td>28 A</td>
<td>44 A</td>
<td>6.4 D</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aminopyralid 280</td>
<td>DRY</td>
<td>14 DE</td>
<td>28 D</td>
<td>6.2 E</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>WET</td>
<td>21 B</td>
<td>29 CD</td>
<td>5.5 G</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMCP Fine 84</td>
<td>DRY</td>
<td>1 G</td>
<td>21 E</td>
<td>6.5 D</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>WET</td>
<td>7 F</td>
<td>26 D</td>
<td>6.0 EF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMCP Coarse 84</td>
<td>DRY</td>
<td>10 E</td>
<td>17 F</td>
<td>6.0 EF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>WET</td>
<td>15 D</td>
<td>21 E</td>
<td>5.9 F</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nontreated</td>
<td>DRY</td>
<td>0 G</td>
<td>0 G</td>
<td>7.3 A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>WET</td>
<td>0 G</td>
<td>0 G</td>
<td>7.2 A</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Abbreviations: NDVI, normalized difference vegetation index; DAT, days after treatment; AMCP, aminocyclopyrachlor; AMCP Fine, aminocyclopyrachlor fine granular; AMCP Coarse, aminocyclopyrachlor coarse granular; g ae ha⁻¹, grams acid equivalent per hectare; DRY, treatments applied in the absence of dew; WET, treatments applied in the presence of dew;

b Pooled analysis over two experimental runs.

c Evaluated visually on a 0 to 100% scale (0% = no injury; 100% = complete plant death).

d Evaluated using TCM 500 Turf Color Meter radiometer on a 1 to 9 scale (1 = poorest quality; 7 = acceptable quality; and 9 = highest quality).

e Means within columns with same letter (A-G) are not significantly different according to Fisher’s Protected LSD (P<0.01).
Table 5: Pearson's correlation coefficients between ‘Tifway’ bermudagrass visual injury and NDVI turfgrass quality 21 DAT\textsuperscript{ab}.

<table>
<thead>
<tr>
<th>Visual Injury\textsuperscript{c}</th>
<th>Turf Quality\textsuperscript{d}</th>
<th>Pr &gt; f</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-0.88</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Abbreviations: NDVI, normalized difference vegetation index; DAT, days after treatment.

\textsuperscript{b} Pooled analysis over two experimental runs.

\textsuperscript{c} Evaluated visually by researcher.

\textsuperscript{d} Evaluated using a TCM 500 Turf Color Meter radiometer.
Figure 1: Schematic of tent structures used for dew exclusion: A. and B.) PVC frame assembly over DRY plots the evening prior to experiment initiation; C.) tarped tent structures the following morning; and D.) DRY vs WET plots prior to herbicide applications.
Synthetic auxin herbicides are utilized for controlling various broadleaf weeds in turfgrass settings. Aminocyclopyrachlor (AMCP) is a newly registered pyrimidine carboxylic acid with a similar chemical mode of action and structure to clopyralid (CLPY). Off-target plant injury has been documented following exposure to compost containing turfgrass clippings previously treated with synthetic auxin herbicides. Due to this issue, AMCP and CLPY labels recommend all treated turfgrass clippings be returned to the turfgrass stand following a mowing event and not be used as a compost or mulch source; however, large quantities of turfgrass clippings are undesirable in golf course, athletic field, and home lawn turf systems because they can interfere with playability and aesthetics. Therefore, alternative uses for synthetic auxin-treated turfgrass clippings are needed. Research was conducted to determine the efficacy of

**Nomenclature:** Aminocyclopyrachlor, 6-amino-5-chloro-2-cyclopropyl-4-pyrimidine-carboxylic acid; clopyralid, 3,6-dichloro-2-pyridinecarboxylic acid; tall fescue, *Lolium arundinaceum* (Schreb.) S.J. Darbyshire.

**Keywords:** Bioavailability, compost, mulch, regrassing

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recycling AMCP and CLPY clippings for white clover control in utility turf settings. AMCP and CLPY (79 g ae ha\(^{-1}\)) were applied to mature tall fescue 56, 28, 14, 7, 3.5, and 1.25 days before clipping collection (DBC). Following collection, previously treated tall fescue clippings were applied to white clover to determine potential for weed control. AMCP clippings provided greater white clover control, lower NDVI, and reduced clover biomass compared to CLPY clippings. White clover control, NDVI values, and biomass followed a linear regression pattern (\(r^2 \geq 0.80; P \leq 0.05\)) from AMCP clippings as herbicide application moved from 56 to 1.25 DBC. CLPY clippings did not demonstrate a linear pattern for white clover control, NDVI, or biomass (\(r^2 \leq 0.32; P = \text{NS}\)). Minimal white clover control from CLPY clippings was likely due to the application rate used in this study, which was less than labeled recommendation (used in this study at equivalent use rates for comparative purposes). These data indicate recycling AMCP clippings could provide additional weed control; however, turfgrass managers must be proactive in properly recycling treated turfgrass clippings in a manner which avoids potential off-target injury to nontargeted plant species.
Tall fescue \( \textit{Lolium arundinaceum} \) (Schreb.) S.J. Darbyshire is a well-adapted cool-season turfgrass species utilized for athletic fields, home lawns, parklands, pastures, roadsides, and various other ground cover uses throughout the United States (Beard 1973; Turgeon 1980). Native to Europe, tall fescue was introduced to the United States (US) likely as a contaminant in imported meadow fescue \( \textit{Lolium pretense} \) (Huds.) Darbysh.] seed during the 19th century (Buckner et al. 1979). Tall fescue was not widely utilized until the commercial release of the ‘Kentucky 31’ cultivar, which was discovered by University of Kentucky professor Dr. E. N. Fergus in 1931 (Hoveland 2009). To date, tall fescue is one of the most prevalent cool-season grasses in the landscape, estimated to cover 405,000 ha in North Carolina and approximately 14 million ha in the US (Buckner et al. 1979; Hoveland 2009; Tredway et al. 2005).

Cultural management practices in actively growing tall fescue systems require multiple mowing events per week to maintain a healthy turfgrass stand (Beard 1973). Turfgrass specialists recommend returning turfgrass clippings following a mowing event, as an estimated 25% of annual required nitrogen is provided as clippings decompose (Bruneau et al. 2008). Equipment failure, inclement weather, or other causes can delay routinely scheduled mowing, often resulting in the accumulation of turfgrass clippings following the next mowing event. Large quantities of turfgrass clippings are undesirable in golf course, athletic field, and home lawn turf because they can interfere with playability and aesthetics, thereby warranting clipping collection and disposal elsewhere (Beard 1973). In 1984, the annual amount of turfgrass clippings collected for landfill disposal was estimated greater than 7 billion kg, leading many US states, including North Carolina, to ban yard waste disposal in
public landfills (Bruneau et al. 2008; Glenn 1989; Goldstein and Riggle 1990). Alternative uses for turfgrass clippings have included composting or utilizing as gardening mulch since clippings contain high moisture and nutrient content (Bahe and Peacock 1995; Bruneau et al. 2008).

Synthetic auxin herbicides, such as phenoxyacetic and pyridine carboxylic acids, have been utilized for broadleaf weed control in tall fescue pastures, rangelands, and turfgrass stands (Senseman 2007). The commercial release of the phenoxyacetic acids 2,4-D and MCPA in 1945 and 1946, respectively, were the first available selective herbicides used extensively for dicotyledonous weed control in monocotyledonous crops (Cobb and Reade 2010; Neal 1990). Synthetic auxin injury can be characterized by a loss in apical dominance, leaf cupping, epinastic curvature, unregulated plant growth, and eventual plant death (Cobb and Reade 2010; Grossmann 2010). Studies conducted by Hoar et al. (1986) suggested a link between phenoxyacetic acids and non-Hodgkins lymphoma, creating public concern and the need for alternative auxin compounds. Applications of pyridine carboxylic acids, such as aminopyralid, clopyralid (CLPY), picloram, and triclopyr, have become widely adopted in tall fescue stands due to their low mammalian toxicity and broad spectrum weed control at lower use rates compared to phenoxy alternatives (Neal 1990; Senseman 2007).

Off-target plant injury via contaminated compost and mulch has been attributed to turfgrass clippings previously treated with synthetic auxin herbicides (Anonymous 2001; Bahe and Peacock 1995; Blewett et al. 2005; Burkhart and Davitt 2002; Lewis et al. 2011; Miltner et al. 2003; Vandervoort et al. 1997). Bahe and Peacock (1995) reported tall fescue
clippings previously treated with 2,4-D (21.5%) + dicamba (11.5%) + MCPP (2.3%) at a total of 5 kg ai ha\(^{-1}\) reduced cucumber (\textit{Cucumis sativus} L.), tomato (\textit{Lycopersicon esculentum} L.), marigold (\textit{Tagetes tenuifolia} Cav.), and salvia (\textit{Salvia splendens} F.) dry mass by 80, 73, 65, and 34%, respectively. In composting studies, 2,4-D, CLPY, and triclopyr residues were found at 0.6, 319, and 0.48 mg kg\(^{-1}\), respectively, 128 days after application (DAA) but only triclopyr was nondetectable 365 DAA (Vandervoort et al. 1997). In spring 2000, CLPY gained public attention as plant injury was reported in Washington where previously treated turfgrass clippings unintentionally entered compost feedstock (Blewett et al. 2005; Burkhart and Davitt 2002; Miltner et al. 2003). Similarly in Pennsylvania, CLPY was found between 10 and 75 ppb in finished compost (greater than 6 months cured) from plant material collected on the Penn State University grounds, leading the university to suspend the use of herbicides containing CLPY (Burkhart and Davitt 2002). Miltner et al. (2003) reported a waiting period greater than or equal to 1 yr could be necessary for CLPY treated turfgrass to be used as compost feedstock. Furthermore, Lewis et al. (2011) reported injury to alligatorweed [\textit{Alternanthera philoxeroides} (Mart.) Griseb.] and parrotfeather [\textit{Myriophyllum aquaticum} (Vell.) Verdc.] when synthetic auxin treated clippings were placed into aquatic settings. These reports indicate synthetic auxin herbicides can be highly persistent in grass clippings and remain bioavailable under variable environmental conditions.

Aminocyclopyrachlor (AMCP) is a newly developed synthetic auxin herbicide belonging to the pyrimidine carboxylic acid herbicide family with similar mode of action and chemical structure to pyridine herbicides (Claus et al. 2008; Senseman 2007). Research has
indicated AMCP has a favorable environmental profile with low mammalian toxicity, minimal volatility potential, and broad spectrum weed control at low application rates compared to available alternatives (Claus et al. 2008; Strachan et al. 2010). These attributes make AMCP a viable tool for broadleaf weed control in turfgrass systems (Claus et al. 2008; Curtis et al. 2009; Flessner et al. 2011; Gannon et al. 2009). AMCP was registered in 2010 by DuPont Crop Protection for broadleaf weed control in commercial and residential fine turf under the trade name Imprelis® (Anonymous 2010). To date, there is limited research regarding AMCP bioavailability in treated turfgrass clippings.

Due to previously mentioned off-target plant injury, many synthetic auxin herbicide labels place restrictions on clipping collection following application (Anonymous 2008a; Anonymous 2008b; Anonymous 2010). However, clipping collection may be required if regular mowing practices are not followed, creating the need to find alternative uses for synthetic auxin-treated turfgrass clippings. Since research has indicated synthetic auxin residues can be released from turfgrass clippings, it is hypothesized clippings from previously treated turfgrass could be collected and recycled for use as an additional weed control practice. Therefore, research was conducted to evaluate recycling AMCP and CLPY clippings for additional weed control in utility turf.

**Materials and Methods**

Research was initiated June 27, 2011 and July 4, 2011, at the North Carolina State University Turf Field Laboratory in Raleigh, NC to determine the efficacy of recycling turfgrass clippings previously treated with synthetic auxin herbicides for white clover
(Trifolium repens L.) control. Commercially available AMCP\(^1\) and CLPY\(^2\) (79 g ae ha\(^{-1}\)) were applied to mature ‘Confederate’ tall fescue (538 m\(^2\) plots; 16.4 m width by 32.8 m length) at at 56, 28, 14, 7, 3.5, and 1.25 days before clipping collection (DBC). AMCP rate was selected according to label recommendation (0.33 L product ha\(^{-1}\)) for broadleaf weed control in tall fescue. CLPY rate was less than label recommendation (1.2 L product ha\(^{-1}\)) and selected to compare with AMCP on an equal active ingredient basis. AMCP and CLPY were applied to with a CO\(_2\)-pressurized sprayer boom\(^3\) equipped four TeeJet 8002 XR flat fan nozzles\(^4\) on 24 cm spacings calibrated to deliver 304 L ha\(^{-1}\) at 224 kPa. Prior to and following herbicide application, tall fescue was mown twice weekly at a 11.4 cm height of cut (HOC) with a push rotary mower\(^5\) equipped with mulching blades, and clippings were returned following a mowing event. To minimize potential herbicide displacement, the mowing deck was cleaned with 25:75 ammonia:water solution before moving to adjacent tall fescue plots. Other cultural practices including fertilization, disease/insect control, and irrigation were conducted according to state extension recommendations for tall fescue (Bruneau 2007). Initial herbicide applications (56 DBC) were made June 27, 2011 and July 4, 2011, respectively, and final herbicide applications (1.25 DBC) were made August 22, 2011 and August 29, 2011. It should be noted no mowing events took place for the 3.5 and 1.25 DBC timings following herbicide application.

At clipping harvest, HOC was lowered to 9 cm and mulched clippings were collected by placing a plastic insert\(^6\) into the mower collection bag. Clippings were spread by hand onto established ‘Dutch’ white clover\(^7\) plots (1.5 m\(^2\); 1 m width x 1.5 m length) at a rate of 454 g mulch (fresh weight) plot\(^{-1}\). This mulching rate was equivalent to the clippings mass
collected from 1.5 m² area of the treated tall fescue. A foliar application of the previously mentioned herbicides (79 g ha⁻¹), nontreated mulch, and nontreated check were included in the experiment for comparative purposes. After mulching was complete, clover was lightly irrigated (2 mm) to prevent clipping displacement into other plots. All mowing practices on clover ceased following experiment initiation to ensure no clipping movement into adjacent plots.

Experimental design was a randomized complete block in a 2x7 factorial arrangement (two herbicide treatments by seven application timings) with four replications and two experimental runs. Clover was rated for visual control on a 0 to 100% scale (0%=no visible injury; 100%=complete plant death) and normalized difference vegetative index (NDVI) was recorded on a weekly basis. NDVI was calculated using a Crop Circle ACS-210 radiometer held at a 1 m height above the clover canopy. NDVI can provide useful indication to overall plant health, as higher NDVI values are associated with greater photosynthetically active radiation being absorbed by plant material (Bell et al. 2002). At 8 weeks after treatment (WAT), white clover was harvested with the previously mentioned mower at a 10 cm HOC with a single mowing pass (0.5 m width) down the center of each plot (1.5 m length) for a total collection across 0.8 m². Biomass samples were placed in a greenhouse for 4 wks and dry weight was recorded.

ANOVA was conducted using mixed model methodology (SAS 2004). Herbicide and application timings were considered fixed variables in the model. Herbicide treatment by clippings collection timing was evaluated to determine if there was an interaction.
Experimental run was considered a random variable, allowing for the comparison of treatment means and interactions over multiple environments (Carmer et al. 1989; Lewis et al. 2010). Experimental run, replication, and interactions between these effects were considered random effects in the model. Means were separated using Fisher’s Protected LSD (P ≤ 0.05) and subject to linear regression analysis in SigmaPlot to determine the effect of herbicide and application timing on visual clover control, NDVI, and harvest weight.

Results and Discussion

The ANOVA determined a significant (P ≤ 0.05) interaction between herbicide treatment and clippings collection timings; therefore, the interaction will be reported rather than the main effects. Foliar AMCP and CLPY applications were omitted from the linear regression analysis, to demonstrate the relationship of herbicide treatment and clipping collection timings.

Visual White Clover Control

At 2 WAT, epinastic symptomology was evident from all herbicide treatments and clippings collection timings. White clover control ranged from 6% to 16% and 9% to 11% from tall fescue clippings treated with AMCP and CLPY, respectively, 56 to 1.25 DBC (Table 1). Foliar AMCP and CLPY applications controlled white clover the greatest at 74 and 67%, respectively, while no herbicidal activity was noted from the nontreated mulch and true nontreated.

At 8 WAT, AMCP clippings demonstrated greater white clover control than CLPY clippings when applied 28 to 1.25 DBC (Table 1). No differences in white clover control
were evident from AMCP or CLPY clippings when applied 56 DBC. White clover control was 33, 73, 86, 92, and 85% from AMCP clippings treated 28, 14, 7, 3.5, and 1.25 DBC, respectively, while CLPY clippings treated at the same respective timings controlled white clover 13, 21, 32, 24, and 16%. AMCP clippings followed a linear regression pattern ($r^2 = 0.91; P < 0.01$), with white clover control increasing as clipping collection timings neared 1.25 DBC; however, CLPY clippings did not follow a similar regression pattern ($r^2 = 0.36; P = \text{NS}$) (Figure 1). Foliar AMCP and CLPY applications both controlled white clover 100%. The nontreated mulch and true nontreated indicated 4 and 3% white clover control, which is likely due to environmental conditions. Based on visual observations, Strachan et al. (2011) calculated 25% growth reduction (GR$_{25}$) to soybean $[Glycine\ max\ (L.)]$, kidney bean $[Phaseolus\ vulgaris\ (L.)]$, and white clover following exposure to AMCP applied preemergence at 2.2, 0.2, and 1 g ha$^{-1}$, respectively, to a silty clay loam soil under greenhouse conditions. Based on our visual observations on white clover, it can be speculated AMCP residues in treated tall fescue are equal to or exceed 1 g ha$^{-1}$ when applied $\leq$ 28 DBC. Fauci et al. (2002) reported visual injury to pinto bean ($Phaseolus\ vulgaris$) trifoliate leaves following exposure 50 ppb of CLPY contaminated mulch but no visual symptomology was detected from compost containing 0.5 to 5 ppb. When grown in presumably contaminated compost, distorted leaf lettuce ($Lactuca\ sativa$ L.) and bell pepper ($Capsicum\ annuum$ L.) vegetation was noted and found to contain 5 and 4.5 ppb CLPY in plant tissue from two independent laboratories (Burkhart and Davitt 2002). These reports indicate AMCP and CLPY can be biologically active at low residue levels and plant response can vary by species.
**White Clover NDVI**

At 2 WAT, turfgrass clippings treated with AMCP 7 to 1.25 DBC had lower NDVI values than CLPY treated clippings applied at the same respective times (Table 1). No differences in NDVI were apparent from AMCP and CLPY clippings applied 56 to 14 DBC. NDVI from CLPY treated clippings were not different than nontreated mulch and true nontreated. Foliar AMCP and CLPY applications resulted in the lowest NDVI values (0.623 and 0.717, respectively), which is indicative to the high level of clover control.

At 8 WAT, clippings treated with AMCP 14 to 1.25 DBC had lower NDVI values than CLPY clippings applied at the same respective time (Table 1). NDVI from AMCP and CLPY clippings from 56 and 28 DBC were not different from the nontreated mulch or true nontreated. AMCP clippings at 14 to 1.25 DBC resulted in lower NDVI than the nontreated mulch and true nontreated, while only CLPY applied 3.5 DBC produced lower NDVI than the nontreated mulch. As observed with visual control, AMCP clippings followed a linear regression pattern \( r^2 = 0.83; P < 0.05 \), with NDVI decreasing as clipping collection timings neared 1.25 DBC (Figure 2). Again, NDVI for CLPY clippings did not follow a linear pattern \( r^2 = 0.03; P = \text{NS} \). Foliar AMCP and CLPY applications continued to have the lowest recorded NDVI, which can be attributed to the high level of clover control. Biewer et al. (2009) utilized field spectroscopy to predict total biomass of a mixed white clover/perennial ryegrass \((Lolium perenne\ L.)\) sward. Their findings suggest a strong relationship \( r^2 = 0.90 \) between NDVI and biomass yield.
White Clover Dry Biomass

Regarding white clover biomass, AMCP clippings applied 14 to 1.25 DBC had less biomass than respective CLPY clippings 8 WAT (Table 1). No differences in white clover biomass were detected from either herbicide applied 56 DBC. Biomass from nontreated mulch and true nontreated were 117 and 116 g, respectively, which was greater than AMCP clippings applied ≤ 14 DBC and CLPY clippings applied 7 DBC. Biomass reduction from previously treated AMCP clippings followed linear regression pattern (r² = 0.84; P < 0.05), while CLPY clippings did not follow a similar pattern (r² = 0.13; P = NS) (Figure 3). White clover biomass was least from foliar AMCP and CLPY applications, with mass measuring 3 and 19 g, respectively. The white clover biomass reduction observed from AMCP clippings is comparable to biomass reduction reported from Bahe and Peacock (1995) who mulched with 2,4-D plus dicamba plus MCPP treated clippings; however, their mulch rate was greater than or equal to 35 mm mulch depth in 20-25 cm pots, which was more concentrated than the mulch rate utilized in this study. While biomass measurements provide insight to synthetic auxin activity, quantifying auxin injury by plant dry weight only can be insensitive to sub-lethal synthetic auxin levels which can still induce significant growth responses (Fauci et al. 2002).

Pearsons Correlation Coefficients

Pearsons correlation coefficients were analyzed between white clover visual control, NDVI, and biomass data taken 8 WAT (Table 2). Visual control illustrated a strong negative relationship with NDVI (r = -0.83; P < 0.001) and biomass (r = -0.80; P < 0.001), indicating an increase in visual control decreased NDVI and biomass. These results are similar to past
research indicating strong correlation between visual ratings and non-subjective rating assessments, supporting visual evaluations for measuring these kinds of effects in the scientific community (Lewis et al. 2010; Yelverton et al. 2009). Correlation between NDVI and biomass showed a strong positive relationship ($r = 0.76; P < 0.001$), indicating increased biomass with greater NDVI values. These results are congruent with those of Biewer et al. (2009) who found NDVI to be predictive of harvestable biomass. However, sub-lethal synthetic auxin levels cannot accurately be quantified by plant dry weight alone and should be supplemented with visual assessments (Fauci et al. 2002).

**Research Implications**

Synthetic auxin herbicides are a viable tool for broadleaf weed control in turfgrass systems. Research has indicated these compounds can be persistent in turfgrass clippings following applications and can become bioavailable as clippings decompose (Bahe and Peacock 1995; Blewett et al. 2005; Burkhart and Davitt 2002; Fauci et al. 2002; Lewis et al. 2011; Miltner et al. 2003; Vandervoort et al. 1997). It is recommended synthetic auxin treated turfgrass clippings be returned following regular mowing events. Where clipping removal is warranted due to large clipping quantities present in turfgrass settings, treated clippings should not be utilized as a composting source as research has noted variable herbicide degradation depending on composting method with detectable levels found greater than 1 yr after application (Briton et al. 2006; Blewett et al. 2005; Miltner et al. 2003; Vandervoort et al. 1997). This research indicates recycling synthetic auxin treated turfgrass clippings can provide additional weed control in utility turfgrass areas. AMCP has activity on white clover at low application rates and can remain bioavailable in treated turfgrass
clippings when applied greater than 56 DBC. While visual symptomology was apparent from all clipping collection timings, the lack of white clover control from CLPY clippings is likely due to the lower application rate utilized in this study which was 5.5x less active ingredient than recommended labeled rate (Anonymous 2008b). Recycling synthetic auxin treated clippings could provide turfgrass managers an alternative method for clipping disposal while providing additional weed control, potentially reducing herbicide applications. Conversely, turfgrass managers need to take upmost care when recycling synthetic auxin treated turfgrass clippings to avoid injury to nontargeted plant species.
Source of Materials

1 Aminocyclopyrachlor herbicide, E. I. DuPont de Nemours, Wilmington, DE 19805 1523.

2 Clopyralid herbicide, Dow AgroSciences, Indianapolis, IN 46268.

3 CO2-pressurized sprayer, Spraying Systems Co., Wheaton, IL 60189-7900.

4 Teejet nozzles, TeeJet Technologies, Springfield, IL 62703.

5 Honda HRC 216, American Honda Motor Co. Inc., Duluth, Georgia 30136-9421.

6 Hefty Ultra Flex 13 Gallon Tall Kitchen Bags, Pactiv Corp., Lake Forest, IL 60045.

7 ‘Dutch’ white clover seed, Wyatt-Quarles Seed, Garner, NC 27529-3559.


10 Sigma Plot, Systat Software Inc., San Jose, CA 95110.
Literature Cited


Table 1: White clover visual control, NDVI, and biomass affected by herbicide-treated clippings and clipping collection timingsab.

<table>
<thead>
<tr>
<th>Clipping Treatment</th>
<th>DBC Timingf</th>
<th>%Controlc</th>
<th>NDVIc</th>
<th>Biomasse (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMCP</td>
<td>56</td>
<td>6</td>
<td>0.818</td>
<td>0.841</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>11</td>
<td>0.817</td>
<td>0.836</td>
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<tr>
<td></td>
<td>14</td>
<td>10</td>
<td>0.785</td>
<td>0.770</td>
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<tr>
<td></td>
<td>7</td>
<td>16</td>
<td>0.775</td>
<td>0.753</td>
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<tr>
<td></td>
<td>3.5</td>
<td>11</td>
<td>0.772</td>
<td>0.740</td>
</tr>
<tr>
<td></td>
<td>1.25</td>
<td>14</td>
<td>0.764</td>
<td>0.755</td>
</tr>
<tr>
<td></td>
<td>Foliar</td>
<td>74</td>
<td>0.623</td>
<td>0.693</td>
</tr>
<tr>
<td>CLPY</td>
<td>56</td>
<td>9</td>
<td>0.822</td>
<td>0.825</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>3</td>
<td>0.814</td>
<td>0.834</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>8</td>
<td>0.808</td>
<td>0.820</td>
</tr>
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<td>11</td>
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<td>0.837</td>
</tr>
<tr>
<td></td>
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<td>9</td>
<td>0.815</td>
<td>0.824</td>
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<tr>
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<td>0.831</td>
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<td>0</td>
<td>0.813</td>
<td>0.854</td>
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<tr>
<td>NT</td>
<td>---</td>
<td>0</td>
<td>0.813</td>
<td>0.837</td>
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<tr>
<td>LSD(P&lt;0.01)</td>
<td>---</td>
<td>4</td>
<td>0.028</td>
<td>0.029</td>
</tr>
</tbody>
</table>

a Abbreviations: NDVI, normalized difference vegetation index; DBC, days before clippings collection; WAT, weeks after treatment; AMCP, aminocyclopyrachlor; CLPY, clopyralid; MLCH, nontreated mulch; NT, true nontreated.

b Pooled analysis over two experimental runs.

c Evaluated visually on a 0 to 100% scale (based on a 0%, no injury; 100%, complete plant death).

d Evaluated using Crop Circle ACS-210 radiometer.

e Biomass reported as dry weight collected from 0.8 m² area of white clover plots.

f Denotes respective days after herbicide application before clipping collection.

g AMCP and CLPY applied at 79 g ae ha⁻¹.
Table 2: Pearson's correlation coefficients between white clover visual control, NDVI, and biomass 8 WAT\(^{ab}\).

<table>
<thead>
<tr>
<th></th>
<th>White Clover</th>
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<tbody>
<tr>
<td></td>
<td>Control (^c)</td>
<td>NDVI (^d)</td>
<td>Biomass (^e)</td>
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<tr>
<td>Control</td>
<td>1</td>
<td>-0.84</td>
<td>-0.8</td>
</tr>
<tr>
<td>Pr &gt; f</td>
<td>---</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NDVI</td>
<td>-0.84</td>
<td>1</td>
<td>0.76</td>
</tr>
<tr>
<td>Pr &gt; f</td>
<td>&lt;0.001</td>
<td>---</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Biomass</td>
<td>-0.8</td>
<td>0.76</td>
<td>1</td>
</tr>
<tr>
<td>Pr &gt; f</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>---</td>
</tr>
</tbody>
</table>

\(^a\) Abbreviations: NDVI, normalized difference vegetation index; WAT, weeks after treatment.

\(^b\) Pooled analysis over two experimental runs.

\(^c\) Evaluated visually by researcher.

\(^d\) Evaluated using Crop Circle ACS-210 radiometer.

\(^e\) Biomass reported as dry weight collected from 0.8 m\(^2\) area of white clover plots.
Figure 1: Linear regression of white clover injury affected by herbicide and clipping collection timings 8 WAT, using the equation $y = mx + b$. 

- AMCP: $r^2 = 0.91$; $P < 0.01$
- CLPY: $r^2 = 0.36$; $P = \text{NS}$
Figure 2: Linear regression of white clover NDVI affected by herbicide and clipping collection timings 8 WAT, using the equation $y = mx + b$. 
Figure 3: Linear regression of white clover biomass affected by herbicide and clipping collection timings 8 WAT, using the equation $y = mx + b$. 
BIOAVAILABILITY OF SYNTHETIC AUXIN RESIDUES FROM TURFGRASS CLIPPINGS IN AQUATIC AND RIPARIAN PLANTS

Dustin F. Lewis, Robert J. Richardson, Fred H. Yelverton, and Thomas R. Wentworth*

---Formatted for Weed Technology---

Synthetic auxin herbicides are widely utilized in golf course settings for selective broadleaf weed control. Aminocyclopyrachlor (AMCP) is a newly registered pyrimidine carboxylic acid with similar chemical mode-of-action and structure to triclopyr (TRIC) and clopyralid (CLPY). Off-target injury on terrestrial plants has been documented following exposure to turfgrass clippings previously treated with TRIC and CLPY. Management practices on golf courses can distribute turfgrass clippings into water bodies; however, research has not evaluated the bioavailability of synthetic auxin residues from turfgrass clippings to aquatic and riparian plants within aqueous environments. A bioassay study was conducted to determine the response of alligatorweed and parrotfeather to tall fescue clippings previously treated with AMCP.

Nomenclature: Aminocyclopyrachlor, 6-amino-5-chloro-2-cyclopropyl-4-pyrimidine-carboxylic acid; clopyralid, 3,6-dichlor-2-pyridinecarboxylic acid; triclopyr, 3,5,6-trichloro-2-pyridinloyxycetic acid; alligatorweed, Alternanthera philoxeroides Mart. Griseb.; parrotfeather, Myriophyllum aquaticum (Vell.) Verdc.; tall fescue, Lolium arundinaceum (Schreb.) S.J. Darbyshire.

Keywords: off-target movement

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treated with synthetic auxin herbicides. AMCP (84 g ae ha\textsuperscript{-1}) and TRIC+CLPY (315 g ha\textsuperscript{-1} plus 105 g ha\textsuperscript{-1}, respectively) were applied to mature tall fescue 14, 7, 3, 1, and 0 days before clippings collection (DBC). Clippings were placed into growth containers containing alligatorweed and parrotfeather. All herbicide treated clippings induced significant growth responses to alligatorweed and parrotfeather growth compared to a nontreated mulch and nontreated control. Alligatorweed control was greater from AMCP clippings collected 14, 7, 3, and 1 DBC than comparative TRIC+CLPY clipping. Alligatorweed shoot lengths were reduced more with AMCP than TRIC+CLPY clippings from 7 and 0 DBC. Similarly, parrotfeather control and shoot lengths were reduced greater from AMCP clippings collected 14, 7, and 3 DBC than similar TRIC+CLPY clipping. Based on these data, synthetic auxin residues can become bioavailable to aquatic and riparian plants within aqueous environments.
Golf courses comprise approximately 908,708 ha within the United States (US) (Lyman et al. 2007). On average, a typical golf course consists of 67% maintained turfgrass (609,000 ha), 16% naturalized areas (140,000 ha), and 7% water bodies (65,250 ha) (Lyman et al. 2007). Many public concerns have been raised about the negative environmental impacts of golf courses such as nutrient runoff, pesticide exposure, and reduced species diversity through habitat loss (Barton 2008). However, the modern philosophy in golf course design, construction, and management has become focused toward environmental sustainability and stewardship (Terman 1997). Naturalistic golf course design focuses on minimal disruption to existing landscapes and uses the natural elements to create a functional playing surface (Doak 1992). The movement toward naturalistic courses has become evident as US golf course managers have increased woodlands, grasslands, riparian borders, and water bodies by 44% (4 ha course\(^{-1}\)) since 1996 (Lyman et al. 2007). Furthermore, membership in conservation groups has risen in popularity as > 2,400 golf courses are currently participating in the Audubon Cooperative Sanctuary Program, which reported natural areas by 9 ha course\(^{-1}\) since receiving certification (Audubon International 2009).

Properly maintained golf courses possess characteristics which allow for key environmental benefits including soil erosion control, synthetic compound biodegradation/dilution, and wildlife habitat (Beard and Green 1994; Bell and Moss 2008; Gannon et al. 2011; Jodice and Humphrey 1992; Terman 1997). Sedimentation from runoff is a major non-point pollutant in US waterways (USDA 1989). Research simulating 76 mm rainfall h\(^{-1}\) over a 30 min period accounted for 10 to 60 kg ha\(^{-1}\) and 223 kg ha\(^{-1}\) sediment loss from a turfgrass stand versus bare ground agronomic system, respectively, indicating
turfgrass is effective at reducing sediment runoff (Gross et al. 1991). Cole et al. (1997) demonstrated bermudagrass \([\text{Cynodon dactylon (L.) Pers.}]\) vegetative buffers significantly reduced nutrient and pesticide runoff from a golf course fairway on a 6% slope. Gannon (2011) reported lower herbicide residues and reduced downward mobility in an established bermudagrass stand compared to a bare ground agronomic system, which was attributed to chemical adsorption within the turfgrass organic layer. Ecological studies have reported golf courses can offer protected habitat for threatened and endangered wildlife species, including the Big Cypress fox squirrel \((\text{Sciurus niger avicennia})\), loggerhead shrike \((\text{Lanius ludovicianus})\), and red-headed woodpecker \((\text{Melanerpes erythrocephalus})\) (Jodice and Humphrey 1992; Rodewald et al. 2005; Smith and Kruse 1992). Furthermore, Tanner and Gange (2004) reported birds and insects showed a higher species richness and abundance in golf course systems compared to neighboring agronomic systems. This research indicates golf courses can present numerous environmental benefits and provide sustainable habitats.

Although aquatic and riparian communities only occupy a small area on golf courses, they are a highly diverse habitat matrix capable of providing shoreline stabilization, water quality improvement, and viable habitat for aquatic and terrestrial organisms (Davis and Lydy 2002; Gregory et al. 1991; Smart et al. 1998; Terman 1997). Rufty et al. (2008) examined nitrate concentrations on four golf courses in the Appalachian region of North Carolina over a 2.5 yr period. The study sites were selected as a potential ‘worst-case’ scenario for environmental risk due to shallow soils, slower turfgrass growth, and reduced microbial activity due to geographic locations. Results indicated nitrate concentrations in streams were higher at course inflow compared to outflow, which the authors attributed in
part to denitrification from aquatic and riparian plants. Kohler et al. (2004) also noted water quality entering a golf course improved after passing through a constructed wetland. Ryals et al. (1998) detected several commonly used herbicides in three North Carolina golf course water bodies but herbicides were nondetectable at course outflows. While herbicide residues in golf course water bodies are a key environmental concern, research has indicated residues are often found at levels below water quality standards (Cohen et al. 1999; Davis and Lydy 2002; Ryals et al. 1998). Turfgrass managers often border golf course water bodies with perennial vegetative buffers to intercept potential pollutants from entering sensitive aquatic and riparian communities (Figure 1) (Bell and Moss 2008; Davis and Lydy 2002). The implementation of vegetative buffers and other site specific best management practices has reduced nutrient and pesticide residues within golf course water bodies (Davis and Lydy 2002). Furthermore, turfgrass managers often exclude herbicide applications from vegetative buffers and aquatic/riparian zones to promote greater biodiversity and protect wildlife habitat.

Synthetic auxin herbicides are used in golf course settings to control dicotyledonous weeds (Senseman 2007). These compounds mimic the natural plant auxin indole-3-yl-acetic acid (IAA) but are not metabolized rapidly in susceptible plant species (Grossmann 2009). Synthetic auxin herbicides such as 2,4-D (2,4-dichlorophenoxyacetic acid), clopyralid [(CLPY); 3,6-dichlor-2-pyridinecarboxylic acid], and triclopyr [(TRIC); 3,5,6-trichloro-2-pyridinyloxyacetic acid], are systemically translocated via the phloem and/or xylem to meristematic plant regions where injury can be characterized by a loss in apical dominance, epinastic curvature of leaves and stems, and unregulated plant growth (Cobb and Reade
2,4-D and TRIC are the only synthetic auxin herbicides labeled for use in aquatic settings to control unwanted vegetation (Senseman 2007). In aqueous environments the half-life of 2,4-D and TRIC range from 1 to 4 d with degradation occurring predominantly through microbial inactivation and/or photolysis, respectively (Senseman 2007).

Aminocyclopyrachlor [(AMCP);6-amino-5-chloro-2-cyclopropyl-4-pyrimidinecarboxylic acid] is a newly developed synthetic auxin herbicide. AMCP belongs to the pyrimidine carboxylic acid herbicide family with mode of action and chemical structure similar to CLPY and TRIC (Claus et al. 2008; Senseman 2007). AMCP was registered in 2010 by DuPont Crop Protection under the trade name Imprelis® for broadleaf weed control in commercial and residential fine turf (Anonymous 2010). Research has indicated AMCP has a favorable environmental profile compared to available auxin alternatives with low mammalian, fish, and daphnia toxicity; minimal volatility potential; and broad spectrum weed control at low application rates (Claus et al. 2008; Finkelstein et al. 2008; Senseman 2007; Strachan et al. 2010). These attributes make AMCP a viable tool for broadleaf weed control in turfgrass systems (Claus et al. 2008; Curtis et al. 2009; Flessner et al. 2011; Gannon et al. 2009). AMCP has shown rapid photodegradation in shallow water bodies (Claus et al. 2008). However, limited research has focused on AMCP biological activity in aquatic environments (Israel 2011).

Bioavailability of synthetic auxin residues from 2,4-D, CLPY, and TRIC treated turfgrass clippings has been documented on numerous terrestrial plants (Bahe and Peacock 1995; Blewett et al. 2005; Burkhart and Davitt 2002; Miltner et al. 2003; Vandervoort et al.
1997). To address this issue, many synthetic auxin herbicide labels suggest clippings be returned to the mown area following an application (Anonymous 2008; Anonymous 2010). Routine golf course management practices include frequent mowing events to maintain a healthy turfgrass stand and desirable playing surface (Beard 1973). Equipment failure, inclement weather, or other causes can postpone regular mowings, resulting in turfgrass clipping accumulation. Large quantities of turfgrass clippings are undesirable on golf courses due to impaired playability (Beard 1973). Turfgrass managers often employ large blowers or other mechanical methods to disperse unwanted clippings from playing surfaces; however these practices can result in copious turfgrass clippings entering sensitive aquatic and riparian communities (Figure 2). Turfgrass previously treated with synthetic auxin herbicides could injure aquatic/riparian plant species if herbicide residues become bioavailable as clippings decompose in aqueous environments.

To date, no published research has evaluated the bioavailability of synthetic auxin residues from turfgrass clippings on aquatic/riparian plants. As past research has indicated, synthetic auxin residues can become bioavailable from previously treated turfgrass clippings in terrestrial plants; therefore, it is hypothesized treated clippings could injure aquatic/riparian plants if allowed to enter water bodies. Conversely, it could also be speculated synthetic auxin residues from treated turfgrass clippings impose negligible impacts on aquatic/riparian plants due to rapid photodegradation of these compounds in aquatic bodies. Based on these premises, research was conducted to evaluate the bioavailability of synthetic auxin residues from turfgrass clippings in aquatic and riparian plants.
Materials and Methods

Experiments were conducted at North Carolina State University, Raleigh, NC, to evaluate the bioavailability of two synthetic auxin herbicides from previously treated turfgrass on aquatic/riparian plants. Herbicide treatments included commercially available Imprelis® 1 [AMCP (84 g ae ha⁻¹)] and Confront® 2 [TRIC (315 g ae ha⁻¹) plus CLPY (105 g ae ha⁻¹); (TRIC+CLPY)]. AMCP and TRIC+CLPY were applied to mature tall fescue [Lolium arundinaceum (Schreb.) S.J. Darbyshire] 14, 7, 3, 1, and 0 days before clipping collection (DBC). Tall fescue plots measured 538 m² (16.4 m width by 32.8 m length) and was grown in a Cecil sandy loam with pH 6.3 and 3% organic matter content. Herbicide applications were made with a CO₂-pressurized sprayer boom ³ equipped four TeeJet 8002 XR flat fan nozzles ⁴ on 24 cm spacings calibrated to deliver 304 L ha⁻¹ at 224 kPa. Prior to and following herbicide application, tall fescue was mown twice weekly at a 11.4 cm height of cut (HOC) with a push rotary mower ⁵ equipped with mulching blades and clippings were returned following a mowing event. To minimize herbicide displacement, the mowing deck was cleaned with 3:1 v/v water/ammonia solution before moving to adjacent tall fescue plots. Treatments applied 14 and 7 DBC received four and two mowing events, respectively, prior to clipping harvest while treatments applied 3, 1, or 0 DBC were unmown until clipping collection. Other cultural practices including fertilization, disease/insect control, and irrigation were conducted according to state extension recommendations for tall fescue (Bruneau 2007). Initial herbicide applications (14 DBC) were made March 12, 2009 and final herbicide applications (0 DBC) were made March 26, 2009. A nontreated mulch and true nontreated control were also collected and included for comparison. Following
collection, clippings were placed into plastic bags\(^6\) and stored in a freezer (-18\(^o\) C) until experiment initiation.

Alligatorweed \([\textit{Alternanthera philoxeroides} \text{ (Mart.) Griseb.}]\) and parrotfeather \([\textit{Myriophyllum aquaticum} \text{ (Vell.) Verdc.}]\) were used as bioassay species to assess AMCP and TRIC+CLPY bioavailability to aquatic/riparian plants. While alligatorweed and parrotfeather are often considered invasive weeds in golf course water bodies, they were utilized in this study because research has documented their sensitivity to various rates of synthetic auxin herbicides (Allen et al. 2007; Hofstra et al. 2006; Hofstra and Champion 2010; Israel 2011; Langeland 1986; Wersal and Madsen 2010). Alligatorweed and parrotfeather previously collected from a local water body (Weldon’s Pond, Vance County, NC; 36.290155 - 78.321344) were propagated in the Weed Science Annex Glasshouses, Raleigh, NC, into 10 cm square pots containing 25 cm\(^2\) layer of potting mix\(^7\) capped with a 25 cm\(^2\) layer of sand to prevent growth media suspension in the water column. Alligatorweed and parrotfeather were allowed to establish in pots until shoot lengths averaged 15 cm. Beginning March 5, 2010, and June 14, 2010, respectively, aforementioned turfgrass clippings (25 g fresh weight) were placed into 15 L growth containers\(^8\) (9.5 L pond water; pH \(\approx 7.8\)) containing both alligatorweed and parrotfeather. Clipping application rate was chosen to lightly cover the water surface and mimic clipping dispersal previously observed within golf course water bodies. These conditions were set as a potential ‘worst-case’ scenario to a lentic wetland ecosystem as water was only replenished to account for evaporative loss. Glasshouse conditions remained at 29 / 21 C day/night temperatures with a
12-h photoperiod supplemented with overhead lighting (490 µE m\(^{-2}\) s\(^{-1}\) photosynthetic photon flux density at plant height) for the remainder of the study.

Treatments were arranged in a 4 x 5 factorial (four clipping treatments by five clipping collection timings) in a randomized complete block design with four replications and two experimental runs. Visual plant injury (0 to 100% scale; 0 = no visible plant injury, 100 = complete plant death) was recorded weekly. Plants were harvested 10 weeks after treatment (WAT) on May 13, 2010, and August 25, 2010, respectively, where plant shoot length (cm) was measured. After shoot lengths were recorded, biomass was placed into a drying oven (65°C) for 14-d and dry weights were recorded. ANOVA was conducted using mixed model methodology (SAS 2004)\(^9\). Due to inherent differences between alligatorweed and parrotfeather, the two species were analyzed separately. Clipping treatment and DBC timings were considered fixed variables in the model. Clipping treatment by DBC timing effects were evaluated to determine if there was an interaction. Experimental run was considered a random variable, allowing for the comparison of treatment means and interactions over multiple environments (Carmer et al. 1989; Lewis et al. 2010). Experimental run, replication, and interactions between these effects were considered random effects in the model. Means were separated using Fisher’s Protected LSD (P ≤ 0.05) to determine the effect of clipping treatment and DBC timing on alligatorweed and parrotfeather visual control, shoot length, and dry mass.

**Results and Discussion**

ANOVA revealed a significant clipping treatment main effect on alligatorweed and parrotfeather control, shoot length, and dry mass at 6 and 10 WAT,
respectively (Table 1). Similarly, a significant DBC timing main effect ($P \leq 0.05$) was evident on alligatorweed control (10 WAT) and parrotfeather control and shoot length (6 and 10 WAT) (Table 2). Finally, significant interactions between clipping treatment and DBC timing main effects ($P \leq 0.05$) were apparent on alligatorweed control and shoot length (10 WAT), as well as parrotfeather control and shoot length (6 and 10 WAT) (Table 3). For the remainder of the paper, only significant main effects and interactions will be discussed; however, non-significant main effects and interactions are listed in the aforementioned tables for comparative purposes. In general, AMCP and TRIC+CLPY clippings demonstrated herbicidal activity on alligatorweed and parrotfeather at all DBC timings compared to the nontreated mulch and true nontreated. Malformed alligatorweed meristematic tissue was noted 1 WAT and epinastic curvature of the stems and leaves at the terminal nodes were obvious 2 WAT (data not shown). Similarly, parrotfeather demonstrated growth responses suggestive to synthetic auxin activity 1 WAT with exaggerated meristematic growth followed by epinastic stem curvature 2 WAT (data not shown). All symptomology observed from AMCP and TRIC+CLPY clippings were indicative to synthetic auxin activity (Grossmann 2009).

**Alligatorweed**

AMCP clippings illustrated greater biological activity than TRIC+CLPY clippings when pooled over DBC timings (Table 1). At 6 WAT, greater alligatorweed control was observed from AMCP clippings (52%) than TRIC+CLPY clippings (39%). Similar results were observed 10 WAT, where AMCP and TRIC+CLPY clippings controlled alligatorweed 70% and 47%, respectively. No herbicidal activity or growth inhibition was noted from the
nontreated mulch and true nontreated at 6 or 10 WAT. Alligatorweed shoot length from the nontreated mulch and true nontreated measured 42 and 50 cm, respectively, whereas shoot length from AMCP and TRIC+CLPY clippings measured 33 and 36 cm, respectively. No difference was observed between AMCP and TRIC+CLPY clippings on alligatorweed dry mass (0.7 and 1.0 g, respectively), but mass was reduced compared to the nontreated mulch and true nontreated (1.4 and 2.0 g, respectively). While biomass data provides useful insight on herbicide activity, quantifying synthetic auxin injury by plant dry weight only can be insensitive to sub-lethal synthetic auxin levels which can still induce significant growth responses (Fauci et al. 2002).

The DBC timing main effect was not as impactful as the clipping treatment main effect on alligatorweed control, shoot length, or dry mass, as F-values reported in ANOVA were much greater for the latter mentioned main effect (data not shown). Pooled across clipping treatments, DBC timing main effect was only significant on alligatorweed visual control 10 WAT (Table 2). Alligatorweed control was greatest following applications made 0 to 3 DBC (37 to 32%, respectively), whereas control was reduced at 7 and 14 DBC (21%). The reduction in control may be due to the influence of mowing events that took place on the 14 and 7 DBC treatments; however, Miltner et al. (2003) reported mowing had no significant effect on clopyralid content in grass clippings. Due to the high water solubility of AMCP and TRIC+CLPY, environmental variables such as dew, rainfall, and/or irrigation may have resuspended unabsorbed herbicide from the turfgrass leaves prior to clipping collection.

Regarding the interaction between clipping treatment and DBC timing main effects, AMCP clippings had greater herbicidal activity than TRIC+CLPY clippings at several DBC
timings (Table 3). AMCP illustrated greater alligatorweed control than TRIC+CLPY when applied 14, 7, 3, and 1 DBC. No differences were observed between AMCP and TRIC+CLPY clippings applied 0 DBC, which controlled alligatorweed 72 and 76%, respectively. However, AMCP and TRIC+CLPY clippings at all DBC timings had greater alligatorweed control compared to the nontreated mulch and true nontreated. Alligatorweed shoot lengths were less for AMCP clippings applied 7 DBC than respective TRIC+CLPY clippings. Interestingly, TRIC+CLPY clippings at 0 DBC had lower shoot length measurements than comparative AMCP clippings (20 and 34 cm, respectively). The nontreated mulch and true nontreated had greater shoot lengths (42 and 50 cm, respectively) than AMCP clippings at 3 DBC (26 cm) and TRIC+CLPY clippings at 0 DBC (20 cm). Past research has evaluated the use of synthetic auxin herbicides for alligatorweed control (Allen et al. 2007; Hofstra and Champion 2010; Israel 2011; Langeland 1986). 2,4-D applied at 0.46 kg 100L⁻¹ controlled alligatorweed 80% 2 WAT but was reduced to 50% 8 WAT due to vegetative regrowth (Langeland 1986). TRIC applied from 1.7 to 5.2 kg ha⁻¹ reduced alligatorweed cover and biomass early after application but regrowth occurred later in the season (Allen et al. 2007; Hofstra and Champion 2010). Israel (2011) was the first to report AMCP for aquatic weed control, in which foliar application of AMCP from 70 to 280 g ha⁻¹ controlled alligatorweed 82 to 95%, respectively. These results indicate AMCP and TRIC+CLPY clippings have herbicidal activity comparable to suggested synthetic auxin use rates for alligatorweed control.
Parrotfeather

Regarding clipping treatment main effect, AMCP clippings had greater herbicidal activity on parrotfeather than TRIC+CLPY clippings (Table 1). Further, both AMCP and TRIC+CLPY clippings had significantly greater growth effects compared to the nontreated mulch and true nontreated when pooled over DBC timings. AMCP clippings controlled parrotfeather greater than TRIC+CLPY clippings at 6 WAT (74 and 28%, respectively) and 10 WAT (83 and 47%, respectively). The nontreated mulch and true nontreated did not inhibit parrotfeather growth at either of the aforementioned rating dates. Parrotfeather shoot length from AMCP and TRIC+CLPY clippings were 20 and 44 cm, respectively, whereas the nontreated mulch and true nontreated produced 75 and 84 cm shoots, respectively. AMCP clippings produced the lowest parrotfeather dry weight (1.8 g) but was not different from TRIC+CLPY clippings (2.0 g). As mentioned previously, dry weights may not accurately quantify synthetic auxin injury as exaggerated plant growth can confound results (Fauci et al. 2002).

Pooled over clipping treatment main effect, greater parrotfeather control was observed from 0, 1, and 3 DBC compared to 7 and 14 DBC at 6 and 10 WAT (Table 2). The increase in control from the 0 to 3 DBC may again be attributed to mowing events or environmental variables that took place within the 7 and 14 DBC. Further research may be warranted to compare these results with those of Miltner et al. (2003) who reported mowing did not reduce synthetic auxin concentration in turfgrass clippings. Parrotfeather shoot length was reduced from 67 to 44 cm as timings decreased from 14 to 0 DBC.
The interactive effect between clipping treatment and DBC timing main effects showed AMCP clippings had greater herbicidal activity on parrotfeather than TRIC+CLPY clippings at several DBC timings (Table 3). AMCP clippings provided greater parrotfeather control than TRIC+CLPY clippings at all DBC timings 6 WAT. All AMCP and TRIC+CLPY clippings had greater parrotfeather control than the nontreated mulch and true nontreated at 6 WAT, with the exception of TRIC+CLPY clippings at 7 and 14 DBC. At 10 WAT, greater parrotfeather control was observed from AMCP clippings at 14, 7, and 3 DBC timings (61, 75, and 97%, respectively) than comparative TRIC+CLPY clippings (26, 14, and 33%, respectively). All AMCP and TRIC+CLPY clippings had greater parrotfeather activity than the nontreated mulch and true nontreated, with the exception of TRIC+CLPY at 7 DBC. As observed with parrotfeather control, AMCP clippings at 14, 7, and 3 DBC reduced parrotfeather shoot lengths greater than comparative TRIC+CLPY clippings. Parrotfeather shoot lengths from the nontreated mulch and true nontreated measured 75 and 84 cm, respectively, which was greater than shoot lengths recorded for AMCP clippings at all DBC timings and TRIC+CLPY at 3, 1, and 0 DBC. Research has demonstrated parrotfeather susceptibility to synthetic auxin herbicides (Hofstra et al. 2006; Israel 2011; Wersal and Madsen 2010). Wersal and Madsen (2010) reported 2,4-D (2.1 kg ha\(^{-1}\)) and TRIC (6.7 kg ha\(^{-1}\)) controlled parrotfeather 85 and 70%, respectively, 5 WAT in an aquatic mesocosm study. Similarly, Hofstra et al. (2006) reported foliar applications of TRIC at 4 kg ha\(^{-1}\) reduced parrotfeather cover under field conditions. Israel (2011) screened parrotfeather to foliar applied AMCP (35 to 560 g ha\(^{-1}\)) and indicated ≥ 80% control from all tested rates.
The findings of Israel (2011) further support the observations of this study on the sensitivity of parrotfeather to AMCP residues in turfgrass clippings.

**Research Implications**

Golf courses occupy nearly 1 million ha in the US with new construction continuing to take place (Brown et al. 2005; Lyman et al. 2007). Research has indicated properly designed and maintained golf courses can offer key environmental benefits and increasing naturalized areas can reduced nutrient and pesticide residues in golf course water bodies, as well as provide significant habitat for numerous species (Beard and Green 1994; Bell and Moss 2008; Davis and Lydy 2002; Gannon 2011; Jodice and Humphrey 1992; Rufty et al. 2008; Terman 1997). Synthetic auxin herbicides are commonly applied for broadleaf weed control; however, research has indicated bioavailable herbicide residues can be released from previously treated turfgrass clippings and cause unintended injury (Bahe and Peacock 1995; Blewett et al. 2005; Burkhart and Davitt 2002; Miltner et al. 2003; Vandervoort et al. 1997). When turfgrass clippings accumulate on the playing surface, golf course managers use mechanical dispersal methods that can deposit clippings into aquatic and riparian settings. Results from this research indicate AMCP and TRIC+CLPY residues in turfgrass clippings can become bioavailable in aqueous environments. The results previously presented coincide with past research demonstrating terrestrial plant injury following exposure to turfgrass clippings previously treated with synthetic auxin herbicides (Bahe and Peacock 1995; Blewett et al. 2005; Burkhart and Davitt 2002; Miltner et al. 2003; Vandervoort et al. 1997).

Examination of published literature indicates this is the first documentation of synthetic auxin bioavailability from turfgrass clippings in aquatic settings. The methods
previously described can provide an effective model for aquatic bioassay studies to determine the bioavailability of synthetic compounds in turfgrass clippings. This study was conducted to represent a ‘worst-case’ scenario to a lentic ecosystem and future research should evaluate aquatic/riparian plant injury within a lotic system where moving water could potentially dilute herbicide concentrations. Regardless, the level of herbicidal activity observed from AMCP and TRIC+CLPY clippings on alligatorweed and parrotfeather was alarming as control was comparable to recommended synthetic auxin applications rates for aquatic weed management (Allen et al. 2007; Hofstra et al. 2006; Hofstra and Champion 2010; Israel 2011; Langeland 1986; Wersal and Madsen 2010). Furthermore, Israel (2011) reported foliar AMCP application $\geq 30$ g ha$^{-1}$ significantly injured lizard tail (*Saururus cernuus* L.) and pickerelweed (*Pontederia cordata* L.), both of which are native aquatic species. These results indicate bioavailable synthetic auxin residues from turfgrass clippings can inhibit plant growth in aqueous settings and could have detrimental impacts on the native flora and existing fauna that inhabit aquatic and riparian communities.

To reduce off-target injury, golf course managers who apply synthetic auxin herbicides should implement clipping dispersal methods which minimize displacement into sensitive natural areas. Recommendations include the use of dew whips, drag hoses, and/or remowing the area to incorporate clippings into the turfgrass canopy (Beard 1973). Furthermore, these results can also be extrapolated to residential turfgrass settings where synthetic auxin herbicides are applied. Homelawn owners often place excess clippings along residential roadways for landfill disposal; however heavy rains may relocate clippings into storm drains where they could enter aquatic environments at wastewater outflows. When
excessive treated clippings cannot be dispersed by the aforementioned methods, synthetic auxin treated clippings may be collected and ‘recycled’ for additional weed control in other turfgrass areas; conversely, this practice would require upmost care to prevent off-target plant injury (Lewis and Yelverton 2012). Based on this research, turfgrass managers should become more cognizant of the potential ecological impacts of synthetic auxin treated turfgrass clippings in aquatic and riparian plant communities and perhaps incorporate management strategies to avoid unintended effects.
Source of Material

1 Imprelis® herbicide, E. I. DuPont de Nemours, Wilmington, DE 19805-1523.

2 Confront® herbicide, Dow AgroSciences, Indianapolis, IN 46268.

3 CO₂-pressurized sprayer, Spraying Systems Co., Wheaton, IL 60189-7900.

4 Teejet nozzles, TeeJet Technologies, Springfield, IL 62703.

5 Honda HRC 216, American Honda Motor Co. Inc., Duluth, Georgia 30136-9421.

6 Hefty Ultra Flex 13 Gallon Tall Kitchen Bags, Pactiv Corp., Lake Forest, IL 60045.

7 4P Mix, Conrad Fafard, Inc., Agawam, MA 01001.

8 4-gallon square bucket, United States Plastic Corp., Lima Ohio, 45801-3196.

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Vandervoort, C., M. J.Zabik, B. Branham, and D. W.Lickfeldt. 1997. Fate of selected
pesticides applied to turfgrass: Effect of composting on residues. Bull.
Environ.Contam.Toxicol. 58:38-45.
Wersal, R. M., and J. D. Madsen. 2010. Comparison of subsurface and foliar herbicide
applications for control of parrotfeather (Myriophyllum aquaticum). Inv. Plant Sci.
and Manage. 3:262-267.
Table 1: Main effect of clipping treatment on alligatorweed and parrotfeather control, shoot length, and dry mass 6 and 10 WAT, respectively, pooled over DBC timings\(^ab\).

<p>| Clipping Treatment | Alligatorweed | | Parrotfeather | | | | | |
|--------------------|---------------|----------------|---------------|----------------|----------------|---------------|----------------|---------------|----------------|---------------|----------------|---------------|----------------|---------------|</p>
<table>
<thead>
<tr>
<th></th>
<th>% Control(^c)</th>
<th>Shoot (cm)</th>
<th>Dry Mass (g)</th>
<th>% Control</th>
<th>Shoot (cm)</th>
<th>Dry Mass (g)</th>
<th>% Control</th>
<th>Shoot (cm)</th>
<th>Dry Mass (g)</th>
<th>% Control</th>
<th>Shoot (cm)</th>
<th>Dry Mass (g)</th>
<th>% Control</th>
<th>Shoot (cm)</th>
<th>Dry Mass (g)</th>
</tr>
</thead>
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<tr>
<td>AMCP</td>
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<td>70</td>
<td>33</td>
<td>0.7</td>
<td>74</td>
<td>83</td>
<td>20</td>
<td>1.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRIC+CLPY</td>
<td>39</td>
<td>47</td>
<td>36</td>
<td>1.0</td>
<td>28</td>
<td>47</td>
<td>44</td>
<td>2.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MULCH</td>
<td>0</td>
<td>0</td>
<td>42</td>
<td>1.4</td>
<td>0</td>
<td>0</td>
<td>75</td>
<td>3.1</td>
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</tr>
<tr>
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<td>0</td>
<td>84</td>
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<td></td>
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<td></td>
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<tr>
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<td>7</td>
<td>6</td>
<td>0.4</td>
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<td>1.4</td>
<td></td>
<td></td>
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<td></td>
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</tbody>
</table>

\(^a\) Abbreviations: DBC, days before clipping collection; WAT, weeks after treatment; AMCP, aminocyclopyrachlor (84 g ae ha\(^{-1}\)); TRIC+CLPY, triclopyr (315 g ha\(^{-1}\)) plus clopyralid (105 g ha\(^{-1}\)); MULCH, nontreated mulch; NT, true nontreated; NS, non-significant.

\(^b\) Pooled analysis over two experimental runs.

\(^c\) Evaluated visually on a 0 to 100% scale (0 = no visible plant injury; 100 = complete plant death).
Table 2: Main effect of DBC timings on alligatorweed and parrotfeather control, shoot length, and dry mass 6 and 10 WAT, respectively, pooled over clipping treatmentab.

<table>
<thead>
<tr>
<th>DBC Timing</th>
<th>Alligatorweed</th>
<th>Parrotfeather</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>% Controlc</td>
<td>Shoot (cm)</td>
</tr>
<tr>
<td></td>
<td>6 WAT 10 WAT</td>
<td>10 WAT 10 WAT</td>
</tr>
<tr>
<td>14</td>
<td>21 21</td>
<td>44 1.4</td>
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<td>7</td>
<td>19 21</td>
<td>43 1.3</td>
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<td>24 36</td>
<td>40 1.2</td>
</tr>
<tr>
<td>0</td>
<td>26 37</td>
<td>37 1.1</td>
</tr>
</tbody>
</table>

LSD (<0.05) NS 8 NS NS 6 7 11 NS

a Abbreviations: DBC, days before clipping collection; WAT, weeks after treatment; NS, non-significant.
b Pooled analysis over two experimental runs.
c Evaluated visually on a 0 to 100% scale (0 = no visible plant injury; 100 = complete plant death).
Table 3: Interaction of clipping treatment and DBC timing on alligatorweed and parrotfeather control, shoot length, and dry mass 6 and 10 WAT, respectively\(^ab\).

<table>
<thead>
<tr>
<th>Clipping Treatment</th>
<th>DBC Timing</th>
<th>% Control</th>
<th>Shoot (cm)</th>
<th>Dry Mass (g)</th>
<th>% Control</th>
<th>Shoot (cm)</th>
<th>Dry Mass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMCP</td>
<td>14</td>
<td>45</td>
<td>49</td>
<td>36</td>
<td>1.1</td>
<td>57</td>
<td>61</td>
</tr>
<tr>
<td>TRIC+CLPY</td>
<td>14</td>
<td>38</td>
<td>33</td>
<td>47</td>
<td>1.1</td>
<td>10</td>
<td>26</td>
</tr>
<tr>
<td>AMCP</td>
<td>7</td>
<td>42</td>
<td>60</td>
<td>33</td>
<td>0.6</td>
<td>66</td>
<td>75</td>
</tr>
<tr>
<td>TRIC+CLPY</td>
<td>7</td>
<td>34</td>
<td>25</td>
<td>48</td>
<td>1.3</td>
<td>9</td>
<td>14</td>
</tr>
<tr>
<td>AMCP</td>
<td>3</td>
<td>63</td>
<td>90</td>
<td>26</td>
<td>0.4</td>
<td>81</td>
<td>97</td>
</tr>
<tr>
<td>TRIC+CLPY</td>
<td>3</td>
<td>35</td>
<td>37</td>
<td>29</td>
<td>1.2</td>
<td>23</td>
<td>33</td>
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<tr>
<td>AMCP</td>
<td>1</td>
<td>58</td>
<td>80</td>
<td>35</td>
<td>0.6</td>
<td>77</td>
<td>85</td>
</tr>
<tr>
<td>TRIC+CLPY</td>
<td>1</td>
<td>38</td>
<td>64</td>
<td>33</td>
<td>0.7</td>
<td>34</td>
<td>77</td>
</tr>
<tr>
<td>AMCP</td>
<td>0</td>
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<td>72</td>
<td>34</td>
<td>0.7</td>
<td>87</td>
<td>97</td>
</tr>
<tr>
<td>TRIC+CLPY</td>
<td>0</td>
<td>51</td>
<td>76</td>
<td>20</td>
<td>0.5</td>
<td>63</td>
<td>86</td>
</tr>
<tr>
<td>MULCH</td>
<td>---</td>
<td>0</td>
<td>0</td>
<td>42</td>
<td>1.4</td>
<td>0</td>
<td>0</td>
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<tr>
<td>NT</td>
<td>---</td>
<td>0</td>
<td>0</td>
<td>50</td>
<td>1.9</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

LSD (\(\text{cm}^5\)) --- NS 15 14 NS 12 14 22 NS

\(^a\) Abbreviations: DBC, days before clipping collection; WAT, weeks after treatment; AMCP, aminocyclopyrachlor (84 g ae ha\(^{-1}\)); TRIC+CLPY, triclopyr (315 g ha\(^{-1}\)) plus clopyralid (105 g ha\(^{-1}\)); MLCH, nontreated mulch; NT, true nontreated; NS, non-significant.

\(^b\) Pooled analysis over two experimental runs.

\(^c\) Evaluated visually on a 0 to 100% scale (0 = no visible plant injury; 100 = complete plant death).
Figure 1: Vegetative buffer surrounding a golf course water body at the Honors Course, Ootlewah, TN. Photo credit: Dustin F. Lewis.
Figure 2: Turfgrass clipping accumulation in a golf course water body. Photo credit: Dustin F. Lewis
CHARACTERIZATION OF AMINOCYCLOPYRACHLOR IN TALL FESCUE
[LOLIUM ARUNDINACEUM (SCHREB.) S.J. DARBYSHIRE]
Dustin F. Lewis*, Rory L. Roten, Wesley J. Everman, Robert J. Richardson, and Fred H. Yelverton

---Formatted for Weed Science---

Synthetic auxin herbicides are commonly used in turfgrass systems for dicotyledonous weed control. Aminocyclopyrachlor (AMCP) is a newly developed pyrimidine carboxylic acid with similar chemical structure and mode of action to the pyridine carboxylic acids aminopyralid, clopyralid, and picloram. Off-target injury has been observed following exposure to monocotyledonous plant material previously treated with pyridine compounds. The absorption, translocation, and metabolism of AMCP has been documented in susceptible broadleaf weeds; however, no information is available regarding AMCP fate in tolerant Poaceae spp. that serve as the source material for off-target plant injury. Based on this premise, research was conducted to characterize radiolabeled AMCP (14C-AMCP) absorption, translocation, and metabolism in tall fescue. 14C-AMCP was applied to single tiller tall fescue under controlled laboratory conditions at North Carolina State University, Raleigh, NC. Radiation was quantified in leaf wash, treated leaf, foliage, crown, roots, and root exudates at 3, 12, 24, 48, 96, and 192 hours after treatment (HAT). 14C-AMCP

Nomenclature: Aminocyclopyrachlor, 6-amino-5-chloro-2-cyclopropyl-4-pyrimidine-carboxylic acid; tall fescue, [Lolium arundinaceum (Schreb.) S.J. Darbyshire].

Keywords: Absorption, metabolism, off-target movement, synthetic auxin, translocation.

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absorbed rapidly in tall fescue, reaching 38 and 68% at 3 and 48 HAT, respectively. Translocation of $^{14}$C-AMCP was limited to the foliage, which reached maximum translocation (34%) 96 HAT. The majority of recovered $^{14}$C-AMCP remained in the leaf wash, treated leaf, or foliage whereas minimal radiation was detected in the crown, roots, or root exudates throughout the 192 hour time period. No AMCP metabolism was found in tall fescue 192 HAT. These data suggest AMCP applied to tall fescue can remain bioavailable and mishandling treated turfgrass clippings could result in off-target plant injury.
Synthetic auxin herbicides are widely used for broadleaf weed control in turfgrass systems. The phenoxyacetic acids 2,4-D (2,4-dichlorophenoxyacetic acid) and MCPA (2-methyl-4-chlorophenoxyacetic acid) were commercially released in 1945 and 1946, respectively, making them the first available selective herbicides used for controlling dicotyledonous weeds in monocotyledonous crops (Cobb and Reade 2010). Pyridine carboxylic acids, such as aminopyralid (4-amino-3,6-dichloro-2-pyridinecarboxylic acid), clopyralid (3,6-dichlor-2-pyridinecarboxylic acid), and picloram (4-amino-3,5,6-trichloropicolinic acid) have also become widely adopted due to their broad spectrum weed control at lower use rates compared to alternative phenoxy herbicides (Senseman 2007). Both phenoxyacetic and pyridine chemistries mimic the natural plant auxin indole-3-yl-acetic acid (IAA) but are not metabolized rapidly in susceptible broadleaf weeds (Grossmann 2009). IAA is found in highest concentrations within meristematic plant tissue, where it stimulates cellular division, differentiation, and plant growth (Cobb and Reade 2010). Once absorbed through the cuticle, synthetic auxin compounds are systemically translocated via the phloem and/or xylem to meristematic plant regions such as shoots and roots, lending effective control against perennial species (Grossmann 2009). Synthetic auxin injury can be characterized by a loss in apical dominance, leaf cuppings, epinastic curvature, and unregulated plant growth (Cobb and Reade 2010). The inactivity of these compounds in monocotyledonous plants is not fully understood but thought to be due to compartmentalization, metabolism, target site insensitivity, and/or irreversible sequestration into cell wall constituents (Cobb and Reade 2010; Grossmann 2009).
Research has characterized the absorption, translocation, and metabolism of several phenoxyacetic and pyridine carboxylic acids in susceptible broadleaf weeds (Bukun et al. 2009; Devine and Vandenborn 1985; Lym and Moxness 1989; Orfanedes et al. 1993; Valenzuela-Valenzuela et al. 2001). Leafy spurge (Euphorbia esula L.) absorbed 14 and 34% of applied 14C-picloram and 14C-2,4-D, respectively, 72 hours after treatment (HAT) with the majority of both herbicides remaining unmetabolized 96 HAT (Lym and Moxness 1989). Bukun et al. (2009) reported 72% 14C-clopyralid absorption in Canada thistle [Cirsium arvense (L.) Scop.] 24 HAT and remaining near 80% absorption 192 HAT. In the same study, 14C-aminopyralid absorption was 34% and 60% 24 and 192 HAT, respectively. 14C-Clopyralid translocation from the treated leaf was 39% 192 HAT, whereas only 17% 14C-aminopyralid translocation was reported over the same time period; however, no metabolism was reported for either compound (Bukun et al. 2009). Similarly, Devine and Vandenborn (1985) found 99% 14C-clopyralid absorption in Canada thistle 144 HAT, with 40% translocation above the treated leaf and 29% to the plant roots. Hemp dogbane (Apocynum cannabinum L.) absorbed 38% of applied 14C-clopyralid 72 HAT, with 75% of absorbed 14C-clopyralid recovered outside the treated leaf; however, minimal metabolism was reported (Orfanedes et al. 1993). Valenzuela-Valenzuela et al. (2001) found yellow starthistle (Centaurea solstitialis L.) rapidly absorbed 14C-clopyralid (75%) 2 HAT and translocation ranged 39% above the treated, 9% below the treated leaf, and 1% to the roots 96 HAT. Interestingly, 14C-clopyralid metabolism was reported to take place as early as 2 HAT in the same study. These data indicate the absorption, translocation, and metabolism of synthetic auxin compounds can vary depending on plant species.
Off-target plant injury through contaminated compost and livestock manure has been linked to synthetic auxin compounds remaining in previously treated Poaceae spp. plant material (Bahe and Peacock 1995; Blewett et al. 2005; Burkhart and Davitt 2002; Davis et al. 2010; Miltner et al. 2003; Vandervoort et al. 1997). Bahe and Peacock (1995) reported tall fescue [Lolium arundinaceum (Schreb.) S.J. Darbyshire] clippings previous treated with 2,4-D+dicamba+MCPP (5 kg ai ha$^{-1}$) used as a gardening mulch reduced growth dry mass of cucumber (Cucumis sativus L.), tomato (Lycopersicon esculentum L.), marigold (Tagetes tenuifolia Cav.), and salvia (Salvia splendens F.) by 80, 73, 65, and 34%, respectively, compared to controls. Clopyralid gained public attention as off-target plant injury was reported from compost containing previously treated turfgrass clippings (Blewett et al. 2005; Burkhart and Davitt 2002). Miltner et al. (2003) studied the effect of mowing on clopyralid concentration within turfgrass clippings and concluded mowing did not reduce herbicide concentrations to levels acceptable for composting. Furthermore, the authors reported a waiting period of greater than 1 yr following a clopyralid application could be necessary for treated turfgrass to be used as compost feedstock. Kates (1965) first documented off-target synthetic auxin injury to tobacco (Nicotiana tabacum L.) from livestock manure, in which mules used for field cultivation had grazed on a right-of-way previously treated with picloram and then defecated in the furrows prior to transplanting. Recently aminopyralid injury was reported in gardens fertilized with contaminated manure from which livestock had grazed on previously treated pastures (Davis et al. 2010). These reports indicate synthetic auxin residues can be persistent in Poaceae spp. following applications and become bioavailable as plant materials decompose.
Aminocyclopyrachlor [(AMCP); 6-amino-5-chloro-2-cyclopropyl-4-pyrimidineneboxylic acid] is a newly developed synthetic auxin herbicide belonging to the pyrimidine carboxylic acid herbicide family with similar mode of action and chemical structure to pyridine herbicides (Claus et al. 2008; Senseman 2007). Research has indicated AMCP has low mammalian toxicity, minimal volatility potential, and broad spectrum weed control at low application rates (70 to 315 g ae ha\(^{-1}\)) due to foliar and root absorption (Claus et al. 2008; Finkelstein et al. 2008; Strachan et al. 2010). These attributes suggest AMCP could become widely utilized for broadleaf weed and brush control in non-cropland, pasture, rangeland, right-of-way, and turfgrass systems (Bukun et al. 2010; Claus et al. 2008; Curtis et al. 2009; Gannon et al. 2009; Roten and Richardson 2009). Developed by DuPont Crop Protection, AMCP received initial registration for broadleaf weed control in commercial and residential turfgrass in 2010 under the trade name Imprelis\(^{®}\) and other markets are currently being pursued (Anonymous 2010; Finkelstein et al. 2008). Physicochemical and environmental properties indicate AMCP is highly water soluble (\(K_s = 4,200\) mg L\(^{-1}\)), poorly adsorbed to soil organic matter (\(K_{oc} = 28\) mL g\(^{-1}\)), and fairly persistent in turfgrass systems (\(T_{1/2} = 37\) to 103 d) (Claus et al. 2008; Finkelstein et al. 2008). While these properties suggest leaching potential, this risk is thought to be negated due to low application rates (Claus et al. 2008; Finkelstein et al. 2008; USEPA 2010).

AMCP has the potential to be widely utilized in turfgrass systems as an alternative to phenoxyacetic and pyridine compounds. Management practices in typical turfgrass systems require multiple mowing events per week and can result in turfgrass clipping accumulation (Beard 1973). To avoid off-target plant injury, label recommendations suggest turfgrass
clippings must be returned following a mowing event if previously treated with AMCP (Anonymous 2010). Due to past issues with synthetic auxin bioavailability in turfgrass clippings, it is critical to understand the fate of AMCP in turfgrass to avoid potential off-target plant injury from clipping displacement. To date, no published research has determined the fate of AMCP in tolerant monocotyledonous plants. Therefore, research was conducted to characterize the absorption, translocation, and metabolism of AMCP in tall fescue.

**Materials and Methods**

**Plant Material**

Research was conducted in 2011 at North Carolina State University in Raleigh, NC to characterize the absorption, translocation, and metabolism of $^{14}$C-AMCP in tall fescue. ‘Confederate’ tall fescue was seeded in 66 ml containers (2.5 cm diameter by 16 cm depth) filled with a sand medium and thinned to one plant per container following germination. Tall fescue seedlings were placed into a greenhouse at 24C/15C day/night temperatures with 12-h photoperiod (490 µE m$^{-2}$ s$^{-1}$ photosynthetic photon flux density at plant height) until establishment. Plants were irrigated once daily and fertilized weekly with a foliar applied 20-20-20 water soluble-fertilizer to provide 1.2 g N-P-K m$^{-2}$. Tall fescue remained in the greenhouse until reaching the 5 to 7 leaf stage (single tiller) then acclimated to a laboratory growth chamber for 3 d prior to experiment initiation.

Following acclimation, the third leaf from the crown on each tall fescue plant was covered with aluminum foil and plants were moved outdoors where the remaining foliage was oversprayed with commercially formulated AMCP at 79 g ae ha$^{-1}$ plus NIS at 0.25%
v/v using a CO₂-pressurized sprayer boom⁶ equipped four TeeJet 8002 XR flat fan nozzles⁷ on 24 cm spacings calibrated to deliver 304 L ha⁻¹ at 224 kPa. Following foliar overspray, plants were immediately returned to the laboratory and five 1 µl droplets of radiolabeled ¹⁴C-AMCP⁸ plus NIS at 0.25% v/v were applied to the foil-covered leaf on the adaxial surface for a total of 4.2 kBq per plant.

**Absorption, Translocation, and Root Exudation**

Plants were harvested 3, 12, 24, 48, 96, and 192 HAT and separated into treated leaf, treated leaf wash, remaining aboveground foliage, crown, roots, and root exudates. The treated leaf was excised into a 20 mL scintillation vial containing 10 mL 50:50 v/v methanol/deionized water and shaken by hand for 1 min to remove any unabsorbed ¹⁴C-AMCP from the leaf surface. Radioactive recovery from the leaf wash was determined by placing a 2mL aliquot of leaf wash solution into 15mL scintillation fluid⁹ and subjected to liquid scintillation spectroscopy¹⁰ (LSS) to calculate total absorption relative to percentage of total applied radioactivity. Following the leaf wash, the treated leaf was removed from the scintillation vial and plants were further dissected into remaining aboveground foliage, crown, and roots. All plant material was oven dried for 48 h at 65 C. Dried plant samples were weighed, combusted using a biological oxidizer¹¹ to collect ¹⁴CO₂ in 15mL of ¹⁴C cocktail¹², and quantified using LSS. Root exudation was determined by dissecting container cells to remove intact root system. Sand medium was removed from the roots by suspension into 130 mL deionized water. Remaining growth media in the container cells were rinsed with 130 mL methanol into shaker jars¹³. Root wash solution was combined with growth media solution for a total of 260 mL 50:50 v/v methanol/deionized water solution. Growth
solution was shaken, allowed to settle for 2 min, and then a 2mL aliquot solution was combined with scintillation fluid for quantifying total $^{14}$C radioactivity using LSS. These methods are similar to previously described research evaluating AMCP absorption and translocation in Canada thistle (Bukun et al. 2010).

Experimental design was a randomized complete block in a six by six factorial arrangement (six plant parts by six harvest periods) with three replications and two experimental runs. Percent absorption of $^{14}$C was calculated by total amount of radioactivity applied minus radioactivity recovered in the leaf wash. Translocation was calculated by measuring the total amount of radioactivity in previously mentioned plant samples (other than treated leaf) and divided by total amount of radioactivity applied.

**Metabolism**

Only the treated leaf, foliage, and crown were used to analyze $^{14}$C-AMCP metabolism. Plant samples were oven dried at 60 C for 48 h and then homogenized by placing plant material in a 1.5 mL micro-sampling vial with 0.1 g glass beads and 1 mL 90:10 v/v methanol/water plus 0.05% v/v formic acid solution. Plant material was pulverized using a dental capsule mixer$^{14}$ at 4,500 rpm for 60 seconds. Following maceration, homogenized plant material was then centrifuged for 5 min at 15,000 rpm and supernatants were transferred to a 10 mL auto-sampler vials. The previously mentioned processes were conducted three times per sample to collect sufficient supernatant. Remaining glass beads and pelleted plant material were oxidized and radiation quantified by LSS using previously described methods.
Supernatant samples were subjected to high-performance liquid chromatography (HPLC) to determine $^{14}$C-AMCP metabolism. The HPLC system included a gradient pump, UV absorbance detector, and a C18 column solid phase coupled with a $^{14}$C radiation detector. The samples were separated with a gradient, with mobile phases consisting of (A) 99.9% ultra-pure water : 0.01% acetonitrile plus 0.05% formic acid (v/v), (B) 50% ultra-pure water : 50% acetonitrile plus 0.05% formic acid (v/v). The separation program included: 10 min column equilibration with A; followed by a linear gradient for 10 min from 100% A to 100% B; then further elution with B for 10 minutes; and a column wash with 100% methanol for 10 min. Experimental design was identical to those described previously for absorption and translocation. Chromatograms from extracted samples were compared to those produced from analytical $^{14}$C-AMCP standards to determine if metabolism had occurred.

**Data Analysis**

ANOVA was conducted using mixed model methodology (SAS 2004). Harvest period and plant sample were considered fixed variables in the model. Harvest period by plant sample was evaluated to determine if an interaction between the main effects was present. Replication, experimental run, and their interaction were considered random effects (Carmer et al. 1989). Treatment means were separated using Fisher’s Protected LSD ($P \leq 0.05$). Absorption and translocation means were plotted using nonlinear regression in SigmaPlot to illustrate the effect of harvest period and plant sample on $^{14}$C-AMCP concentrations within tall fescue.
Results and Discussion

Absorption and Translocation

Harvest period main effect was non-significant ($P > 0.05$), indicating $^{14}$C-AMCP levels were homogeneous at all harvest timings. Percent $^{14}$C-AMCP recovery at 3, 12, 24, 48, 96, and 192 HAT was 86, 89, 95, 99, 97 and 83%, respectively, when radioactivity detected from the leaf wash was combined with radioactivity found within each plant part (Table 1). This recovery is well within acceptable levels and comparable to past research examining synthetic auxin absorption and translocation (Bukun et al. 2009; Bukun et al. 2010).

Plant part main effect was significant ($P < 0.01$) on $^{14}$C-AMCP radiation when pooled over harvest period (Figure 1). Leaf wash, treated leaf, and foliage contained 36, 30, and 19%, respectively, of applied $^{14}$C-AMCP. The crown, roots, and root exudates contained only 3, 1, and 3%, respectively. These results indicate the majority of AMCP can remain unabsorbed on tall fescue leaves or within the aboveground foliage during a 192 hour time period.

Rapid foliar absorption of $^{14}$C-AMCP was noted, as 38% radioactivity was detected in tall fescue 3 HAT (Figure 2). Maximum absorption (68%) was achieved 48 HAT and remained unchanged up to 192 HAT. The rate of absorption observed in tall fescue is similar to previous research with AMCP, aminopyralid, and clopyralid in Canada thistle, which reached maximum at 24, 24, and 96 HAT, respectively, and total absorption ranged from 56 to 80% (Bukun et al. 2009; Bukun et al. 2010). Converse to foliar absorption, $^{14}$C-AMCP in
the leaf wash reached minimum levels 48 HAT and remained unchanged until 192 HAT (Figure 2).

Translocated $^{14}$C-AMCP from the treated leaf attained highest concentration within foliage (34%) 96 HAT (Figure 3). Bukun et al. (2009 and 2010) also reported similar foliar translocation of $^{14}$C-AMCP, $^{14}$C-aminopyralid, and $^{14}$C-clopyralid in Canada thistle. Minimal $^{14}$C-AMCP translocation was detected in the tall fescue crown, roots, or root exudates over the 192 hour time period and did not follow a similar regression pattern compared to foliage translocation. Interestingly, $^{14}$C-AMCP detected in root exudates reached an 8% maximum 48 HAT but was $\leq$ 3% at all other harvest periods. Past research has also indicated minimal translocation of synthetic auxin herbicides to belowground plant tissue (Bukun et al. 2009; Bukun et al. 2010; Lym and Moxness 1989).

A significant interaction between harvest period and plant part main effects ($P < 0.01$) was apparent (Table 1). In general, $^{14}$C-AMCP recoveries were greater in the leaf wash, treated leaf, and foliage than levels detected in the crown, roots, and root exudates throughout the 192 time period. At 3 HAT the leaf wash and treated leaf contained 49 and 25% $^{14}$C-AMCP, respectively, whereas recovery was $\leq$ 5% in the foliage, crown, roots, and root exudates. No differences in $^{14}$C-AMCP were detected between leaf wash and treated leaf 12 and 24 HAT but were greater than radioactivity recovered within foliage at the same respective time periods. At 48 HAT the leaf wash, treated leaf, and foliage contained 32, 35, and 21% $^{14}$C-AMCP. No differences were apparent between $^{14}$C-AMCP concentrations in the leaf wash, treated leaf, and foliage 96 or 192 HAT.
Metabolism

HPLC analysis determined $^{14}$C-AMCP remained intact as the parent compound for the duration of the 192 hour time period with no formation of metabolites (Figure 4). Pelleted plant material contained $\leq 2\%$ of $^{14}$C-AMCP, indicating sample extraction methods were valid. Similarly, Bukun et al. (2010) and Roten (2011) reported no metabolism of AMCP in Canada thistle or loblolly pine (*Pinus taeda* L.) at 48 HAT. Other research has indicated minimal metabolism of pyridine carboxylic acids in susceptible plants (Bukun et al. 2009; Lym and Moxness 1989; Orfanedes et al. 1993). As previously mentioned, the chemical structure of AMCP is very similar to pyridine herbicides so it is not unexpected metabolism did not occur in tall fescue.

Research Implications

AMCP has potential to be widely adopted by turfgrass managers for broadleaf weed control. Research has indicated similar synthetic auxin compounds can be persistent in turfgrass clippings following applications (Bahe and Peacock 1995; Blewett et al. 2005; Burkhart and Davitt 2002; Fauci et al. 2002; Miltner et al. 2003; Vandervoort et al. 1997). Many synthetic auxin herbicide labels, including AMCP, suggest returning turfgrass clippings if the turfgrass stand has been previously treated (Anonymous 2008a; Anonymous 2008b; Anonymous 2010). Due to the high solubility, low soil adsorption, and relatively persistent half-life of AMCP in turfgrass systems, unabsorbed herbicide could potentially resuspend from tall fescues leaves following irrigation or rainfall events and relocate into the soil profile where it could become bioavailable (Claus et al. 2008; Finkelstein et al. 2008). Furthermore, the aboveground foliage (including treated leaf) contained considerable AMCP
and returning previously treated turfgrass clippings following a mowing event could release herbicide residues into the soil as clippings decompose. Since AMCP is foliar and root absorbed, it is probable tall fescue could reabsorb herbicide from the soil profile and translocate it back to the aboveground foliage. Future research should investigate this proposed ‘cyclic’ AMCP movement within tall fescue turfgrass systems, as it may contribute to the longevity associated with synthetic auxin residues in turfgrass clippings (Miltner et al. 2003; Vandervoort et al. 1997).

Review of published literature suggests this is the first research characterizing $^{14}$C-AMCP absorption, translocation, and metabolism in a tolerant Poaceae spp. The findings could further be extrapolated to pasture and rangeland systems containing tall fescue, where DuPont Crop Protection is currently seeking AMCP registration (Claus et al. 2008; Finkelstein et al. 2008). Regardless of system, results presented in this study indicate the majority of AMCP will remain unabsorbed on the tall fescue leaf surface or readily absorb/translocated within aboveground foliage where it remains unmetabolized. Herbicide applicators should be aware of AMCP persistence in tall fescue and manage treated plant material in a manner which avoids off-target plant injury.
Sources of Materials

1 ‘Confederate’ tall fescue seed, Wyatt-Quarles Seed, Garner, NC 27529-3559.

2 RLC4 Pine Ray Leach Cone-tainers, Stuewe and Sons, Inc., Corvallis, OR 97333.

3 Peters Professional 20-20-20 Water Soluble Fertilizer; Scotts-Sierra Horticultural Products Co., Marysville, OH 43041.


5 Induce® surfactant, Helena Chemical Co., Memphis, TN 38137.

6 CO₂-pressurized sprayer, Spraying Systems Co., Wheaton, IL 60189-7900.

7 Teejet nozzles, TeeJet Technologies, Springfield, IL 62703.

8 Radiolabeled aminocyclopyrachlor herbicide, E. I. DuPont de Nemours, Wilmington, DE 19805-1523.

9 Ultima Gold LLT (6013371), PerkinElmer Life and Analytical Sciences, Inc., Waltham, MA 02451.


11 OX-500 Biological Material Oxidizer, R. J. Harvey Instrument Co., Tappan, NY 10983.

12 Carbon 14 cocktail, R. J. Harvey Instrument Co., Tappan, NY 10983.
13 Ball® 16 oz canning jar, Jarden Home Brands, Daleville, IN 47334.

14 Silamat S5, Ivacar Vivadent, Amherst, NY 14228.

15 Hitachi L-6200A Intelligent Pump, Hitachi, Ltd., Chiyoda-ku, Tokyo 100-8280.

16 Waters 486 Tunable Absorbance Detector. Waters Corp., Milford, MA, 01757.


18 Flo-one® Beta Radiomatic Flow Scintillation Analyzerb Packard Instrument Co., Downers Grove, IL 60515.


20 Sigma Plot, Systat Software Inc., San Jose, CA 95110.
Acknowledgements

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Literature Cited


Table 1: Interaction of plant part and hour after treatment main effects on percent $^{14}$C-aminocyclopyrachlor recovered in tall fescue$^{ab}$.

<table>
<thead>
<tr>
<th>Hours After Treatment</th>
<th>Leaf Wash$^d$</th>
<th>Treated Leaf</th>
<th>Foliage</th>
<th>Crown</th>
<th>Roots</th>
<th>Root Exudates</th>
<th>Total Recovery$^e$</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>49 A</td>
<td>25 EFG</td>
<td>5 IJ</td>
<td>4 IJ</td>
<td>0 J</td>
<td>3 J</td>
<td>86</td>
</tr>
<tr>
<td>12</td>
<td>38 ABC</td>
<td>35 BCDE</td>
<td>11 HIJ</td>
<td>4 IJ</td>
<td>0 J</td>
<td>1 J</td>
<td>89</td>
</tr>
<tr>
<td>24</td>
<td>37 BCD</td>
<td>41 AB</td>
<td>14 GHI</td>
<td>3 J</td>
<td>0 J</td>
<td>0 J</td>
<td>95</td>
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<tr>
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<td>32 BCDEF</td>
<td>35 BCDE</td>
<td>21 FGH</td>
<td>2 J</td>
<td>1 J</td>
<td>8 J</td>
<td>99</td>
</tr>
<tr>
<td>96</td>
<td>29 CDEF</td>
<td>30 BCDEF</td>
<td>34 BCDE</td>
<td>2 J</td>
<td>1 J</td>
<td>1 J</td>
<td>97</td>
</tr>
<tr>
<td>192</td>
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<td>26 DEFG</td>
<td>25 EFG</td>
<td>1 J</td>
<td>2 J</td>
<td>3 J</td>
<td>83</td>
</tr>
</tbody>
</table>

$^a$ Pooled analysis over two experimental runs.

$^b$ Means within rows and columns with same letter (A-J) are not significantly different according to Fisher’s Protected LSD (P<0.01).

$^c$ Based on 4.2 kBq $^{14}$C-aminocyclopyrachlor per plant.

$^d$ Treated leaf washed in 50:50 v/v methanol/deionized water solution.

$^e$ Total recovery based on sum of radiation recovered in each plant part at corresponding harvest period.
Figure 1: Main effect of plant part on percent $^{14}$C-aminocyclopyrachlor concentration pooled over 192 hour time period.
Figure 2: Percent $^{14}$C-aminocyclopyrachlor absorbed in tall fescue and remaining in leaf wash over a 192 hour time period based on the amount of radioactivity applied. Data points are means and standard errors. Absorption regression based on nonlinear equation $y = 63.9 \left(1 - e^{-0.7x}\right)^{0.3}$. Leaf wash based on nonlinear equation $y = \frac{[11.2 + (100 - 11.2)]}{[1 + \left(x/1.1\right)^{0.3}]}$. 
Figure 3: Percent $^{14}$C-aminocyclopyrachlor translocated in tall fescue plant parts over a 192 hour time period based on the amount of radioactivity applied. Data points are means and standard errors. Foliage regression based on nonlinear equation $y = 3.5 (1 - e^{-3.3x})$. 
Figure 4: Chromatograms from reverse-phase high performance liquid chromatography (HPLC) on (A.) analytical $^{14}$C-aminocyclopyrachlor standard and (B.) $^{14}$C-aminocyclopyrachlor extracts from tall fescue plants 192 hours after treatment.
EFFECT OF SIMULATED AMINOCYCLOPYRACHLOR DRIFT ON FLUE-CURED TOBACCO
D.F. Lewis, S.T. Hoyle, L.R. Fisher, F.H. Yelverton, and R.J. Richardson*

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Flue-cured tobacco is sensitive to foliar and soil residues of off-target synthetic auxin drift. Aminocyclopyrachlor is a newly developed synthetic auxin herbicide that may be utilized in right-of-way applications for broadleaf weed and brush control. Aminocyclopyrachlor is considered a reduced risk alternative in rights-of-way compared to similar compounds due to its low application rate and volatility risk. However, no research is available on the response of field grown flue-cured tobacco to aminocyclopyrachlor drift exposure. Research was conducted in 2009 and 2010 at the Border Belt Tobacco Research Station in Whiteville, NC to determine the response of ‘NC 71’ flue-cured tobacco to five aminocyclopyrachlor (0.31, 1.6, 3.1, 15.7, and 31.4 g ae ha\(^{-1}\)) and one aminopyralid (6.1 g ae ha\(^{-1}\)) simulated drift rates applied pre-transplant

**Nomenclature:** Aminocyclopyrachlor, 6-amino-5-chloro-2-cyclopropyl-4-pyrimidine-carboxylic acid; aminopyralid 4-amino-3,6-dichloro-2-pyridinecarboxylic acid; tobacco, *Nicotiana tabacum* L. ‘NC-71’.

**Keywords:** Off-target movement, synthetic auxin.

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incorporated, pre-transplant unincorporated, three weeks post-transplant, and six weeks post-
transplant. All herbicide rates and application timings caused significant visual tobacco
injury, ranging from slight to severe with increasing herbicide drift rates. Tobacco plant
heights and fresh weights were reduced at all application timings receiving ≥15.7 g ha⁻¹
aminocyclopyrachlor and the comparative aminopyralid rate.
Flue-cured tobacco (Nicotiana tabacum L.) is a high value crop in North Carolina. In 2009, North Carolina producers planted flue-cured tobacco on approximately 70,445 ha and generated >$730 mil. in production value, which accounted for greater than 80% of flue-cured production in the United States (Fisher et al. 2010). Tobacco fields are often located within close proximity to state-owned rights-of-way. The North Carolina Department of Transportation manages 242,000 ha of maintained rights-of-way, including approximately 127,000 km of highways, 103,000 km of secondary roads, and 6,000 km of railway lines.

Synthetic auxin herbicides are often utilized for vegetation management and weed control in rights-of-way; however, these compounds can move off-site and injure nontarget plant species (Sciumbato et al. 2004). Off-target drift movement of synthetic auxin herbicides, such as 2,4-D, aminopyralid, clopyralid, picloram, and triclopyr have been reported to injure numerous crop species, including tobacco (Fung et al. 1973; Kates 1965; Klingman and Guedez 1967; Sheets and Harrell 1986; Sheets and Worsham 1991; Yelverton et al. 1991), cotton (Hutchins 1953; Marple et al. 2007; Snipes et al. 1991), and soybean (Kelley et al. 2005; Wax et al. 1968). Potential for synthetic auxin drift in tobacco is compounded because right-of-way applications often coincide with the entire tobacco season, including spring field preparation and transplanting, and summer growth and harvest. Synthetic auxin injury to tobacco can be characterized by a loss in apical dominance and epinastic curvature of the stalk and main stem, stunted growth, calloused leaf edges, downward cupping of leaves (referred to as “hooding”), reduced lateral leaf expansion (Fung et al. 1973). Differentiating between specific synthetic auxin herbicides by examining visual
tobacco symptomology has proven difficult and can create problems with determining who is legally responsible for the off-target application (Sheets and Worsham 1991).

Research has demonstrated tobacco sensitivity to foliar exposure and soil residues from synthetic auxin drift. Kates (1965) first documented off-target movement of picloram into surface water from a utility right-of-way application. The picloram-contaminated surface water was used to irrigate tobacco fields, resulting in substantial injury and crop loss. Interestingly, Kates (1965) also reported synthetic auxin injury on tobacco associated from livestock manure degradation, in which mules used to cultivate a tobacco field had previously grazed a picloram treated right-of-way and then defecated in the furrows during field conditioning. Klingman and Guedez (1967) reported complete tobacco yield loss from ≥12.4 g ai ha⁻¹ picloram incorporated into the row ridge prior to tobacco transplant and 50% yield loss from 1.2 g ha⁻¹ picloram applied overtop shortly after transplanting. Sheets and Worsham (1991) reported minimal tobacco injury from 2,4-D (1.6 to 102.4 g ha⁻¹) and triclopyr (0.4 to 25.6 g ha⁻¹) and moderate to severe tobacco injury from dicamba (25.6 to 410 g ha⁻¹) and picloram (0.1 to 1.6 g ha⁻¹) incorporated prior to tobacco transplant. Fung et al. (1973) reported differing tobacco sensitivities to overtop 2,4-D amine applications as a function of plant maturity, with highest levels of injury occurring in less mature plants.

Residual soil carryover of synthetic auxin herbicides is dependent on herbicide physicochemical properties including field half-life (T₁/₂), ionizability (pKa), lipophicity (K₀w), portioning coefficient (Kₐ or Kₒc), and water solubility (Kₘ) (McCarty et al. 2010). Edaphic conditions including soil moisture, soil type, soil texture, pH, microbial population, and organic matter content, as well as environmental conditions, contribute to herbicide
persistence in soils (Cheng 1990). Sheets and Harrell (1986) reported picloram dissipation to be slowest in soils with coarser texture and low organic matter content, which are typical characteristics of most soils used in flue-cured tobacco production in the southeastern US. Furthermore, soil-applied picloram (25 g ha\(^{-1}\)) caused visual tobacco injury and yield reductions 4 yrs after the respective application (Sheets and Harrell 1986). Remediation techniques using activated charcoal has proven effective at limiting herbicide availability in contaminated soils (Coffey and Warren 1969; Strek et al. 1981; Yelverton et al. 1992). Yelverton et al. (1992) determined applications of activated carbon to the tobacco root zone were effective in remediating imazaquin-and chlorimuron-contaminated soils but ineffective in reducing the phytotoxic effects of dicamba.

Aminocyclopyrachlor is a newly developed synthetic auxin herbicide belonging to the pyrimidine carboxylic acid herbicide family with similar chemical structure and mode of action to the pyridine herbicides aminopyralid, clopyralid, fluroxypyr, and picloram (Claus et al. 2008; Senseman 2007). Aminocyclopyrachlor may be utilized for brush and broadleaf weed control in forestry, noncropland, pasture, rangeland, right-of-way, and turfgrass management systems. Initial reports indicate aminocyclopyrachlor has a favorable environmental profile with low use rates, low mammalian toxicity, and activity on a broad spectrum of weed species (Claus et al. 2008). Early-stage testing was conducted with the methyl-ester form of aminocyclopyrachlor; however, production has moved to the free acid form due its low volatility risk (Strachan et al. 2010). Studies have indicated field dissipation of aminocyclopyrachlor varies by management system. Terrestrial field dissipation studies in turfgrass systems had a half-life ranging from 37 to 103 d, compared to bare ground
application half-life ranging from 72 to 128 d; however, aminocyclopyrachlor was detected >365 d following application to a bare ground system, indicating its persistence can vary greatly (Claus et al. 2008; US EPA 2010).

Aminocyclopyrachlor has potential to be widely utilized in right-of-way applications. However, no research has determined the sensitivity of flue-cured tobacco to off-target aminocyclopyrachlor drift. The objective of this study was to determine the response of flue-cured tobacco to different simulated aminocyclopyrachlor drift applications at various production stages and evaluate the quality of the cured crop.

**Materials and Methods**

Research was conducted in 2009 and 2010 at the Border Belt Tobacco Research Station in Whiteville, NC on ‘NC 71’ flue-cured tobacco. The soil type was a Norfolk sandy loam (fine-loamy, siliceous, thermic Typic Paleudult). Soil pH and organic matter content ranged from 5.3 to 6.0 and 1.0 to 2.5%, respectively. Raised ridge rows were prepared on 122 cm spacings prior to herbicide applications and tobacco transplanting. Tobacco transplanting took place on April 23, 2009 and April 13, 2010. Field density was 14,573 plants ha\(^{-1}\) with plants 56 cm apart within the row. Experimental plots consisted of 22 plants in a single herbicide treated row with non-treated buffer rows on either side to reduce drift from adjacent plots. To eliminate potential for herbicide carryover, experimental plots were placed in adjacent fields in 2009 and 2010. Cultural practices, including fertilization, cultivation, pest control, topping, irrigation and other production practices were conducted following regional guidelines according to state extension recommendations over the duration of the study (Fisher 2010; Smith and Fisher 2001).
Aminocyclopyrachlor\textsuperscript{1} was applied at 0.31, 1.6, 3.1, 15.7, and 31.4 g ae ha\textsuperscript{-1} selected as 1/1000th, 1/200th, 1/100th, 1/20th, and 1/10th, respectively, of the suggested maximum use rate (315 g ha\textsuperscript{-1}) for right-of-way weed control (Anonymous 2011a; Anonymous 2011b; Anonymous 2011c). Aminopyralid\textsuperscript{2} was also applied at 1/10\textsuperscript{th} of the suggested maximum use rate (6.1 g ae ha\textsuperscript{-1}) to compare with the 1/10\textsuperscript{th} rate of aminocyclopyrachlor (Anonymous 2008). All herbicide treatments were compared to a nontreated check. Application timings included: pre-transplant incorporated (PTI); pre-transplant unincorporated (PTU); post-transplant I (POST I); and post-transplant II (POST II). PTI applications were immediately incorporated to an approximate depth of 7 to 9 cm using a rotary cultivator and ridge rows were reformed. PTI and PTU applications were made three weeks before transplanting while POST I and POST II applications were made three and six weeks after transplanting, respectively. Average tobacco height at the POST I and POST II applications was 12 and 64 cm, respectively. Herbicide applications were made with a CO\textsubscript{2} pressurized spray boom\textsuperscript{3} equipped four TT 11003 TeeJet nozzles\textsuperscript{4} calibrated to deliver 187 L ha\textsuperscript{-1} and applied under calm wind conditions to further reduce drift potential.

Experimental design was a randomized complete block with four replications in a factorial arrangement (seven herbicide treatments by four application timings). Tobacco injury was visually rated on a 0 to 100\% scale (0\%=no visible injury; 100\%=complete plant death) (Table 1) and plant heights were recorded each rating date. Harvested tobacco was to be graded by a licensed U.S. Government Grader; however, it was apparent after the first run of the study no tobacco from any herbicide-treated plots would be acceptable for marketing.
due to visual injury from a non-labeled herbicide. Therefore, tobacco plants were harvested at maturity (twelve weeks after transplanting date) and fresh weights were recorded.

ANOVA (P = 0.05) was conducted using mixed model methodology (SAS 2004). Herbicide treatments and application timings were considered fixed variables in the model. Yr was considered an environmental run sampled at random, allowing for the comparison of treatment means and interactions over multiple environments (Carmer et al. 1989). Yr, replication, and interactions between these effects were considered random effects in the model. Herbicide treatment by application timing was evaluated to determine if there was an interaction. Means were subject to nonlinear regression analysis in SigmaPlot to determine the effect of aminocyclopyrachlor rate and application timing on tobacco visual injury, plant height, and fresh weight.

Results and Discussion

The ANOVA determined a significant interaction (P<0.05) between herbicide treatment and application timing; therefore, the interaction is reported rather than the main effects.

Tobacco Visual Injury.

Regarding visual flue-cured tobacco visual injury, all herbicide treatments at all application timings displayed characteristic synthetic auxin symptomology, ranging from slight downward leaf cupping to complete abortion of upper stem meristem. In general, tobacco injury increased as aminocyclopyrachlor rate increased (Table 2). Nonlinear logarithmic regression analyses indicated tobacco visual injury increased as influenced by aminocyclopyrachlor rate six and twelve weeks after transplanting (Figures 1 and 2).
Eight weeks after transplanting aminocyclopyrachlor applied at the lowest test rate (0.31 g ha\(^{-1}\)) injured tobacco <20% at all application timings, with greater injury occurring in the POST II (20%) vs. PTI (11%) application (Table 2; Figure 1). Injury ranged from 29 to 39% for all application timings receiving 1.6 g ha\(^{-1}\) of aminocyclopyrachlor, with greater injury occurring in the POST II (39%) vs. PTU (31%) and POST I (29%) application timings. POST II applications of aminocyclopyrachlor at 3.1 g ha\(^{-1}\) resulted in greater tobacco injury (56%) than all other application timings receiving the same herbicide rate. No statistical separation was apparent between application timings receiving 15.7 g ha\(^{-1}\) of aminocyclopyrachlor, with tobacco injury ranging from 68 to 75%. Aminocyclopyrachlor applied at the highest rate (31.4 g ha\(^{-1}\)) injured tobacco 71 to 88%, with greater injury in PTU (88%) and POST I (85%) vs. PTI (77%) and POST II (71%) application. Aminopyralid injured tobacco 73 to 85%, showing greater injury in POST I vs. PTU and POST II applications; however, no difference between the comparative aminocyclopyrachlor rate were observed.

At twelve weeks after transplant, tobacco injury continued to follow the same pattern of increased injury with increased aminocyclopyrachlor rate (Table 2; Figure 2). Tobacco injury ranged from 12 to 32% for application timings receiving the lowest rate of aminocyclopyrachlor (0.31 g ha\(^{-1}\)), with greater injury occurring in the POST II, PTU, and POST I vs. PTI application. Klingman and Guedez (1967) reported 4 and 13% tobacco injury following PTI and POST II applications, respectively, of a similar picloram rate (0.123 g ha\(^{-1}\); 1/1000\(^{th}\) labeled rate) in Clayton, NC; however, injury increased to 16 and 17%, respectively, for the same applications at Oxford, NC. POST I application of
aminocyclopyrachlor at 1.6 g ha\(^{-1}\) resulted in greater tobacco injury (53\%) than PTI (38\%) and PTU (35\%) application. POST II applications of aminocyclopyrachlor at 3.1 g ha\(^{-1}\) caused greater tobacco injury (57\%) than PTI (46\%) and PTU (40\%) applications. Klingman and Guedez (1967) reported 54 and 43\% tobacco injury from a similar picloram (1.2 g ha\(^{-1}\); 1/100\(^{th}\) labeled rate) PTI and POST II application timings at the Oxford location, but \(\leq 13\%\) injury for applications at the Clayton location. Aminocyclopyrachlor applied at 15.7 g ha\(^{-1}\) resulted in 64 to 80\% tobacco injury, with greater injury in POST I vs. PTI applications. Similarly, picloram (6.2 g ha\(^{-1}\)) applied POST I caused greater tobacco injury (63\%) than PTI applications (35\%) (Klingman and Guedez 1967). No statistical separation was apparent between application timings receiving the highest rate of aminocyclopyrachlor (31.4 g ha\(^{-1}\)), with injury ranging from 79 to 87\%. Similarly, picloram (12.4 g ha\(^{-1}\); 1/10\(^{th}\) labeled rate) applied PTI injured tobacco 79\% at the Oxford location (Klingman and Guedez 1967).

POST I and POST II aminopyralid applications had greater tobacco injury than PTI and PTU applications. Interestingly, PTI and PTU applications of aminopyralid had less tobacco injury than the same application timings made with the comparative rate of aminocyclopyrachlor. Sheets and Worsham (1991) reported mild tobacco injury from soil-applied picloram (0.025 g ha\(^{-1}\)) and dicamba (6.4 g ha\(^{-1}\)) shortly after transplant; however, symptoms increased from moderate to severe as rates increased to 0.1, 0.4, and 1.6 g ha\(^{-1}\) of picloram and 25.6, 102, and 410 g ha\(^{-1}\) of dicamba. Yelverton et al. (1992) also reported significant tobacco stunting from PTI applications of dicamba applied at 300 and 600 g ha\(^{-1}\).
**Tobacco Plant Height.**

Flue-cured tobacco plant height decreased with increasing aminocyclopyrachlor rate eight and twelve weeks after transplanting (Table 2). Nonlinear polynomial regression analysis confirmed tobacco plant height decreased with increasing aminocyclopyrachlor rate (Figure 3 and 4).

Eight weeks after transplanting, no differences were observed in tobacco plant heights between the plots receiving aminocyclopyrachlor at 0.31, 1.6, and 3.1 g ha⁻¹ and the nontreated (69 cm), except for POST II applications at 3.1 g ha⁻¹ (Table 2; Figure 3). Aminocyclopyrachlor applied at 15.7 g ha⁻¹ reduced tobacco plant heights, ranging from 23 to 45 cm with greater heights in PTI vs. POST I and POST II applications. No statistical separation was apparent between application timings receiving the highest rate of aminocyclopyrachlor (31.4 g ha⁻¹), with tobacco plant height ranging from 15 to 21 cm. Aminopyralid reduced tobacco plant height to 13 to 25 cm, with greater plant heights in PTI vs. POST I applications; however, no differences were observed between the comparative aminocyclopyrachlor rate.

Twelve weeks after transplanting, no differences were observed in tobacco plant heights between the plots receiving aminocyclopyrachlor at 0.31, 1.6, and 3.1 g ha⁻¹ and the nontreated (79 cm), except for POST II applications at 3.1 g ha⁻¹ (Table 2; Figure 4). This is congruent with work of Klingman and Guedez (1967), who also reported no reduction in tobacco plant height following similar picloram rates and application timings. Aminocyclopyrachlor at 15.7 g ha⁻¹ reduced tobacco plant heights, ranging from 20 to 45 cm with greater heights in PTI vs. PTU and POST I applications. These results differ from
similar picloram applications, which did not significantly reduce tobacco plant height compared to the nontreated (Klingman and Guedez 1967). No statistical separation was apparent between application timings receiving the highest aminocyclopyrachlor rate (31.4 g ha\(^{-1}\)) and comparative aminopyralid rate, with tobacco plant height ranging from 12 to 19 cm.

**Tobacco Fresh Weight.**

Flue-cured tobacco fresh weight decreased as aminocyclopyrachlor rate increased from 3.1 to \(\geq 15.7\) g ha\(^{-1}\). Nonlinear polynomial regression analyses verified tobacco fresh weight decreased with increasing aminocyclopyrachlor rate (Figure 5).

Twelve weeks after transplanting, no differences were observed in tobacco fresh weight between the plots receiving aminocyclopyrachlor at 0.31, 1.6, and 3.1 g ha\(^{-1}\) and the nontreated (1215 g) (Table 2; Figure 5). Aminocyclopyrachlor (15.7 g ha\(^{-1}\)) applied PTI had greater tobacco fresh weight (895 g) than POST II (625 g) and POST I (285 g) applications. At the highest aminocyclopyrachlor rate (31.4 g ha\(^{-1}\)), tobacco fresh weight ranged from 165 to 470 g, with the POST II having the greatest fresh weight. Aminopyralid had the broadest range of fresh weight reduction (195 to 890 g), with greater fresh weight in PTI vs. POST I application. Klingman and Guedez (1967) reported picloram applied PTI (0.1 to 6.2 g ha\(^{-1}\)) and POST II (0.1 to 1.2 g ha\(^{-1}\)) did not reduce tobacco yield; however, complete yield loss occurred from 12.4 g ha\(^{-1}\) of picloram applied PTI. Yelverton et al. (1992) also reported reduced tobacco fresh weight and harvest yield from PTI applications of dicamba (600 g ha\(^{-1}\)).

**Research Implications**

Based upon results, aminocyclopyrachlor is injurious to flue-cured tobacco regardless of evaluated herbicide rate or application timing. While tobacco plant heights and fresh
weights were not reduced by all applications, visible herbicide injury was apparent from all herbicide treatments thereby causing a total yield loss due to a nonmarketable crop. When comparing aminocyclopyrachlor and aminopyralid applications on a percentage of labeled rate (1/10th), no differences were observed at POST I and POST II applications but significantly less visual injury and greater fresh weights were observed from aminopyralid PTI and PTU applications. This may indicate aminopyralid is less persistent in soils, likely to shorter field half-life than aminocyclopyrachlor (Senseman 2007). However, comparing both compounds by amount of applied active ingredient indicates aminopyralid may be as or more active on tobacco than aminocyclopyrachlor. In recent yrs, applicators have been forced to pay severance to tobacco producers who lost crops due to off-target synthetic auxin drift from right-of-way applications. While it is difficult to distinguish specific synthetic auxin herbicides based on tobacco injury, analytical laboratory analyses may be able to differentiate between individual compounds. These procedures can be expensive but may provide useful information in determining fault for an off-target application. Due to the acute sensitivity of tobacco to synthetic auxin herbicides and soil-persistence of aminocyclopyrachlor, it could require multiple growing seasons for soil concentrations to reach levels where tobacco injury would not be prevalent. While this study did not measure residual carryover from aminocyclopyrachlor applications from one season to the next, research has demonstrated auxin compounds can continue to injure tobacco several yrs following the initial application, depending on soil texture, organic matter content, and herbicide rate (Sheets and Harrell 1986; Sheets and Worsham 1991). Increased organic matter concentration can result in a faster dissipation period of synthetic auxin herbicides.
(Sheets and Harrell 1986); however, annual cultivation of tobacco fields inherently reduces organic matter accumulation. Furthermore, attempts to remediate synthetic auxin contaminated soil with activated carbon amendments has been inconsistent, possibly forcing growers to abandon tobacco production until herbicide concentrations have diminished to a nontoxic level (Yelverton et al. 1992). To eliminate the potential risk for off-target synthetic auxin drift, right-of-way applicators must be cognizant of the surrounding areas and only make applications when drift conditions are not present.
Source of Materials

1 Aminocyclopyrachlor herbicide, E. I. DuPont de Nemours, Wilmington, DE 19805-1523.

2 Milestone® herbicide, Dow AgroSciences, Indianapolis, IN 46268.

3 CO2-pressurized sprayer, Spraying Systems Co., Wheaton, IL 60189-7900.

4 Teejet nozzles, TeeJet Technologies, Springfield, IL 62703.


6 Sigma Plot, Systat Software Inc., San Jose, CA 95110
Acknowledgements

The authors wish to express appreciation to the entire staff of the Border Belt Tobacco Research Station for production and maintenance of the tobacco plots and technical assistance. We also wish to acknowledge the numerous undergraduate and graduate students for their hard work and assistance. Partial funding was provided by DuPont Crop Protection and the North Carolina Tobacco Research Commission.
Literature Cited


Table 1: Visual rating guidelines for synthetic auxin injury on flue-cured tobacco.

<table>
<thead>
<tr>
<th>Percent Visual Tobacco Injury</th>
<th>Tobacco Symptomology</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10</td>
<td>No obvious symptoms to slight downward leaf cupping; no growth reduction</td>
</tr>
<tr>
<td>10-30</td>
<td>Slight to moderate leaf cupping with callous formation in younger leaf margins; slight growth reduction</td>
</tr>
<tr>
<td>30-50</td>
<td>Moderate leaf thickening and callusing with prominent 'hooding'; epinastic curvature of immature leaves; slight to moderate growth reduction</td>
</tr>
<tr>
<td>50-70</td>
<td>Moderate to severe 'hooding'; Prominent epinasty and reduced lateral leaf expansion in upper stem region; moderate to severe growth reduction</td>
</tr>
<tr>
<td>70-95</td>
<td>Severe epinasty and complete reduction of lateral leaf expansion in upper stem region to abortion of meristem; severe growth reduction</td>
</tr>
<tr>
<td>95-100</td>
<td>Near to complete plant death</td>
</tr>
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</table>
Table 2: Flue-cured tobacco visual injury, plant height, and fresh weight affected by herbicide rate and application timing\textsuperscript{a}.

<table>
<thead>
<tr>
<th>Herbicide\textsuperscript{c}</th>
<th>Rate\textsuperscript{d}</th>
<th>Application\textsuperscript{d}</th>
<th>Injury\textsuperscript{b} 8 WAT\textsuperscript{d}</th>
<th>12 WAT</th>
<th>Plant Height 8 WAT</th>
<th>12 WAT</th>
<th>Fresh Weight 12 WAT</th>
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<td></td>
<td>-</td>
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\textsuperscript{a} Pooled analysis of 2009 and 2010.

\textsuperscript{b} Evaluated visually on a 0 to 100\% scale, based on a 0\% = no injury and 100\% = complete plant death.

\textsuperscript{c} Herbicide treatments included a nonionic surfactant at 0.25\% V/V ratio and expressed as g acid equivalent hectare\textsuperscript{-1} (g ae ha\textsuperscript{-1}).

\textsuperscript{d} Abbreviations: PTI, Pre-transplant Incorporated; PTU, Pre-transplant Unincorporated; POST I, Post-transplant I (Three weeks after transplanting); POST II, Post-transplant II (Six weeks after transplanting); WAT, Weeks After Transplanting.
Figure 1: Non-linear logarithmic regression of flue-cured tobacco visual injury affected by aminocyclopyrachlor rate and application timing eight weeks after transplanting. Regression conducting using the equation $y = a \ln(x)$. 
Figure 2: Non-linear logarithmic regression of flue-cured tobacco visual injury affected by aminocyclopyrachlor rate and application timing twelve weeks after transplanting. Regression conducting using the equation $y = a \ln(x)$. 
Figure 3: Non-linear polynomial regression of flue-cured tobacco height affected by aminocyclopyrachlor rate and application timing eight weeks after transplanting. Regression conducting using the quadratic equation $y = a + bx^2$. 
Figure 4: Non-linear polynomial regression of flue-cured tobacco height affected by aminocyclopyrachlor rate and application timing twelve weeks after transplanting. Regression conducting using the quadratic equation $y = a + bx^2$. 
Figure 5: Non-linear polynomial regression of flue-cured tobacco fresh weight affected by aminocyclopyrachlor rate and application timing twelve weeks after transplanting. Regression conducting using the quadratic equation $y = a + bx^2$. 