

## Potential Damage to Sensitive Landscape Plants from Wood Chips of Aminocyclopyrachlor Damaged Trees

Aaron J. Patton, Gail E. Ruhl, Tom C. Creswell, Ping Wan, David E. Scott, Joe D. Becovitz, and Daniel V. Weisenberger\*

Applications of aminocyclopyrachlor in 2011 to turf resulted in brown and twisted shoots, leaves, and needles; shoot dieback; and in some cases, death of trees and ornamental plants adjacent to treated turf areas. Our research objective was to determine if a sensitive plant could be injured from wood chips (mulch) obtained from aminocyclopyrachlor-damaged trees, and to quantify movement of aminocyclopyrachlor from contaminated wood chips into soil and its subsequent uptake by roots into landscape plant tissues. Tomatoes were grown under greenhouse conditions and mulched with chipped tree branches collected from honey locust and Norway spruce damaged 12 mo previously by aminocyclopyrachlor. Analysis of tomato tissue for aminocyclopyrachlor residues 32 d after mulching found aminocyclopyrachlor in all mulched tomato plants, which was consistent with observations of epinasty on tomato leaflets. Aminocyclopyrachlor residues ranged from 0.5 to 8.0 ppb in tomato plants while chipped tree branches contained 1.7 to 14.7 ppb. Aminocyclopyrachlor residues in the potting soil below the mulch ranged from below the quantifiable limit to 0.63 ppb, indicating that aminocyclopyrachlor can leach from wood chips into soil, causing plant injury. These results indicate that trees damaged by aminocyclopyrachlor should not be chipped and used for mulch or as an ingredient in compost.

**Nomenclature:** Aminocyclopyrachlor; honey locust, *Gleditsia triacanthos* L.; Norway spruce, *Picea abies* (L.) Karst.; tomato, *Solanum lycopersicum* L.

**Key words:** Growth regulator, herbicide, lawn care, mulch, off-target, ornamentals, shrubs, turf.

En 2011, aplicaciones de aminocyclopyrachlor en céspedes resultó en tejido aéreo y hojas café y enrolladas, muerte del tejido aéreo, y en algunos casos, la muerte de árboles y plantas ornamentales adyacentes a las áreas tratadas en el césped. El objetivo de nuestra investigación fue determinar si una planta sensible podría ser dañada por una cobertura de chips de madera (mulch) que se obtuvo a partir de árboles dañados con aminocyclopyrachlor, y cuantificar el movimiento de aminocyclopyrachlor desde chips de madera hacia el suelo y su subsiguiente absorción por las raíces de plantas presentes en el paisaje. Plantas de tomate fueron crecidas en invernadero y con cobertura de chips hecha a partir de ramas colectadas de árboles de *Gleditsia triacanthos* y *Picea abies* dañados 12 meses antes con aminocyclopyrachlor. El análisis de aminocyclopyrachlor en el tejido de tomate 32 d después de poner la cobertura encontró aminocyclopyrachlor en todas las plantas de tomate con cobertura, lo cual fue consistente con observaciones de epinastia en las hojas de tomate. Los residuos de aminocyclopyrachlor variaron entre 0.5 y 8.0 ppb en plantas de tomate mientras que en las ramas de los árboles fue de 1.7 a 14.7 ppb. Los residuos de aminocyclopyrachlor en la mezcla de suelo de las macetas debajo de la cobertura varió desde niveles por debajo del límite de cuantificación a 0.63 ppb, indicando que aminocyclopyrachlor puede lixiviarse desde los chips de madera al suelo, causando daño en las plantas. Estos resultados indican que árboles dañados con aminocyclopyrachlor no deberían ser usados para producir coberturas o como ingrediente en compost.

Aminocyclopyrachlor (previously sold under the trade name Imprelis® for weed control in turf) is a selective growth regulator herbicide in the pyrimidine carboxylic acid class, that provides selective control of broadleaf weeds (Strachan et al. 2010). Imprelis was used by turf professionals for control of troublesome turf weeds including ground ivy (*Glechoma hederacea* L.), Virginia buttonweed (*Diodia virginiana* L.), and wild violet (*Viola sororia* Willd.) (Anonymous 2010; Gannon et al. 2009; Patton et al. 2012b). Imprelis labeled use rates were < 79 g ae ha<sup>-1</sup> (Anonymous 2010) and this low use rate

coupled with its control of troublesome weeds and low mammalian toxicity (Turner et al. 2009) made it a desirable herbicide for turf. Additionally, aminocyclopyrachlor has both foliar and soil residual activity and is absorbed by the target plant's leaves, stems, and roots (USEPA 2010). Aminocyclopyrachlor has a long soil half-life (Conklin and Lym 2013; USEPA 2010), which extends the duration of residual activity and weed control (Lindenmayer et al. 2013; Strachan et al. 2011).

Following aminocyclopyrachlor use in the fall of 2010 and spring of 2011 in turf, lawn care companies and golf courses across the U.S. began reporting damage to trees and ornamental shrubs adjacent to treated turf areas (Patton et al. 2011). Symptoms included shoot dieback and brown and twisted shoots, leaves, and needles that were most noticeable in tree tops and most severe on new growth (Figure 1). Trees with moderate to severe damage did not recover and in most cases symptoms worsened (Patton et al. 2012a) with trees dying. Aminocyclopyrachlor injury occurred rapidly, often within 2 to 4 wk of application. The most commonly

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\* First and seventh authors: Associate Professor and Research Agronomist, Department of Agronomy, Purdue University, 915 W. State Street, West Lafayette, IN 47907-2054; second and third authors: Senior Plant Disease Diagnostician and Director, Plant and Pest Diagnostic Laboratory, Department of Botany and Plant Pathology, Purdue University, 915 W. State Street, West Lafayette, IN 47907-2054; fourth, fifth, and sixth authors: Pesticide Laboratory Supervisor, Manager Pesticide Section, and Pesticide Program Specialist, Office of Indiana State Chemist, Department of Biochemistry, Purdue University, 175 S. University Street, West Lafayette, Indiana 47907-2063. Corresponding author's E-mail: ajpatton@purdue.edu



Figure 1. Injury from aminocyclopyrachlor at locations where tree samples were collected. Honey locust at location 1 with initial symptomology in July 2011 (A–B) and limited regrowth in May 2012 (C–D). Norway spruce injury at location 1 (E), location 2 (F), and location 3 (G). Photo E was taken in July 2011, photo F in June 2011, and photo G in May 2012. In photo G, notice the range of spruce injury from killed Norway spruce (left side of photo) where applications were made to turf on both sides of the tree and mild injury to Norway Spruce (right side of photo) where applications were made to turf on only one side of the tree (turf in background not visible) because of a mulched landscape bed without turf immediately in front of the trees and behind the neighborhood entrance sign. Samples were collected from damaged trees at each of these different locations and used to create the various wood chip lots for this experiment. (Color for this figure is available in the online version of this paper.)

damaged trees were Norway spruce [*Picea abies* (L.) Karst.], Colorado blue spruce (*Picea pungens* Engelm.), honey locust (*Gleditsia triacanthos* L.), and eastern white pine (*Pinus strobus* L.); however, many other tree and shrub species were also affected (Ruhl 2012).

Investigations by the Office of Indiana State Chemist (OISC) concluded that damage observed was not caused by applicator error or misapplications by the turf professionals (Patton et al. 2011) and determined that injury to trees resulted from aminocyclopyrachlor uptake by tree roots present in treated area following applications to turf according to label instructions. After tree damage was initially reported, DuPont issued a statement on June 17, 2011, that cautioned applicators: “do not apply Imprelis where Norway spruce or white pine are present on, or in close proximity to, the property to be treated” (DuPont 2011). The original herbicide label, however, did not specify this caution to applicators (Anonymous 2010; Patton et al. 2011).

The amount of landscape plant damage from aminocyclopyrachlor treatments to turf was so extensive that within

54 d of the first damage report (June 2011), the OISC issued to DuPont a stop sale, use or removal order (SSURO) of the herbicide in Indiana to halt the distribution and use of Imprelis (Patton et al. 2011). This was followed by the U.S. Environmental Protection Agency (EPA) issuing a federal SSURO 10 days later (Ferdas 2011). Aminocyclopyrachlor is no longer registered by the EPA for turf although it is registered for use in land and vegetation management (Anonymous 2013a, b, c). DuPont then implemented a voluntary suspension of sale for Imprelis and initiated a return and refund program in October 2011 and provided instructions on the disposal of damaged trees and shrubs. In addition, because of the rapid response of extension specialists in publicizing information on the detrimental effects of aminocyclopyrachlor (Patton et al. 2011), few applications occurred after June 2011.

During this process, Purdue University extension specialists were repeatedly asked how to properly remove and dispose of damaged trees and specifically whether they could be safely chipped for mulch. Although Purdue University Extension

Table 1. Treatment descriptions, tree species, coordinates of sample collection, and ppb of aminocyclopyrachlor measured in tissues of affected trees in 2011 and 2012.

| Treatment description                               | Location           | Aminocyclopyrachlor concentration <sup>a</sup> |                 |
|-----------------------------------------------------|--------------------|------------------------------------------------|-----------------|
|                                                     |                    | 2011                                           | 2012            |
|                                                     |                    | ppb                                            |                 |
| Honey locust 1                                      | 40.464°N, 86.935°W | 9.6                                            | NA <sup>b</sup> |
| Honey locust 2                                      | 40.417°N, 86.877°W | 32                                             | 4.7             |
| Honey locust 3                                      | 39.654°N, 86.139°W | 42                                             | 29              |
| Norway spruce 1                                     | 40.464°N, 86.935°W | 25                                             | 5.4             |
| Norway spruce 2                                     | 40.215°N, 86.917°W | 83                                             | 7.1             |
| Norway spruce 3                                     | 39.974°N, 85.918°W | 276                                            | 8.8             |
| Norway spruce 4                                     | 39.958°N, 85.955°W | 63                                             | 15              |
| Nontreated                                          |                    |                                                |                 |
| Norway spruce                                       | 40.396°N, 86.745°W | BDL <sup>c</sup>                               | BDL             |
| Nontreated                                          |                    |                                                |                 |
| Freeman maple                                       | 40.396°N, 86.745°W | BDL                                            | BDL             |
| Aminocyclopyrachlor at 0.015 kg ae ha <sup>-1</sup> | —                  | —                                              | —               |

<sup>a</sup> Samples collected and analyzed by the Office of the Indiana State Chemist. Samples collected in 2012 were taken at the same location as in 2011, however, not necessarily from the same affected tree or limb as the 2011 samples (Anonymous 2012).

<sup>b</sup> NA = Not sampled in 2012.

<sup>c</sup> Below Detection Limit (BDL).

and DuPont recommend that people not chip and shred (mulch) aminocyclopyrachlor-damaged trees, many landscape professionals were concerned about what might happen if they used wood chips produced from trees killed by aminocyclopyrachlor. These concerns were from previous experiences using herbicide treated lawn clippings as a mulch on ornamental plants (Branham and Lickfeldt 1997). To help answer this question, we initiated research with the objective to determine if a sensitive plant could be injured from wood chips obtained from aminocyclopyrachlor-damaged trees, and to quantify movement of aminocyclopyrachlor from contaminated wood chips into soil and its subsequent uptake by roots into plant tissues.

## Materials and Methods

A greenhouse study was conducted at Purdue University to assess the effect of wood chips from trees damaged by aminocyclopyrachlor 12 mo previously on the growth of 'Rutgers' tomato. Tomato was selected for this bioassay based on its sensitivity to growth regulator herbicides (Branham and Lickfeldt 1997; Busey et al. 2003; Gomez De Barreda 1993). Tomato plants were grown from 15 cm tall seedlings that were transplanted into 15 cm diam pots (Hummert International, Earth City, MO 63045) filled with a Whitaker silt loam (fine-loamy, mixed, active, mesic Aeric Endoaqualf) with a pH of 6.8 and 3.1% organic matter. One tomato plant was transplanted into each pot and plants were fertilized after transplanting with 29 kg ha<sup>-1</sup> nitrogen (32-10-10, Infinity Fertilizers, Inc., Milan, IL 61264) and subsequently watered as needed until initiation of the experiments.

Wood chips were obtained in May 2012 from seven trees damaged by aminocyclopyrachlor applied in May 2011 (Figure 1; Table 1). The OISC confirmed aminocyclopyra-

chlor presence in plant tissues collected in July 2011 from the outer 0.2 m of the growing points on branches of these trees with concentrations ranging from 9.6 to 270 ppb (Table 1). The OISC also detected aminocyclopyrachlor residues in the top 10 to 15 cm of lawn soils adjacent to the trees in July 2011. By May 2012, trees were completely dead or had few living branches. Two samples were collected from each of these damaged trees in May 2012; one sample was used for creating wood chips/mulch applied in these experiments and the second was sent to the OISC laboratory to be analyzed for aminocyclopyrachlor residues as part of their continuing investigation. Branches were harvested from the periphery of each of the seven trees by pruning off lengths 0.5 to 1.0 m in size. Samples from the growing tips (outer 0.2 m) were used by OISC for verification and quantification of aminocyclopyrachlor. Following collection, the larger branches were chipped by feeding branches through a chipper shredder (Yard Machines, Valley City, OH 44280) to simulate the common practice of chipping and shredding trees used by arborists following tree removal. Woods chips were approximately 2 to 4 cm in size following the shredding of branches with Norway spruce having slightly smaller particle sizes because of the presence of needles.

Branches from one healthy, living conifer (Norway spruce) and one healthy, living hardwood tree (Freeman maple, *Acer × freemanii* A.E. Murray) species where no aminocyclopyrachlor had been applied were collected as nontreated negative controls. These branches were also chipped and shredded, dried for 72 h at 60 C and used as nontreated mulch standards (Table 1). Tree samples collected from aminocyclopyrachlor-treated properties were not dried after chipping and shredding because there was little to no moisture present in the dead branches. Control pots of soil were mulched with either the Norway spruce or Freeman maple (negative control treatments). A positive control of aminocyclopyrachlor was soil applied in a single 100 ml water volume at a rate equal to 0.015 kg ae ha<sup>-1</sup> equal to 20% of the high label rate of Imprelis (Anonymous 2010). Following treatment with aminocyclopyrachlor, Norway spruce wood chips from the control treatments were applied to the surface of these pots so that all treatments were mulched.

The experiment was conducted twice with treatment applications initiated on May 11, 2012 and June 6, 2012. At the start of the first experiment, tomato plants were approximately 30 cm in height and had developed their third leaf truss. The second experiment used 20 cm tall tomato plants at their first leaf truss. An equal volume of 400 cm<sup>3</sup> of wood chips for each treatment (Table 1) was placed in each pot around the base of the tomato plants to a depth of approximately 3 cm. Plants were watered 24 h prior to application of the treatments and again following treatment application. Plants were monitored daily thereafter and watered as needed by applying 200 ml water. This volume of water provided adequate moisture with no leaching of irrigation water from the pots. Day/night temperatures and photosynthetically-active radiation (PAR) following herbicide application were collected continuously (every 1 h) with a mini-weather station (WatchDog 2475 Plant Growth Station, Spectrum Technologies, Inc., Plainfield, IL 60585) and



Figure 2. Epinasty rating used in greenhouse experiments. Epinasty was assessed on a 0 to 4 scale where 0 = no epinasty, 1 = one to two new leaflets with epinasty (leaf bending, twisting, and/or curling), 2 = three to nine new leaflets with epinasty, 3 = 10 to 20 new leaflets with epinasty, and 4 = considerable epinasty with 21 or more new leaflets displaying epinasty symptoms. (Color for this figure is available in the online version of this paper.)

averaged 31/17 C with an average daily light integral of 37 mol m<sup>-2</sup> d<sup>-1</sup> during the first experimental run and 34/20 C with an average daily light integral of 38 mol m<sup>-2</sup> d<sup>-1</sup> during the second experimental run. Relative humidity was measured and averaged 56% and 60% for the first and second experiments, respectively.

The severity of plant epinasty was assessed as a measure of symptom development approximately 20 d after the initiation of each experiment. Epinasty symptoms observed included bending and twisting of leaves. Epinasty was assessed on a 0 to 4 scale where 0 = no epinasty, 1 = one to two new leaflets with epinasty, 2 = three to nine new leaflets with epinasty, 3 = 10 to 20 new leaflets with epinasty, and 4 = considerable epinasty with 21 or more new leaflets displaying epinasty symptoms (Figure 2). Symptoms of epinasty on new growth included twisting or bending with minor strapping of leaves. Adventitious rooting (swellings protruding from stem) was assessed at the same time as epinasty on a 0 to 4 scale where 0 = no adventitious roots (smooth stem 'no swellings'), 1 = swellings slightly raised and visible on lower 5 cm of stem, 2 = swellings raised on lower 5 cm of stem, 3 = swellings raised > 5 cm up the stem, and 4 = many adventitious roots ('swellings') forming at 10 cm or higher up the stem. Thirty-

two days after the initiation of each experiment, the upper 10 cm of each plant (comprised of the leaflets exhibiting visible epinasty) was collected, frozen at -20 C, and assayed by the OISC for aminocyclopyrachlor residues. Tissues were pooled across replications for each treatment to obtain enough plant material for aminocyclopyrachlor residue analysis. One pooled soil sample of each treatment was sent to the OSIC laboratory for aminocyclopyrachlor analysis as well. Two separate wood chip samples were tested during the second experimental run. Prior to experiment initiation, a subsample of wood chips created from the branches harvested at each field location was tested for aminocyclopyrachlor residues and at the conclusion of the experiment, a second subsample of wood chips was collected from the tomato pots prior to harvesting soil. These samples were pooled within treatment, and tested for aminocyclopyrachlor residues. This analysis allowed insight regarding whether aminocyclopyrachlor leached from wood chips during the experiment.

Measurement of aminocyclopyrachlor was based on the analytical method developed by the Minnesota Department of Agriculture (Anonymous 2011) and Nanita et al. (2009) and modified by OISC to allow for accurate quantification of aminocyclopyrachlor to a lower quantification limit of 0.2

Table 2. Leaf epinasty on tomato mulched with wood chips from aminocyclopyrachlor-damaged trees, a treated aminocyclopyrachlor control, and two nontreated control treatments following growth in the greenhouse for 32 d.

|                                  | Leaf epinasty of new growth |
|----------------------------------|-----------------------------|
|                                  | 0 to 4 <sup>a</sup>         |
| Honey locust 1                   | 2.6 b <sup>b</sup>          |
| Honey locust 2                   | 2.8 b                       |
| Honey locust 3                   | 3.5 a                       |
| Norway spruce 1                  | 1.8 cd                      |
| Norway spruce 2                  | 1.5 d                       |
| Norway spruce 3                  | 1.4 d                       |
| Norway spruce 4                  | 2.3 bc                      |
| Nontreated Norway spruce         | 0.0 e                       |
| Nontreated Freeman maple         | 0.0 e                       |
| Treated with aminocyclopyrachlor | — <sup>c</sup>              |
| Treatment ( <i>P</i> -value)     | < 0.0001                    |
| Honey locust                     | 2.9 a                       |
| Norway spruce                    | 1.7 b                       |
| Species ( <i>P</i> -value)       | < 0.0001                    |

<sup>a</sup> Epinasty was assessed on a 0–4 scale where 0 = no epinasty, 1 = one to two new leaflets with epinasty, 2 = three to nine new leaflets with epinasty, 3 = 10 to 20 new leaflets with epinasty, and 4 = considerable epinasty with 21 or more new leaflets displaying epinasty symptoms (Figure 1).

<sup>b</sup> Data was combined across experimental runs. Means followed by the same letter are not significantly different according to Fisher's protected LSD ( $\alpha = 0.05$ ).

<sup>c</sup> New growth was suppressed by this treatment and no new leaves formed which prevented measurements of leaf epinasty. In both runs of the experiment, pots treated with aminocyclopyrachlor had stunted shoot growth, shoot epinasty, and fewer leaves.

ppb (tomato plant fresh weight basis), with a limit of detection (LOD) of approximately 0.06 ppb. Briefly, the technique involved assessing residues of aminocyclopyrachlor extracted from 10 g fresh tomato plant leaflets or 10 g air dried wood chips with 50 ml of acetonitrile (ACN)/0.2% formic acid 70 : 30 (v/v) by sonicating for 10 min and shaking at high speed for 30 min. Aliquots (10.0 ml) of the extracts were evaporated to 3 ml using a nitrogen evaporator and then diluted with 0.5% formic acid (aq) to 8 ml. Samples were loaded onto an Envi-Carb SPE cartridge and washed with 3 ml of water. The analytes were eluted with 10 ml of 10 mM ammonium acetate (aq) in methanol into tubes containing 50  $\mu$ l of 0.2% formic acid (aq). Samples were evaporated to 1 ml using a nitrogen evaporator with the water bath set at 40 C and then diluted to 5.0 ml with 0.01% formic acid (aq), filtered through a 0.45- $\mu$ m PTFE filter and analyzed by UPLC/MS/MS.

Soil samples were measured using a similar procedure as described above. The limit of quantification (LOQ) was 0.04 ppb (soil wet weight basis) and the LOD was approximately 0.01 ppb. Residues of aminocyclopyrachlor were extracted from 10 g soil samples as described above. Extract aliquots (10 ml) were evaporated to 1 ml using a nitrogen evaporator as above, then diluted with 1 ml methanol and 4.0 ml of 0.01% formic acid (aq), filtered through a 0.45- $\mu$ m Nylon filter and analyzed by UPLC/MS/MS.

Each experiment run was arranged on the greenhouse bench as a randomized complete block design with four replications. Leaf epinasty data were subjected to a combined analysis of variance across experimental runs as variances were

homogeneous between experiments and there was no experimental run-by-treatment interaction. Data were analyzed using PROC GLM, REG, and TTEST (SAS v. 9.2, SAS Institute Inc., Cary, NC) and means were separated with a Fisher's protected least significant difference (LSD,  $\alpha = 0.05$ ) when *P*-values < 0.05 (Saxton 1998). Experiment was considered a fixed variable and treatments considered a random variable during the analysis of variance and the appropriate F-tests were conducted (McIntosh 1983). Statistical analysis was not completed on residues from wood chips, tomato, or soil, since these samples were pooled across replication.

## Results and Discussion

Aminocyclopyrachlor was confirmed in these 2012 experiments in tree branches and soil collected from the same field locations of initial detection in 2011 (Table 1). Overall, plant tissue and soil residues decreased from 2011 to 2012 (Table 1) (Anonymous 2012).

Leaf epinasty was observed on all tomato plants mulched with wood chips from aminocyclopyrachlor-damaged honey locust and Norway spruce treatments while no visible epinasty occurred on tomato plants mulched with nontreated Norway spruce or Freeman maple wood chip negative controls (Table 2). Epinasty was first observed 19 days after treatment in experiment one and 10 days after treatment in experiment two. There was a correlation ( $r = 0.70$ ,  $P = 0.08$ ) between observed epinasty (Table 2) and aminocyclopyrachlor residues in wood chips (Table 3). Tomato plants mulched with aminocyclopyrachlor affected honey locust wood chips had more epinasty than plants mulched with aminocyclopyrachlor affected Norway spruce wood chips (Table 2). New growth was suppressed where aminocyclopyrachlor (positive control) was soil applied and accurate leaf epinasty measurements were unable to be obtained as too few leaves formed. In both experiments, positive control plants with soil applied aminocyclopyrachlor had stunted shoot growth, stunted root growth, and fewer leaves than all other treatments, and these were the only plants that produced adventitious roots at the base of the stem (data not shown).

Laboratory analysis of tomato tissues confirmed that aminocyclopyrachlor was present in all tomato plants mulched with wood chips from aminocyclopyrachlor-damaged honey locust and Norway spruce trees, which was consistent with observations of epinasty. Aminocyclopyrachlor residues ranged from 0.5 to 8.0 ppb in tomato plants (Table 3). Treatments with more leaf epinasty (Table 2) generally had higher aminocyclopyrachlor residues (Table 3) in tomato tissues ( $R^2 = 0.31$ ,  $P = 0.038$ ). The amount of aminocyclopyrachlor quantified in tomato plants was greater in the second run of the experiment. In the case of plants mulched with wood chips from aminocyclopyrachlor-damaged trees, growth regulator-type herbicide symptoms were visible in tomato leaflets containing as little as 0.5 ppb of aminocyclopyrachlor.

Both the Purdue University sample collections consisting of larger branches (0.5 to 1.0 m) and OISC samples that consisted of smaller, apical portions of branches (0.2 m)

Table 3. Aminocyclopyrachlor residues at 32 d after treatment in the top 10 cm of foliage from greenhouse grown tomato plants, wood chips from aminocyclopyrachlor-damaged honey locust and Norway spruce trees, and soil in which tomato plants were grown.

| Treatment description            | Aminocyclopyrachlor concentration |       |                                 |                      |                   |       |
|----------------------------------|-----------------------------------|-------|---------------------------------|----------------------|-------------------|-------|
|                                  | Tomato                            |       | Mulch (wood chips) <sup>a</sup> |                      | Soil              |       |
|                                  | Run 1                             | Run 2 | Before planting tomatoes        | After tomato harvest | Run 1             | Run 2 |
|                                  | ppb                               |       |                                 |                      |                   |       |
| Honey locust 1                   | 1.2                               | 1.2   | 2.0                             | 2.1                  | 0.05              | 0.07  |
| Honey locust 2                   | 1.9                               | 5.3   | 6.1                             | 6.1                  | 0.20              | 0.22  |
| Honey locust 3                   | 2.4                               | 8.0   | 14.7                            | 13.3                 | 0.52              | 0.63  |
| Norway spruce 1                  | 2.9                               | 4.2   | 8.9                             | 6.0                  | 0.20              | 0.16  |
| Norway spruce 2                  | 0.6                               | 1.5   | 1.7                             | 2.0                  | DBQL <sup>b</sup> | DBQL  |
| Norway spruce 3                  | 1.0                               | 5.9   | 2.0                             | 4.2                  | 0.08              | 0.15  |
| Norway spruce 4                  | 0.5                               | 2.3   | 3.2                             | 3.5                  | 0.07              | 0.11  |
| Nontreated Norway spruce         | BDL <sup>c</sup>                  | BDL   | BDL                             | BDL                  | BDL               | BDL   |
| Nontreated Freeman maple         | BDL                               | BDL   | BDL                             | BDL                  | BDL               | BDL   |
| Treated with aminocyclopyrachlor | 4.3                               | 33    | BDL                             | 6.5 <sup>d</sup>     | 5.6               | 2.1   |

<sup>a</sup> Means are from experimental run 2.

<sup>b</sup> Detected Below Quantifiable Limit (DBQL). DBQL was  $\leq 0.04$  ppb with our analytical methodology.

<sup>c</sup> Below Detection Limit (BDL).

<sup>d</sup> Aminocyclopyrachlor was soil applied at  $0.015 \text{ kg ae ha}^{-1}$  in 100 ml water volume at the initiation of the experiment. Following treatment with aminocyclopyrachlor, nontreated conifer wood chips were applied to the surface of the soil similar to the other treatments so that all treatments were mulched. Residues detected in the wood chips in this treatment were from transfer of aminocyclopyrachlor from soil to mulch.

contained aminocyclopyrachlor when measured in May of 2012 approximately 1 yr after the initial application to turf (Tables 1 and 3). There was little change in aminocyclopyrachlor concentrations in wood chips at the start of the experiment (before) compared to concentrations at the end of the experiment (after) after several irrigations to promote tomato plant growth (Table 3). Soil aminocyclopyrachlor residues ranged from detectable but below quantifiable limit (DBQL) to 0.63 ppb in greenhouse pots mulched with wood chips from aminocyclopyrachlor-damaged honey locust and Norway spruce trees.

Tomato was a sensitive bioassay plant in our experiment that confirmed the presence of aminocyclopyrachlor in wood chips and soil and the sensitivity of tomato to growth regulator-type herbicides (Branham and Lickfeldt 1997; Busey et al. 2003; Gomez De Barreda 1993). The amount of quantified aminocyclopyrachlor in tomato plants varied some between experiments, but this was likely the result of our use of younger plants in the second experiment than the first as it has been documented that tomato plant tolerance to growth regulator herbicide increases as plants age (Fagliari et al. 2005). Leaf epinasty was noted on tomato plants with as little as 0.5 ppb aminocyclopyrachlor in plant tissues and in tomato plants growing in soil with aminocyclopyrachlor concentrations below quantifiable limits ( $< 0.04$  ppb with our methodology). Our data clearly show that an extremely sensitive analytical and bioassay method is necessary for aminocyclopyrachlor quantification in cases of suspected herbicide presence and that there is potential for plant injury to nontarget species from low aminocyclopyrachlor residues. A previous report by Strachan et al. (2011) indicated that the analytical method (Nanita et al. 2009) was sensitive to 0.1 ppb in soil which was more than sufficient when working with agronomic crops. However, leaf epinasty was visible in tomato when soil aminocyclopyrachlor concentrations were  $< 0.1$

ppb in our experiment indicating that in some instances a more sensitive analytical method is needed.

Leaf epinasty caused by aminocyclopyrachlor was limited to new, top-growth of the tomato plants, which is consistent with that known to be caused by other growth regulator herbicides (Monaco et al. 2002). These results are consistent with previous reports that translocation of aminocyclopyrachlor is primarily to the meristematic sink tissues of plants (Bell et al. 2011; Lindenmayer et al. 2013). Honey locust wood chips containing aminocyclopyrachlor caused greater epinasty on tomato plants than wood chips containing aminocyclopyrachlor from Norway spruce trees (Table 2). Two possible explanations for this effect are that average aminocyclopyrachlor concentrations were higher in wood chip mulch from honey locust than Norway spruce (Table 3) or that aminocyclopyrachlor more readily leached from honey locust wood chip mulch than Norway spruce wood chip mulch. Plants are known to vary in their sensitivity to aminocyclopyrachlor (Bell et al. 2011; Flessner et al. 2012) and Norway spruce and honey locust were both among the tree species most susceptible to aminocyclopyrachlor injury at the Indiana locations investigated.

Aminocyclopyrachlor is known to have soil activity (Strachan et al. 2011; USEPA 2010). In this experiment, epinasty of tomato leaflets was likely a result of root uptake of aminocyclopyrachlor that leached into the soil from wood chips during irrigation. However, some uptake could have also occurred where wood chips came in contact with tomato stems. Aminocyclopyrachlor has limited mobility in soil (USEPA 2010), so any leaching from contaminated wood chips would remain in upper regions of the soil where root uptake by sensitive plant species could occur (Oliveira et al. 2011). Testing by the OISC, at locations where aminocyclopyrachlor was applied 12 mo prior to sampling, showed that aminocyclopyrachlor residues were higher in the top 5 cm of soil than in the 5 to 10 cm deep soil layer (Anonymous

2012). Since little or no irrigation water leached from the pots during the tomato experimental period, virtually all the aminocyclopyrachlor that leached from the wood chips remained in the potted soil. Further, since no epinasty occurred on nontreated control plants in our experiment, we attribute the epinasty to root uptake and not volatilization (Strachan et al. 2010).

Our primary research objective was to answer whether wood chips produced from trees damaged by aminocyclopyrachlor 12 mo previously can injure sensitive plants when used as mulch. Results demonstrate that wood chips produced from aminocyclopyrachlor-damaged trees can cause injury to highly sensitive species, such as tomato; however, additional research would be required to confirm similar injury potential for bedding plants or woody ornamental landscape species from use of these wood chips. Additionally, we confirmed that aminocyclopyrachlor can move from contaminated wood chips into soil. These results are similar to previous findings on the use of clopyralid treated grass clippings as mulch around sensitive ornamentals and the ability of clopyralid to persist in the environment (Branham and Lickfeldt 1997; Vandervoort et al. 1997). Our results do support Purdue University extension and DuPont precautionary recommendations (Anonymous 2013a) to properly dispose of (use as lumber or firewood) aminocyclopyrachlor-damaged trees and not chip them for producing mulch or as an ingredient in compost.

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