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Vapor Movement of Synthetic Auxin Herbicides: Aminocyclopyrachlor, Aminocyclopyrachlor-Methyl Ester, Dicamba, and Aminopyralid

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Aminocyclopyrachlor, a newly discovered synthetic auxin herbicide, and its methyl ester, appear to control a number of perennial broadleaf weeds. The potential volatility of this new herbicide and its methyl ester were determined under laboratory conditions and were also compared to dicamba and aminopyralid with the use of enclosed chamber and open-air plant bioassays. Bioassays consisting of visual estimates of epinastic responses and kidney bean and soybean leaf-width measurements were developed to measure vapor release from glass and plastic. Vapor release of aminocyclopyrachlor from glass surfaces was undetectable under laboratory conditions, and no phytotoxic responses were observed when plants were exposed to vapors emanating from various surfaces. Results were similar to those of aminopyralid, indicating the risk of plant injury from vapor movement of aminocyclopyrachlor and aminopyralid was very low. When combined with 1% methylated seed oil, vapor release of aminocyclopyrachlor-methyl ester reached 86% 192 h after application to glass surfaces. Phytotoxic responses of plants exposed to vapors emanating from various surfaces treated with aminocyclopyrachlor-methyl ester were similar to responses to dicamba under enclosed incubation conditions, but were less in outdoor, open-air environments. Studies are needed to understand better the risk of injury to nontarget plants due to vapor movement of aminocyclopyrachlor-methyl ester under field applications.

Nomenclature: Aminocyclopyrachlor; aminopyralid; kidney bean, *Phaseolus vulgaris* 'Green potted bush'; soybean, *Glycine max* (L.) Merr. 'Pioneer 94B53'.

Key words: Auxinic herbicides, bioassay, vapor drift, volatility.

Off-target movement of synthetic auxin herbicides due to volatilization and subsequent vapor drift can injure many nontarget, broadleaf plants (Behrens and Lueschen 1979; van Rensburg and Breeze 1990; Wax et al. 1969). Applications have been restricted by time of day, air temperature, application method, and type of formulation in order to limit the potential for nontarget herbicidal activity on susceptible ornamental plants and agronomic crops (Texas Agricultural Code 1984).

Injury to nontarget plants continues to occur for several reasons: (1) synthetic auxin herbicides are commonly used for weed control (U.S. Environmental Protection Agency [USEPA] Office of Pesticide Programs 2002), and they provide an alternative mode of action to control weeds resistant to other herbicide modes of action, (2) plant responses are distinctive and easily recognized (Andersen et al. 2004; Sciumbato et al. 2004a), and (3) the amount of herbicide necessary to injure a susceptible plant is very small (Sciumbato et al. 2004b). For example, 0.01% of the labeled application rate of dicamba is sufficient to cause visible phytotoxicity to soybean (Steckel et al. 2005), and 0.001% of the label rate of 2,4-D butyl ester is sufficient to cause visible phytotoxicity to tomato (*Lycopersicon esculentum* Mill. 'Marglobe') and lettuce (*Lactuca sativa* L.) (van Rensburg and Breeze 1990).

Aminocyclopyrachlor, a newly discovered herbicide, and its methyl ester, can control a number of invasive, broadleaf weed species (Claus et al. 2008). This new herbicide has utility for broadleaf weed control in pastures, rangeland, and industrial rights of way, and for selected control of unwanted brush and trees in forestry. Aminocyclopyrachlor is the first pyrimidine carboxylic acid herbicide with a chemical structure similar to the pyridine herbicides picloram, clopyralid, aminopyralid, and fluroxypyr. The response of sensitive broadleaf plants to aminocyclopyrachlor and its methyl ester is consistent with synthetic auxin herbicides (Claus et al. 2008). This compound may also elicit volatility and vapor drift concerns similar to those of currently available synthetic auxin herbicides. The goals of this research are to determine if aminocyclopyrachlor and its methyl ester are volatile in a laboratory environment and then to compare the potential for volatilization and subsequent vapor movement to dicamba, a relatively volatile synthetic auxin herbicide, and aminopyralid, a relatively nonvolatile compound (Senseman 2007).

Materials and Methods

Volatility from Glass Surfaces. Experiments to determine the potential volatility of aminocyclopyrachlor[1] and its methyl ester[2] were conducted by first testing technical and formulated versions of both products alone and in combination with 1% methylated seed oil[3] (MSO, v/v). Application solutions of technical and formulated herbicide were prepared at a concentration equivalent to applying 0.14 kg ha^{-1} in 187 L ha^{-1}. Radiolabeled aminocyclopyrachlor-methyl ester (pyrimidine 2-^{14}C) had a specific activity of 3.72×10^8 kBq $mmol^{-1}$, whereas radiolabeled aminocyclopyrachlor (pyrimidine 2-^{14}C) had a specific activity of 3.50×10^8 kBq $mmol^{-1}$. Radiolabeled aminocyclopyrachlor and its methyl ester were added to the nonradioactive application solutions to provide a final concentration of 166 Bq per 5 µl.

Glass microscope slides were cut into 1.5-cm lengths. Ten 0.5-µl droplets of aminocyclopyrachlor or its methyl ester were combined with radiolabeled herbicide and applied directly to the slides. Aminocyclopyrachlor and its methyl ester were evaluated as technical, formulated, and formulated plus 1% MSO solutions. Glass slides were then placed in a

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fume hood at ambient temperatures with a simulated wind speed of 2.2 km hr^{-1}. At 0, 24, 48, 96, and 192 h after treatment (HAT), three replicate slides were randomly selected from the hood and placed in scintillation vials filled with 10 ml of scintillation cocktail.[4] Vials containing glass slides were mixed by vortexing for 1 min, and then radioactivity remaining on the slide was determined by liquid scintillation spectroscopy (LSS). In a parallel experiment, a duplicate set of slides were treated with nonradioactive aminocyclopyrachlor-methyl ester, selected as previously described, and analyzed by high-performance liquid chromatography with detection by tandem mass spectrometry (HPLC/MS/MS) to determine if the herbicide was still present as the methyl ester.

In a second experiment, the influence of surfactants on aminocyclopyrachlor-methyl ester volatility was determined by methods previously described. Nonradiolabeled treatment solutions were prepared as before, but different surfactants were added: 1% MSO (v/v), 0.25% nonionic surfactant[5] (NIS, v/v), 1% crop oil concentrate[6] (COC, v/v), 0.125% nonionic organosilicone[7] (v/v), and 1% blended surfactant[8] (v/v). Radiolabeled aminocyclopyrachlor-methyl ester was added to each treatment solution, and glass slides were prepared as previously described. At 0, 24, 48, 96, and 192 HAT, three slides were randomly selected and placed in scintillation vials containing 10 ml of scintillation cocktail. The amount of radioactivity remaining on the glass slide was determined by LSS.

Data were subject to Levene's test for homogeneity of variance to determine if data from repeated experiments could be combined (SAS version 9.2[9]). SigmaPlot 10[10] was used to analyze time-course experiments with the use of nonlinear regression.

Selecting Bioassay Species. Twenty-seven plant species were grown under greenhouse conditions to select appropriate species with similar responses to both aminocyclopyrachlor-methyl ester and aminopyralid. Seeds were planted into 10-cm pots containing a pH 6.5 Tama silt loam soil (Typic Argiudolls) with 2.9% organic matter. Each pot contained a single plant species. Plants were thinned to a specific population per pot and to uniform sizes prior to herbicide application to ensure uniform plant responses. Aminocyclopyrachlor-methyl ester, formulated as a 25% wettable powder, and aminopyralid, formulated as a 40.6% soluble concentrate, were dissolved/suspended in water, and applied postemergence with the use of a belt sprayer calibrated to spray 280 L ha^{-1} at 262 kPa. Herbicide application rates were 0, 0.25, 1, 4, 16, and 64 g ae ha^{-1}. Plants were grown in a greenhouse balanced with supplemental lighting to maintain a 16-h photoperiod and set to maintain 25 C daytime and 19 C nighttime temperatures. Plant responses were recorded on a 0 to 100% visual response scale, where zero is no visible response and 100 equated to plant death. Evaluations were made approximately 14 d after treatment (DAT).

Amount of herbicide required to produce a 50% phytotoxic response (GR_{50}) was estimated with SAS PROBIT, version 9, correlating plant response to Log_{10}(dose) (SAS version 9.2). This experiment was replicated four times during the months of September 2006 to February 2007.

Bioassay Studies. Formulated samples of aminocyclopyrachlor (70 g ae ha^{-1}), aminocyclopyrachlor-methyl ester (70 g ae ha^{-1}), aminopyralid (70 g ae ha^{-1}), and dicamba (560 g ae ha^{-1}) were dissolved/suspended in water, and applied to 10-cm-diam watch glasses or black plastic (surface area = 300 cm^2) using a belt sprayer calibrated to spray 280 L ha^{-1} at 262 kPa. These herbicide application rates were either label rates or anticipated label rates for weed control in pastures and rangeland. Spray droplets were allowed to dry for 1 h before the treated surfaces were placed inside enclosed chambers. Allowing spray droplets to dry reduced the potential of herbicide movement via liquid transfer (Behrens and Lueschen 1979; Grover et al. 1972). Elimination of chemical transfer via direct contact with treated surfaces and via liquid transfer ensured that any chemical movement within the enclosed chambers was via vapor transfer only (volatility) during the 6-h exposure (Behrens and Lueschen 1979).

Figure 1. Picture of enclosed chamber with heating apparatus, kidney bean, and soybean bioassay plants (see Materials and Methods section).

Kidney bean and soybean (one plant per pot) were grown in 6-cm pots containing potting media.[11] Three kidney bean and three soybean plants, with the first set of trifoliolate leaves approximately one-fourth to one-third fully expanded, were carefully positioned inside the chambers so that no plant material touched any herbicide-treated surface.

All exposure studies were conducted in 39-L glass aquariums (51 by 26 by 31 cm [length by width by height]) covered with clear plexiglass. Small holes were drilled to allow access for electrical cords and temperature sensors (Figure 1). Two types of chambers were constructed, one that remained at room temperature (20 C) during the entire 6-h exposure time, and one that slowly increased the temperature of the treated surface to simulate increasing outdoor surface temperatures as a typical summer day progresses from midmorning to midafternoon. For the heated chamber, treated surfaces were placed on a calibrated hot plate that was turned on just before the chamber was sealed. Surface temperatures of the treated materials and kidney bean and soybean leaves were monitored with an infrared laser thermometer for both exposure studies to ensure temperatures remained as desired. For temperature references, black plastic, kidney bean, and soybean plants were placed outside on August 20, 21, and 22, 2008 (cloudless summer days), in Newark, DE, and surface temperatures were monitored over a 6-h interval from 9 A.M. to 3 P.M. each day. Average hourly

values of these temperatures served as reference values for desired temperatures in the heated chambers.

After exposure, plants were allowed to grow for 7 to 8 d before visual phytotoxic responses and leaf-width measurements were recorded. Visual phytotoxicity responses were recorded on a 0 to 100 scale, where zero was ''no visible phytotoxic response'' and 100 was ''plant death'' (Sciumbato et al. 2004a). Leaf widths at the widest portions of the middle leaflets of the first, second, and third trifoliolate leaves were recorded to the nearest millimeter. All measurements were taken on leaves in their ''natural epinastic state.'' Data were analyzed via either fixed models or mixed models with the use of Tukey's mean separation ($P = 0.05$) to establish significant differences among test results (SAS version 9.2).

For outdoor exposure studies, black plastic trays (total treated surface area = 0.45 m^2) were sprayed with either 70 g ae ha^{-1} of aminocyclopyrachlor, aminocyclopyrachlor-methyl ester, or aminopyralid, or 560 g ae ha^{-1} of dicamba with the use of the same spray parameters as previously described. Smaller black plastic trays (0.03 m^2 of treated surface area) were also sprayed at the same time as the larger black plastic trays. All trays were allowed to dry for 1 h. The smaller trays were bioassayed in enclosed chambers under both environmental conditions previously described. The large trays were placed outdoors in a rectangle completely surrounding, but not touching, three pots each of kidney bean and soybean. Exposure times were from approximately 9 A.M. to 3 P.M. on bright, cloudless summer days during 1 wk in August, 2008.

Outdoor test units were placed at least 15 m from all other test units to reduce potential of vapor drift from the other treatments (Behrens and Lueschen 1979). Plastic surface and leaf surface temperatures were monitored with the use of techniques previously described. During these outdoor studies, winds were generally from the east, with an average speed of 4 km hr^{-1}, and temperatures ranged from a low of 19 C at midmorning, when exposure studies were initiated, to a high of 29 C at midafternoon, when exposure studies were concluded. Relative humidity ranged from a high of 65% at midmorning to a low of 36% at midafternoon.

Bioassay plants were then allowed to grow, phytotoxic responses were measured, and data were analyzed with the use of methods previously described. In addition, visual plant responses were compared to corresponding leaf-width measurements for kidney bean and soybean via linear regression.

Results and Discussion

Volatility from Glass Surfaces. Levene's test for homogeneity of variance indicated that data from repeated experiments could be combined for analysis (SAS version 9.2). Technical and formulated aminocyclopyrachlor showed no volatility over the 192-h time course, whereas aminocyclopyrachlor-methyl ester volatility was significant for both technical and formulated herbicide (Figure 2a). The greatest amount of vapor loss was with formulated aminocyclopyrachlor-methyl ester combined with 1% MSO. Over the 192-h time course approximately 80% of the applied herbicide was lost due to volatility. HPLC/MS/MS analyses indicated that at all time points, herbicide remaining on the glass slides was still present as the intact methyl ester (data not shown).

Some surfactants did significantly reduce aminocyclopyrachlor-methyl ester volatility; however, volatility losses were significant for all surfactants, ranging from 50 to 90% of applied herbicide (Figure 2b). NIS, COC, and the blended adjuvant had the lowest volatility, whereas MSO and the organosilicone surfactant resulted in the highest losses. For the MSO and silicone surfactant, volatility losses were between 80 and 90% over the 192-h time course.

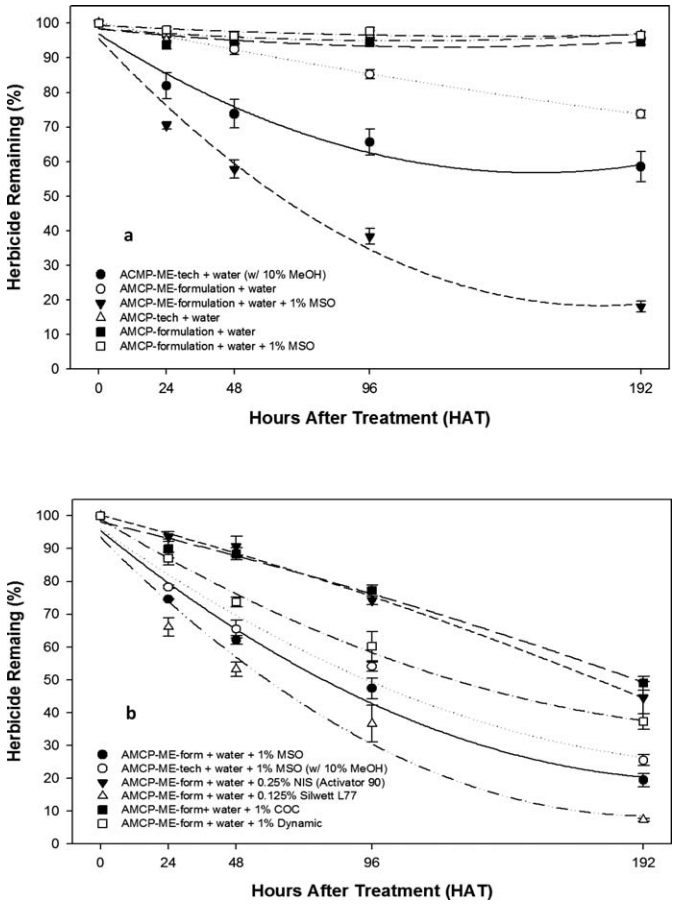
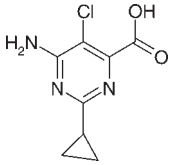
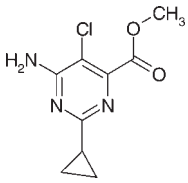
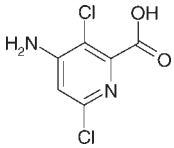
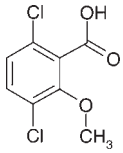


Figure 2. Losses of technical and formulated ^{14}C aminocyclopyrachlor and its methyl ester from glass slides under laboratory conditions. (a) Comparisons of technical and formulated herbicides with and without methylated seed oil at 1% (v/v), (b) comparison of aminocyclopyrachlor-methyl ester losses when applied with different surfactants.

Selecting Bioassay Species. Plant responses to aminocyclopyrachlor-methyl ester, epinasty of newly developing leaves and stems primarily on broadleaf species, were similar to those for aminopyralid, indicating aminocyclopyrachlor's mode of action is consistent with a synthetic auxin herbicide. In general, relative sensitivities of plant species were similar for the two herbicides, with grasses showing greater tolerance than broadleaf species (data not shown). GR_{50} values for grass species were greater than 64 g ae ha^{-1}, the highest dose applied in this experiment. Kidney bean, soybean, and tomato were the broadleaf species most sensitive to aminocyclopyrachlor-methyl ester and aminopyralid, with GR_{50} values of approximately 1 g ae ha^{-1}. Kidney bean and soybean were chosen as bioassay species for all subsequent studies, because plant growth for these two species was consistent across all sample times (data not shown).

Bioassay Studies. The synthetic auxin herbicides, dicamba and aminopyralid, were selected as standards for this study

Table 1. Chemical structures and vapor pressures of aminocyclopyrachlor and its methyl ester, aminopyralid, and dicamba.

Common name	Chemical structure	Vapor pressure (Pa)
Aminocyclopyrachlor[a]		4.89×10^{-6}
Aminocyclopyrachlor methyl ester[a]		4.46×10^{-4}
Aminopyralid[b]		2.56×10^{-8}
Dicamba[b]		4.5×10^{-3}

[a] Cecilia Hirata, DuPont Crop Protection, personal communication.
[b] Senseman (2007).

because the reported vapor pressure of dicamba is higher than aminocyclopyrachlor and its methyl ester, and the reported vapor pressure of aminopyralid is lower (Table 1). In these studies, kidney bean and soybean responses were consistent and statistical analysis indicated that data could be pooled across species. Biological activity of aminocyclopyrachlor-methyl ester was similar to that of the more volatile herbicide, dicamba, and aminocyclopyrachlor's biological activity was similar to that of the less volatile herbicide, aminopyralid (Figure 3). In addition, bioassay responses to dicamba and aminocyclopyrachlor-methyl ester were greater when these

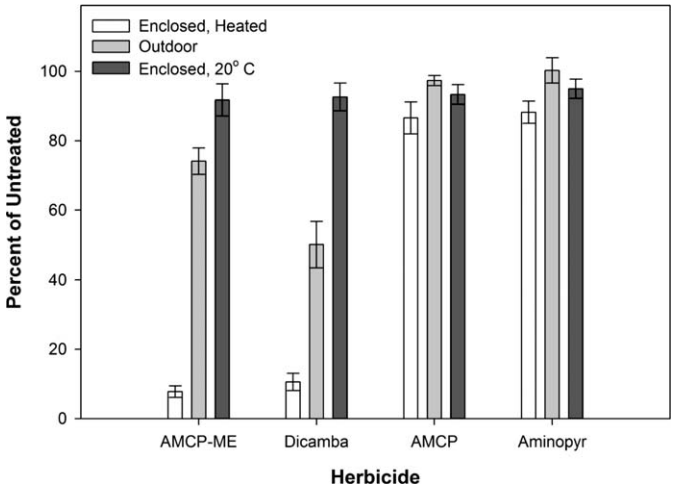


Figure 3. Effect of environment on herbicide volatility for outdoor and enclosed environments (aminocyclopyrachlor–methyl ester [AMCP-ME], dicamba, aminocyclopyrachlor [AMCP], and aminopyralid [Aminopyr]). The sum of the leaf widths from the second and third trifoliolate were averaged over the two species and normalized as a percent of nontreated plants.

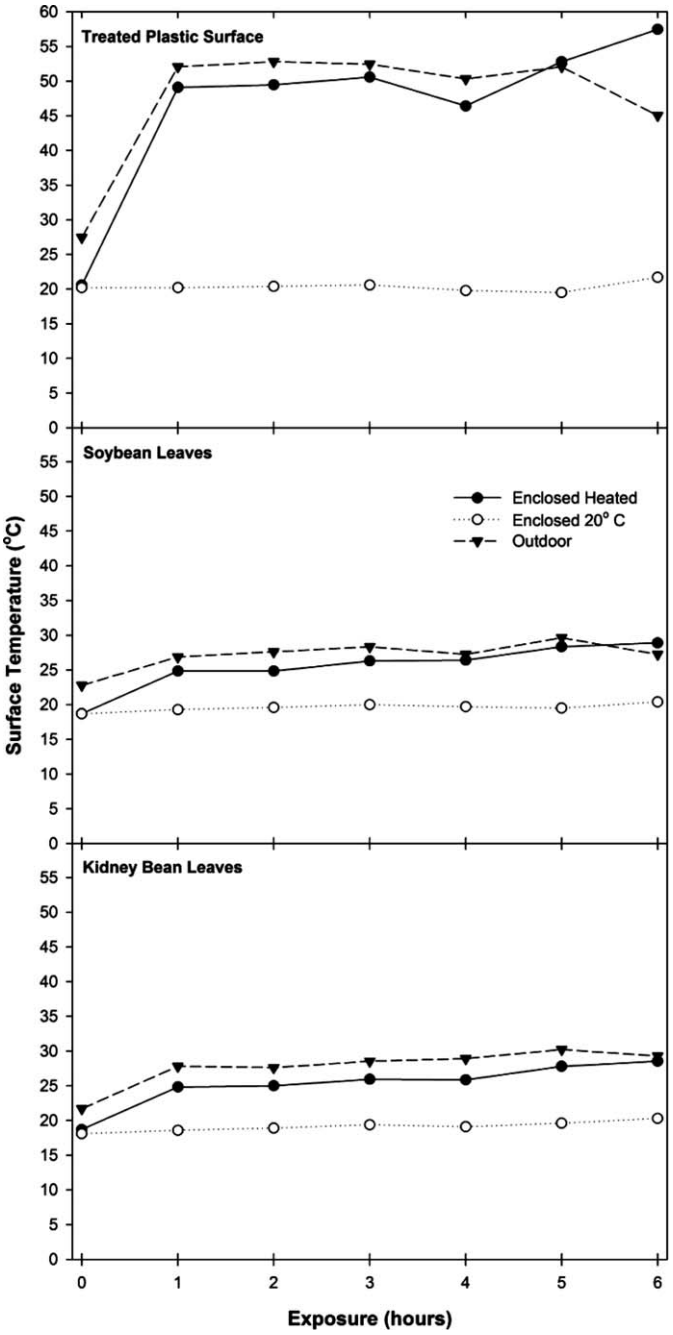


Figure 4. Surface temperatures of plastic, kidney bean leaves, and soybean leaves during exposure.

compounds were placed on heated surfaces. The influence of temperature on dicamba volatility was previously reported in a study on an enclosed system, with soybean as the indicator species (Behrens and Lueschen 1979). Surface temperatures of treated plastic placed inside enclosures and heated were similar to temperatures recorded outside from 9 A.M. to 3 P.M. on a cloudless summer day (Figure 4). This was strong evidence that the heated laboratory exposures approximated conditions that might occur under field conditions. For the exposure parameters used in these studies, when plant injury did occur, plant responses outdoors to treated plastic under full sunlight were nearly always intermediate to response values for the 20 C and the heated-surface enclosed chamber environments (Figure 3).

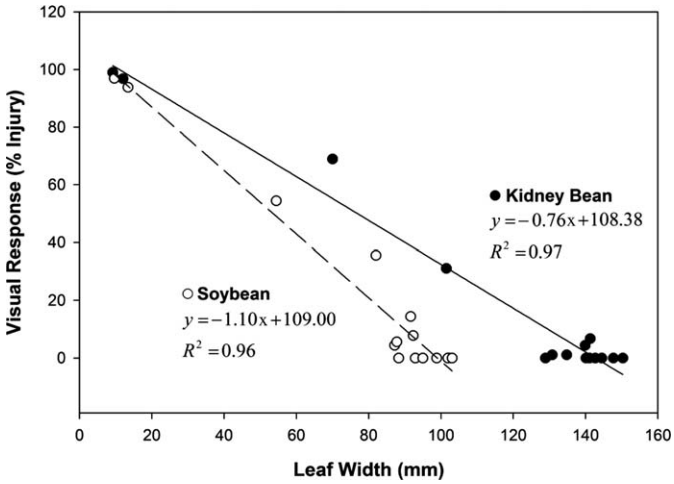


Figure 5. Comparison of soybean and kidney bean visual phytotoxicity responses (percent injury) to leaf-width measurements for plants exposed to dicamba, aminocyclopyrachlor-methyl ester, aminocyclopyrachlor, and aminopyralid.

Epinastic response to synthetic auxin herbicides is predominantly expressed in rapidly dividing cells and new plant growth (Ross and Lembi 1985). Measuring leaf widths of the middle leaflets of the first, second, and third trifoliolate leaves showed the greatest epinastic response occurred on the second and third trifoliolate leaves in kidney bean and soybean (data not shown). For both species, the first trifoliolate leaves were about one-fourth to one-third fully expanded when bioassay plants were exposed to vapors emanating from treated surfaces. After exposure, leaflets of the first trifoliolate grow primarily through cell expansion, whereas growth of leaflets comprising the second and third trifoliolate leaves grow primarily through cell division, differentiation, and subsequent cell expansion (Salisbury and Ross 1978). Responses across the different leaves were therefore consistent with the mode of action of this herbicide chemistry. Vapors of volatile herbicides may penetrate anywhere into the whole plant (van Rensburg and Breeze 1990), and all leaves exposed to dicamba and aminocyclopyrachlor-methyl ester exhibited some degree of epinasty. When compared across both surfaces studied, we found the most sensitive physical measurement of plant response was the combined widths of the middle leaflets from the second and third trifoliolate leaves.

Visual observations of plant phytotoxicity were consistent with reductions in combined widths of middle leaflets of the second and third trifoliolate leaves for kidney bean and soybean. Visual observations were highly correlated with combined widths of the middle leaflets on the second and third trifoliolate leaves for both kidney bean ($R^2 = 0.97$) and soybean ($R^2 = 0.96$), indicating visual assessment of plant growth was a rapid and accurate measure of phytotoxic effects resulting from vapor movement of these herbicides (Figure 5).

Results from laboratory and enclosed chamber bioassay studies were consistent for aminocyclopyrachlor and its methyl ester. Both types of experiments found that aminocyclopyrachlor had very low volatility, whereas the methyl ester could volatilize from nonporous surfaces such as glass and plastic. Although aminocyclopyrachlor-methyl ester volatility could be reduced with some surfactants, volatility losses at best were still 50% or higher under laboratory conditions.

The outdoor bioassay studies provided a slightly different picture for potential impacts to nontarget plants. Consistent with laboratory and chamber experiments, the potential for nontarget plant responses from aminocyclopyrachlor vapor drift was very low; however, results for aminocyclopyrachlor-methyl ester were not as consistent across the three test environments. Kidney bean and soybean bioassay responses to aminocyclopyrachlor-methyl ester and dicamba applied to plastic in enclosed laboratory environments showed vapor activities for the two herbicides were similar; however, in an open, outdoor environment under full sunlight, vapor activity of aminocyclopyrachlor-methyl ester appears to be less than that of dicamba (Figure 3). This discrepancy is consistent with observations of field evaluations, where we have seen epinastic responses of sensitive plants immediately surrounding research plots treated with dicamba, whereas sensitive plants immediately surrounding plots treated with the aminocyclopyrachlor-methyl ester show no epinastic response.

We hypothesize that kidney bean and soybean could be significantly more sensitive to dicamba than to aminocyclopyrachlor-methyl ester. In addition, the greater chemical stability, the higher vapor pressure and application rate of dicamba (eight times the rate of aminocyclopyrachlor-methyl ester) may allow greater opportunity for dicamba molecules to deposit onto kidney bean and soybean during the 6-h exposure. Previous research has shown that the amount of 2,4-D butyl ester absorbed by tomato and lettuce correlates well with the concentration of 2,4-D butyl ester in the vapor phase (van Rensburg and Breeze 1990). In the outdoor environment, aminocyclopyrachlor-methyl ester may be hydrolyzing to the lower vapor pressure, aminocyclopyrachlor, or photolytic reactions might be degrading aminocyclopyrachlor-methyl ester to inactive compounds, reducing the opportunity for molecules of aminocyclopyrachlor-methyl ester to deposit onto kidney bean and soybean. Research within DuPont has shown that approximately 50% of applied aminocyclopyrachlor-methyl ester, applied as a thin film onto a glass surface, degrades to inactive breakdown products during 24-h exposure to a Xenon arc lamp simulating natural sunlight (D. L. Perkins, DuPont Crop Protection, personal communication). Because ester formulations of aminocyclopyrachlor could be useful in certain applications, additional studies are needed to understand vapor movement of aminocyclopyrachlor esters in commercial applications and the risk of injury to nontarget plants better.

Sources of Materials

[1] Aminocyclopyrachlor (6-amino-5-chloro-2-cyclopropyl-pyrimidine-4-carboxylic acid), DuPont, 1007 Market Street, Wilmington, DE 19898.

[2] Aminocyclopyrachlor-methyl ester (6-amino-5-chloro-2-cyclopropyl-pyrimidine-4-carboxylic acid methyl ester), DuPont, 1007 Market Street, Wilmington, DE 19898.

[3] Methylated Seed Oil Concentrate with LECI-Tech®, Loveland Industries, Inc., Greeley, CO 80537.

[4] Ultima Gold LLT scintillation cocktail, PerkinElmer, 940 Winter Street, Waltham, MA 02451.

[5] Activator 90® Non-Ionic Surfactant, Loveland Industries, Inc., Greeley, CO 80537.

[6] Maximizer® Crop Oil Concentrate, Loveland Industries, Inc., Greeley, CO 80537.

[7] Nonionic organosilicone, Silwet L77®, Helena Chemical Co., 225 Schilling Boulevard, Collierville, TN 38017.

[8] Blended surfactant, Dyne-Amic®, Helena Chemical Co., 225 Schilling Boulevard, Collierville, TN 38017.

[9] SAS Version 9.2, SAS Institute, Inc., 100 SAS Campus Drive, Cary, NC 27513-2414.

[10] SigmaPlot 10, Systat Software, Inc., 225 West Washington St., Suite 425, Chicago, IL 60606.

[11] Redi-earth potting mix, Sun Gro Horticulture, Bellevue, WA 98008.

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