Paper chromatography of the chloroform extract indicated that two radioactive compounds were present. One of these compounds was prometryne, while the other component had an $R_F$ value that corresponded to hydroxy prometryne, which is identical to hydroxy propazine. Thus, the carrot appears to degrade prometryne in a manner analogous to that in which corn degrades propazine.

### Proposed Degradation Sequence

There has been considerable interest and speculation in the sequence of reactions leading to complete destruction of the triazine nucleus. The intervening reactions between formation of the 2-hydroxy compounds and complete oxidation to carbon dioxide have not been determined. Figure 1 shows the authors' proposed sequence of reactions in the route of degradation of the triazine herbicides, with herbicide labeled with $C^{14}$ in the two position.

The hydroxy compound in the brackets is shown in the keto form, since the infrared spectrum of this compound indicates a carbonyl group is present.

Evidence for the formation of the suggested degradation products was obtained by paper chromatography of the extracts of corn plants treated with propazine (experiment reported in Table III). The developing solvent was isoamyl alcohol saturated with 3*M* hydrochloric acid. With this developer, the parent triazine has an $R_F$ value of about 0.88, while that of hydroxy propazine is 0.55 to 0.60. Chromatography of the extract of propazine-treated corn 3 days following exposure showed three radioactive components to be present. Most of the radioactivity was present as propazine, with some hydroxy propazine and a small amount of a third component being present. The extract of propazine-treated corn 8 days following exposure, contained four radioactive components, three of which were found in the earlier extract.

This later extract contained appreciable amounts of the two unidentified compounds. The $R_F$ values of these two compounds were much smaller than propazine or hydroxy propazine, which suggests they are basic compounds. Similarity of the $R_F$ values of a substituted biguanide tested in this laboratory, analogous to the one postulated in the degradation scheme, and of one of the degradation products found in corn indicates that one of the radioactive components may very well be the postulated biguanide. Also, the difference in the $R_F$ values for unsubstituted biuret and the second unidentified component is only 0.08, indicating that the second compound may be the substituted biuret. If the substituted biguanide is formed, it probably undergoes hydrolysis to a substituted biuret or a substituted guanidine and a substituted urea.


### Acknowledgment

A grant-in-aid from the Geigy Agricultural Chemical Corp., which made this work possible, is gratefully acknowledged.


### Literature Cited


(1) Ashton, F. M., Zweig, G., Mason, G. W., *Weeds* **8,** 448 (1960).
(2) Bartley, C., *Farm Chem.* **122,** 113 (1957).
(3) Burschel, P., *Weed Res.* **1,** 131 (1961).
(4) Castelfranco, P., Foy, C. L., Deutch, D. B., *Weeds* **9,** 580 (1961).
(5) Cooke, A. R., *Ibid.*, **4,** 397 (1956).
(6) Davis, D. E., Funderburk, H. H., Sansing, N. G., *Proc. Southern Weed Conf.* **12,** 172 (1959).
(7) Davis, D. E., Funderburk, H. H., Sansing, N. G., *Weeds* **7,** 300 (1959).
(8) Exer, B., *Experientia* **14,** 135 (1958).
(9) Freed, V. H., Oregon State University, Corvallis, unpublished data.
(10) Funderburk, H. H., Davis, D. E., *Weeds* **11,** 101 (1963).
(11) Gast, A., *Experientia* **14,** 134 (1958).
(12) Gentner, W. A., Shaw, W. C., 1957 Field results, USDA Progress Report, Plant Industry Station, Beltsville, Md., 1958.
(13) Gysin, H., Knülsi, E., *Advan. Pest Control Res.* **3,** 289 (1960).
(14) Hamilton, R. H., Moreland, D. E., *Science* **135,** 373 (1962).
(15) Montgomery, M., Freed, V. H., *Res. Prog. Rep. Western Weed Control Conf.* **71,** 1960.
(16) Montgomery, M., Freed, V. H., *Weeds* **9,** 231 (1961).
(17) Montgomery, M., Freed, V. H., Fang, S. C., *Res. Prog. Rep. Western Weed Control Conf. Ibid.*, **92,** 1958.
(18) Moreland, D. E., Gentner, W. A., Hilton, J. L., Hill, K. L., *Plant Physiol.* **34,** 432 (1959).
(19) Moreland, D. E., Hill, K. L., *Weeds* **10,** 229 (1962).
(20) Roth, W., *Compt. Rend.* **245,** 942 (1957).
(21) Roth, W., *Experientia* **14,** 137 (1958).
(22) Roth, W., Knülsi, E., *Ibid.*, **17,** 312 (1961).
(23) Sheets, T. J., *Proc. Weed Soc. Amer.* **44,** 1960.
(24) Wessels, J. S. C., Van der Veen, R., *Biochem. Biophys. Acta* **19,** 548 (1956).



*Received for review August 19, 1963. Accepted December 9, 1963. Division of Agricultural and Food Chemistry, 144th Meeting, ACS, Los Angeles, Calif., April, 1963. Published as Oregon Agricultural Experiment Station Technical Paper No. 1712.*


**METABOLISM OF HERBICIDES**

# Tolerance of Several Grass Species to 2-Chloro-s-triazine Herbicides in Relation to Degradation and Content of Benzoxazinone Derivatives


**ROBERT H. HAMILTON**

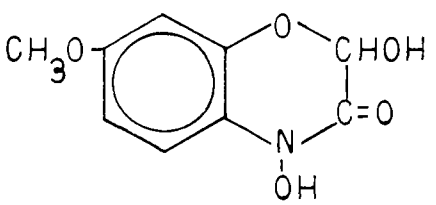
**Department of Botany, Pennsylvania State University, University Park, Pa.**


THE 2-chloro-4,6-dialkylamino-*s*-triazine herbicides are extensively used as pre-emergence sprays for weed control in corn. Corn is not injured by applications up to 10 times the amount required for control of annual weeds. These herbicides are also used for control of weeds in sorghum, sugar cane, and a few other crops. In general, other cereal grains are susceptible to these herbicides. Photosynthesis and specifically the Hill reaction has been implicated as a sensitive site of action (*4, 13, 14*) of these herbicides. However, chloroplasts from corn are as sensitive to inhibition of the Hill reaction as those from susceptible species (*15*). It has been suggested (*5*) that metabolism of these herbicides by corn and other resistant species may be the mechanism for their tolerance. The two most widely used derivatives are 2-chloro-4,6-bis-(ethylamino)-*s*-triazine (simazine) and 2-chloro - 4 - ethylamino - 6 - isopropylamino-*s*-triazine (atrazine). The latter derivative is more water-soluble and thought to be somewhat less selective in the field than simazine.

Extracts of corn degrade simazine in a nonenzymatic manner (*3, 5, 16*). The compound responsible for the apparent catylitic degradation is 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (I) or its 2 glucoside (*8, 17*), and the structure has recently been established (*6, 7, 11, 18, 19*). The major product of the degradation reaction (*8*) is 2-hydroxy-4,6-bis(ethylamino)-*s*-triazine (hydroxy simazine), which is nonphytotoxic (*5*)

**The tolerance of several species of Gramineae to atrazine was not related to the ability of their excised roots to metabolize $C^{14}$-simazine. The content of benzoxazinone derivatives was directly related to the ability of excised roots to form hydroxysimazine and another minor metabolite. Roots not containing benzoxazinone derivatives did not form hydroxysimazine but other minor metabolites were detected.**

Hydroxysimazine is also a major product in corn seedlings treated with $C^{14}$-simazine (*8*). Thus, resistance of corn to simazine might be related to this nonenzymatic detoxification reaction. However, Virtanen and coworkers (*18*, *19*) report that susceptible rye and wheat seedlings also contain the glucoside of this benzoxazinone derivative, and that rye seedlings contain an analog which lacks the 7-methoxy group.



I

It seemed desirable to determine whether dechlorination of 2-chloro-*s*-triazine herbicides was, in fact, directly related to resistance and, if so, the role of benzoxazinone derivatives in this reaction in vivo. In the present study the tolerance of several species of Gramineae to atrazine are compared with the simazine-degrading ability of their excised roots as well as the content of benzoxazinone derivatives in such roots.

### Materials and Methods

**Growth of Seedlings and Tolerance Studies.** Seeds of Redcoat Wheat [*Triticum vulgare* (Vill.)], Tetra Petkus Rye [*Secale cereale* (L.)], Dwarf Yellow Milo [*Sorghum vulgare* (Pers.)], Kafer-60 Sorghum [*Sorghum vulgare* (Pers.)], Clinton Oats [*Avena sativa* (L.)], Hudson Barley [*Hordeum vulgare* (L.)], and Jobs-Tears [*Coix lacryma-jobi* (L.)] were planted in moist sand. For the tolerance studies, uniform seedlings 7 to 10 days old were transferred to one-half strength Hoagland's No. 1 solution (*10*) in 1-quart containers. Three plants were placed in each culture, and three cultures were used per treatment. After about 5 days, atrazine was added to the solution. The nutrient solutions were renewed every 5 days to maintain the atrazine levels. Atrazine was used in place of simazine in the tolerance studies because of its greater solubility in water. The tolerance of these species to either simazine or atrazine is in fact known to be similar from field observations. The tolerance test was repeated with most of the species. In the case of species with some tolerance, fresh weights of roots and tops were secured after about 4 weeks.

**Table I. The Inhibition of Fresh Weight Resulting from a 30-Day Exposure of Sorghum, Corn, and *Coix* to Atrazine in the Culture Solution**

| Species and Variety | Av. Fresh Weight per Plant, Grams | % Inhibition of Fresh Weight at Atrazine Concentration (P.P.M.) 0.1 | 1.0 | 5.0 | 10.0 |
|---|---|---|---|---|---|
| Dwarf Yellow Milo[a] | 15.3 | 81.6 | 97.4 | ... | ... |
| Kafer-60 Sorghum | 8.0 | 79.1 | 90.0 | ... | ... |
| Pa. 812 Corn | 94.9 | ... | ... | 56.4 | 64.2 |
| *Coix lacryma-jobi* | 53.5 | ... | 11.1 | ... | 63.9 |

[a] Selected because of possible susceptibility to propazine.

**Degradation of $C^{14}$-Simazine by Excised Roots.** Seedlings of the above species were harvested when 7 to 10 days old and the roots washed free of sand. The excised roots were blotted, weighed, and placed in 1-liter Erlenmeyer flasks that held 100 ml. of one-half strength Hoagland's solution containing 200 $\mu$g. of $C^{14}$-simazine (random ring labeled with 1.02 mc. per mmole specific activity). The flasks were placed on a horizontal shaker for 6 hours at 25° C. in a dark room. The incubation solutions were removed by decantation and the excised roots were washed several times with tap water. The roots were frozen and ground in a blender at −10° C. in 95% ethanol, to which was added 2 mg. of unlabeled simazine. The residue was removed by filtration and washed with an additional 40 ml. of 80% ethanol. Aliquots of the filtrate and washings were removed for determination of the radioactivity. Portions of the dried residue were ground in a Wiley Mill (40 mesh) and spread on 1-inch planchets for counting. The ethanol filtrate was reduced in volume under reduced pressure in a rotating evaporator at 40° C. for 30 minutes. (Volatility products could have been lost at this stage; however, occasional radioactivity measurements on the ethanol extract and the concentrate indicated fair agreement.) An aliquot of the aqueous concentrate (0.5–1.0 ml.) was removed for paper chromatography. A control was run in which the $C^{14}$-simazine was added to corn roots just prior to grinding.

In some instances, the ethanol concentrate was adjusted to pH 4.0 with 1*N* HCl and extracted with 3 volumes of chloroform to remove $C^{14}$-simazine. The aqueous $C^{14}$ fraction was retained on a Dowex 50W × 4 ($H^+$) 1.5- × 7-cm. column, and the column was washed with water. The radioactivity was eluted with 1*N* $NH_4OH$. Recovery of radioactivity for the column step was 80 to 100%.

Liquid samples were counted in duplicate on 1-inch glass, sand-blasted planchets. The activity of ground residue samples was corrected to infinite thinness. All counting was in a gas flow Geiger-Muller windowless counter, and under these conditions $C^{14}$-simazine had a relative activity of 3100 c.p.m. per $\mu$g. The relative proportions of simazine to hydroxysimazine were determined by direct counting on the paper chromatograms.

**Chromatography.** Paper chromatography was by the ascending technique with Whatman No. 1 or 3MM paper. The solvents used for separation of simazine and hydroxysimazine were *i*-amyl alcohol saturated with 3*N* HCl, 65% 2,6-lutidine, and *n*-butanol–acetic acid–water (4:1:5). The latter solvent was sometimes used with Whatman No. P-20 cation exchange paper for better separations. $C^{14}$-hydroxysimazine for standards was prepared by hydrolysis of $C^{14}$-simazine in equal volumes of 6*N* HCl and 95% ethanol at 50° C. for 8 hours.

**Determination of Benzoxazinone Content.** Seedlings 7 to 10 days old were ground with boiling 95% ethanol in a blender. Following filtration and concentration in vacuo, the 80% ethanol concentrate (10 ml.) was extracted twice with 10-ml. portions of petroleum ether (b.p. 30°–60° C.). The aqueous portion was concentrated to about 1 ml. in vacuo, and aliquots were chromatographed on Whatman 3MM paper as a

**Table II. The Ability of Excised Roots of Several Species of Gramineae to Degrade $C^{14}$-Simazine**

| Species and Variety | Wt. Roots[a] (Grams) | Radioactivity (Total C.P.M.) 80% Ethanol-soluble | Residue | % Hydroxysimazine in 80% Ethanol |
|---|---|---|---|---|
| Pa-812 Corn | 10.0 | 32,850 | 6300 | 43.7 |
| Pa-812 Corn CK[b] | 10.8 | 513,200 | 700 | 0[c] |
| Redcoat Wheat | 10.8 | 21,100 | 6400 | 9.5 |
| Tetra Petkus Rye | 10.4 | 27,200 | 8300 | 25.9 |
| *Coix lacryma-jobi* | 10.0 | 76,050 | ... | 39.8 |
| Kafer-60 Sorghum | 10.5 | 34,650 | 4650 | 0[c] |
| Clinton Oats | 9.8 | 51,500 | 4600 | 0[c] |
| Hudson Barley | 10.1 | 53,550 | 6750 | 0[c] |

[a] Number of seedlings, respectively, was 30, 30, 100, 130, 30, 200, 168, and 140.
[b] The $C^{14}$-simazine was added to the excised roots just prior to grinding in ethanol.
[c] No hydroxysimazine was detected.

**Table III. The Content of Benzoxazinone Derivatives in Several Species of Gramineae**

| Species and Variety | Roots, μmoles per Gram Fresh Wt. | Shoots, μmoles per Gram Fresh Wt. |
|---|---|---|
| Pa-812 Corn | 3.47 | 9.21 |
| Redcoat Wheat | 0.56 | 0.83 |
| Tetra Petkus Rye | 1.12 | 2.90 |
| *Coix lacryma-jobi* | 3.84 | 13.00 |
| Kafer-60 Sorghum | 0[a] | 0[a] |
| Clinton Oats | 0[a] | 0[a] |
| Hudson Barley | 0[a] | 0[a] |

[a] Below the limit for detection (about 0.05 μmole per gram fresh weight).

streak. The chromatograms were developed ascending with *n*-butanol–acetic acid–water (4:1:5, v./v.). The ultraviolet light-absorbing (2537 A.) spots at about $R_f$ 0.6 (glucoside) (*7, 19*) and at $R_f$ 0.8 (aglucone) (*7, 19*) were eluted with 95% ethanol–0.1*N* HCl (equal volumes). The eluted samples were diluted with the acidic aqueous ethanol to 1.9 ml. The blue color intensity which developed upon addition of 0.1 ml. of 0.1*N* $FeCl_3$ was read immediately in a Klett colorimeter with a 59 filter. The same standard curve was found with either crystalline aglucone (*7*) or isolated glucoside. Under these conditions, the assay conformed to Beer's law and was usable from about 0.2 to 2.0 μmoles in 2 ml. It was assumed the analog from rye would give an equivalent color on a molar basis.

Although the ferric chloride reaction is not specific for these benzoxazinone derivatives, they do give a characteristic intense blue color because of the cyclic hydroxamate structure. Standards of the glucoside and the aglucone were included on most chromatograms for reference. The characteristic ferric chloride reactive components in extracts of roots also corresponded with the standards when chromatographed in the *i*-amyl alcohol–HCl solvent. In fact, the major ferric chloride-reactive materials in these seedlings correspond to the benzoxazinone derivatives. However, in older corn leaves, a substance with an $R_f$ similar to glucoside interferes with the determination.

## Results

In tolerance studies, wheat, rye, barley, and oats were killed by a 1-week exposure to 0.5 p.p.m. atrazine in the culture solution. Corn and *Coix lacryma-jobi* (Table I) were inhibited but showed no other symptoms following an exposure to 5 or 10 p.p.m. atrazine for 1 month. Sorghum was intermediate in tolerance (Table I), without acute toxicity symptoms.

The ability of excised roots to degrade $C^{14}$-simazine to $C^{14}$-hydroxysimazine is apparent in the case of corn, *Coix*, rye, and wheat. The results shown (Table II) are for typical experiments. In other experiments there was considerable variation in the ability of excised roots to convert simazine to hydroxysimazine, but no conversion was ever noted for Kafer-60 Sorghum, oats, or barley. In each species, but especially those containing hydroxysimazine, an additional $C^{14}$-component ran at an $R_f$ lower than hydroxysimazine in all three solvents ($R_f$ 0.30 with *n*-butanol–acetic acid–water on P-20 paper). This component corresponded in $R_f$ to a minor component obtained when $C^{14}$-simazine was hydrolyzed with acid. This metabolite was noted previously (*8*) upon incubation of $C^{14}$-simazine with 2,4-dihydroxy - 7 - methoxy - 1,4 - benzoxazin-3-one or the 2-glucoside. Prolonged acid hydrolysis of hydroxysimazine, however, did not yield this derivative. Traces of two other low $R_f$ $C^{14}$-components ($R_f$ 0.14 and 0.05) from wheat concentrates were noted on chromatograms developed with *n*-butanol–acetic acid–water on P-20 paper.

Ethanolic concentrates of excised roots from Kafer-60 Sorghum, Clinton Oats, or Hudson Barley contained still other metabolites in trace amounts. When the concentrate at pH 4.0 was extracted with 3 volumes of chloroform, some radioactivity remained in the aqueous phase. Following purification with the cation exchange resin, and paper chromatography (P-20 paper), one or two additional metabolites were detected. The $C^{14}$-simazine used did not contain any of these substances.

The benzoxazinone determinations (Table III) indicated the presence of these derivatives in wheat and rye as previously reported (*18, 19*). *Coix* also contains high concentrations as was postulated on the basis of the isolation of 6-methoxybenzoxazolinone from it (*12*). The shoots contain higher concentrations than the roots in all these seedlings. The concentration is low in rye, and especially in wheat, compared to corn or *Coix*.

## Discussion

The converting ability of excised roots was found to be directly correlated with the content of benzoxazinone derivatives (Tables II and III). Although benzoxazinone derivatives may play a part in the resistance of corn and *Coix* to 2-chloro-4,6-dialkylamino-*s*-triazine herbicides, other factors appear to be important. Wheat and rye, which are susceptible, contain benzoxazinone derivasives, and their excised roots convert simazine to hydroxysimazine. On the other hand, Kafer-60 Sorghum does not contain benzoxazinone derivatives in detectable amounts, and its excised roots do not appear to convert simazine to hydroxysimazine. Kafer-60 Sorghum, however, is resistant to at least a considerable degree. One may pose the question as to whether any direct correlation between degradation and tolerance does in fact exist. Previous studies have shown that cotton has some tolerance for 2-chloro-4,6-dialkylamino-*s*-triazine herbicides (*9*). Yet no definite evidence for ring cleavage or conversion of 2-chloro-4-diethylamino-6-isopropylamino-*s*-triazine to the 2-hydroxy analog could be obtained (*9*).

Chloroplasts from barley, corn, soybeans, or turnip greens are of about the same sensitivity in regard to inhibition of the Hill reaction by simazine (*15*). The toxic response may be, in part, prevented by supplying sugar or amino acids (*1, 14*) to barley. On the other hand, shading or absence of light (*1*) protects against the acute lethal action of simazine. This clearly indicates that the acute toxicity of susceptible species

is not due to starvation. It is a secondary result of inhibition of the photochemical reaction. Ashton suggested (*2*) that the action spectrum of the acute response is similar to the absorption spectrum of the chlorophylls. Apparently a vital cellular component may be destroyed by photooxidation in sensitive plants when the Hill reaction is blocked. This component may be replaced by synthesis if the plants are supplied a sugar or an amino acid. Although sorghum, *Coix*, and corn may show severe growth inhibition following treatment with atrazine, no acute toxicity symptoms ever become apparent.

Since barley, oats, and sorghum roots appear to contain water-soluble metabolites other than hydroxysimazine, the possibility of other degradation mechanisms must be considered. Also short-term studies with excised roots may not preclude the ability of the intact plant to degrade simazine to hydroxysimazine over longer time intervals.

Another kind of selectivity mechanism would be the differential ability of the several species to take up the herbicide and translocate it to chloroplast-containing leaf mesophyll tissue. Even within a mesophyll cell, and especially along the transport route, binding or deposition at inactive sites may take place. In the case of excised roots, there was no consistent difference in the ability of different species of Gramineae to take up $C^{14}$-simazine. There were also no evident trends in the amount of $C^{14}$ found in the 80% ethanol-insoluble residue.


### Acknowledgment

The author thanks the Geigy Agricultural Chemicals Co., Ardsley, N. Y., for the atrazine and $C^{14}$-simazine.


### Literature Cited


(1) Allen, W. S., Palmer, R. D., *Weeds* **11,** 27 (1962).
(2) Ashton, F. M., *Plant Physiol. Suppl.* **37,** XXV (1962).
(3) Castelfranco, P., Foy, C. L., Deutsch, D. B., *Weeds* **9,** 580 (1961).
(4) Exer, B., *Experientia* **14,** 136 (1958).
(5) Gysin, H., Knüsli, E., *Advan. Pest Control Res.* **3,** 289 (1960).
(6) Hamilton, R. H., Ph.D. thesis, Michigan State University, 1960.
(7) Hamilton, R. H., Bandurski, R. S., Reusch, W. H., *Cereal Chem.* **39,** 107 (1962).
(8) Hamilton, R. H., Moreland, D. E., *Science* **135,** 373 (1962).
(9) Hamilton, R. H., Moreland, D. E., *Weeds* **11,** 213 (1963).
(10) Hoagland, D. R., Arnon, D. I., *Calif. Univ. Agr. Expt. Sta. Circ.* **347,** (1950).
(11) Honkanen, E., Virtanen, A. I., *Acta Chem. Scand.* **19,** 504 (1960).
(12) Koyama, T., Yamato, M., Kubota, K., *J. Pharm. Soc. Japan* **77,** 989 (1957).
(13) Moreland, D. E., Gentner, W. A., Hilton, J. L., *Abstracts, Weed Soc. Amer.* **40** (1958).
(14) Moreland, D. E., Gentner, W. A., Hilton, J. L., Hill, K. L., *Plant Physiol.* **34,** 432 (1959).
(15) Moreland, D. E., Hill, K. L., *Weeds* **10,** 229 (1962).
(16) Roth, W., *Compt. Rend.* **245,** 942 (1957).
(17) Roth, W., Knüsli, E., *Experientia* **17,** 312 (1961).
(18) Virtanen, A. I., Hietala, P. K., *Acta Chem. Scand.* **14,** 499 (1960).
(19) Wahlroos, Ö., Virtanen, A. I., *Ibid.*, **13,** 1906 (1959).



*Received for review May 15, 1963. Accepted September 27, 1963. Division of Agricultural and Food Chemistry, 144th Meeting, ACS, Los Angeles, Calif., April, 1963. Contribution No. 307 from the Dept. of Botany, and Journal series paper No. 2763 from the Pennsylvania Agricultural Experiment Station.*


**METABOLISM OF HERBICIDES**

# The Metabolism and Translocation of 3-Amino-1,2,4-triazole by Canada Thistle


**RICHARD A. HERRETT and WILLIAM P. BAGLEY**

**Agricultural Research Station, Chemicals Division, Union Carbide Corp., Clayton, N. C.**



**The metabolism of 3-amino-1,2,4-triazole (amitrole) by Canada thistle results in the formation of at least three chromatographically distinct derivatives. Two compounds, (Unknowns I and II) are similar to metabolites reported by other workers and relatively inactive. A third metabolite (Unknown III) is herbicidally more active than amitrole. Furthermore, Unknown III will translocate out of leaves under a condition (light starvation) unfavorable for the translocation of amitrole. The evidence is consistent with the hypothesis that amitrole must undergo metabolism to an active form prior to translocation within the phloem.**


Previous studies (*3, 8, 11, 13, 15, 17*) have indicated a rapid metabolism of 3-amino-1,2,4-triazole (amitrole) in a wide variety of species. Two metabolites, Unknowns I and II, were observed (*8*) in Canada thistle (*Cirsium arvense* L.). One of these metabolites was isolated and reapplied to thistle and found to be inactive.

These same studies with thistle (*8*) demonstrated the existence of a lag between penetration of amitrole into the treated leaf and its subsequent translocation from that leaf. Under normal conditions, amitrole applied to a leaf is translocated via the phloem (*1, 9, 12*) to aerial and subterranean portions of the plant. In the absence of light, however, translocation of amitrole is inhibited (*1, 9, 12*). It has been considered that translocation of amitrole (*1*) and other synthetic plant growth regulators, such as 2,4-D (2,4-dichlorophenoxyacetic acid) (*14*), is associated with the photosynthetic food stream.

It was hypothesized (*8*) that for amitrole to be transported in the phloem it must undergo a chemical reaction to an active transport form. The present communication provides additional evidence in support of this hypothesis.

### Materials and Methods

Canada thistle (*Cirsium arvense* L.) was grown under controlled environmental conditions (1500 foot-candles supplied by incandescent and fluorescent lamps) with alternating 12-hour light