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Absorption, Translocation, and Metabolism of ^{14}C-Clomazone in Soybean (*Glycine max*) and Three *Amaranthus* Weed Species

William K. Vencill,* Kriton K. Hatzios, and Henry P. Wilson

Laboratory of Molecular Biology of Plant Stress, Department of Plant Pathology, Physiology and Weed Science, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061-0330; and Eastern Shore Research Center, Painter, Virginia 23420, U.S.A.

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Abstract. The patterns of clomazone (2-[(2-chlorophenyl)methyl-4,4-dimethyl-3-isoxazolidinone) absorption, translocation, and metabolism and their contribution to the plant selectivity of this herbicide were studied in tolerant soybean [*Glycine max* (L.) Merr.] and *Amaranthus hybridus* and in susceptible *A. retroflexus* and *A. lividus*. Differential root absorption appeared to play a significant role in the differential response of these four plant species to clomazone. Absorption of root-applied ^{14}C-clomazone was greater by the two sensitive *Amaranthus* weeds than by the tolerant soybean and *A. hybridus*. Following application of ^{14}C-clomazone to roots, most of the absorbed radioactivity was translocated to the leaves of all four species. Approximately 50% of the absorbed ^{14}C-clomazone was metabolized by all four plant species as early as 12 h after treatment. Thin layer chromatographic (TLC) analysis of plant tissue extracts from all four species revealed the formation of two major metabolites of clomazone. These unidentified metabolites had Rf values of 0.4 and 0.8, respectively, in a butanol:acetic acid:water (12:3:5, vol/vol/vol) developing system. The Rf value of unaltered clomazone in this system was 0.95. Differential metabolism or differential rate of metabolism of clomazone was not observed in this study and did not seem to account for the tolerance of soybean and *A. hybridus* or the suceptibility of *A. retroflexus* and *A. lividus* to this herbicide.

Clomazone is a recently introduced herbicide for the selective control of many grass and broadleaf weeds in soybeans, following preemergence applications at rates ranging from 0.6 to 1.1 kg a.i./ha. Species controlled include large crabgrass (*Digitaria sanguinalis* L.), foxtails (*Setaria spp.*), velvetleaf (*Abutilon theophrasti* Medik.), and common lambsquarters (*Chenopodium album* L.) (Anonymous 1986, Liebl and Norman 1989, Wallinder et al. 1986).

Clomazone is an isoxazolidinone derivative which reduces carotenoid and chlorophyll levels causing a bleached appearance in treated susceptible plant species (Duke and Paul 1986, Duke et al. 1985). Sandmann and Böger (1986, 1987) reported that clomazone inhibits the enzymes isopentenyl pyrophosphate isomerase and prenyltransferase and disrupts the formation of key components of the isoprenoid pathway needed for the synthesis of plant pigments, such as carotenoids and chlorophylls.

Amaranthus species are among the 10 most important weeds in soybeans produced in Virginia (Hagood 1986). *Amaranthus hybridus* (smooth pigweed) is the predominant weed species of the genus *Amaranthus* in Virginia, while *Amaranthus retroflexus* (redroot pigweed) is a weed problem only in some areas (Harville et al. 1981). Field studies have indicated that clomazone only sporadically controls *Amaranthus* species and that *A. retroflexus* is more susceptible to clomazone than *A. hyrbidus* (Wallinder et al. 1986). *Amaranthus lividus* (livid amaranth) has also been observed to be susceptible to clomazone under field conditions. The basis for the observed differential sensitivity of these three *Amaranthus* weed species to clomazone is unknown.

Differential absorption, translocation, and metab-

Plant Pathology, Physiology, and Weed Science Department, Contribution No. 600.

* Present address: Department of Agronomy, University of Georgia, Athens, Georgia 30602, USA.

olism have long been recognized as important factors contributing to herbicide selectivity (Hatzios and Penner 1982, Shimabukuro 1985). Weston and Barrett (1989) reported recently that differential metabolism does not appear to contribute to the selectivity of the herbicide clomazone. Differences in clomazone metabolite levels between tolerant bell pepper (*Capsicum annum* L.) and susceptible tomato (*Lycopersicon esculentum* L.) were negligible. Similar observations have been reported also by Norman and Liebl (1989) who found that differential metabolism alone could not account for the selectivity of clomazone in photomixotrophic cell suspensions of tolerant soybean and susceptible cotton (*Gossypium hirsutum* L.).

The objectives of this research were (1) to study the absorption, translocation, and metabolism of radiolabeled clomazone in tolerant soybean and *A. hyridus* and in susceptible *A. retroflexus* and *A. lividus* following root application of this herbicide and (2) to determine the potential contribution of differential absorption, translocation, and metabolism in the selectivity of this herbicide.

Materials and Methods

Chemicals

Technical (95% pure) and radiolabeled samples of clomazone were provided by FMC Chemical Corporation (Princeton, NJ, USA). Radiolabeled clomazone was uniformly labeled with ^{14}C on the aromatic ring with a specific activity of 28.0 mCi/mmol. Clomazone was dissolved in acetone and made up to volume with distilled water.

Plant Material

Seeds of each species were planted in a potting soil mixture of vermiculite:weblite:sphagnum peat moss (2:2:1, vol/vol/vol) in 400-ml cups. Plants were grown in a growth chamber at a constant temperature of 25°C, and a photoperiod of 14 h light at 400 $\mu E \cdot m^{-2} \cdot s^{-1}$ provided by both incandescent and fluorescent lamps. At the 1- to 2-leaf stage of growth of the *Amaranthus* species and unifoliate stage of soybeans, seedlings were transferred to half-strength Hoagland's solution (pH 6.0) in foil-wrapped 150-ml glass containers, one plant per container (Hoagland and Arnon 1950). After 2 days, plants were grown in full-strength Hoagland's nutrients solution.

Uptake and Distribution of [^{14}C]-clomazone

At the 3- to 4-leaf stage of *Amaranthus* species and first trifoliate stage of soybeans, plants were exposed to radiolabeled clomazone following root application. Seedlings of all species were placed in nutrient solution containing 5 μCi/L radiolabeled clomazone adjusted to 100 μM concentration with technical clomazone.

Plants were harvested at 12, 48, and 96 h after treatment. The roots of treated plants were washed with distilled water to remove any radioactivity from their surface. Radioactivity in root washes was determined by liquid scintillation spectrometry (Beckman LS 8100 Model). At the end of each harvesting period, a 1-ml sample of the remaining nutrient solution was used to determine the amount of radioactivity remaining in the solution by means of liquid scintillation spectrometry. Harvested plants were separated into roots, shoots, and leaves. Plant parts were weighed and ground to a fine powder with liquid nitrogen. Then they were combusted in a biological sample oxidizer (Tricarb Model B0306, Packard Instruments, Downers Grove, IL, USA), and the released $^{14}CO_2$ was trapped and counted by liquid scintillation spectrometry to quantitatively determine absorption and translocation of the radiolabeled clomazone.

Autoradiography

A set of seedlings of all species treated with radiolabeled clomazone was used for analysis by autoradiography following the procedures of Crafts and Yamaguchi (1964). The plant samples were dried after harvest and placed on x-ray film (Kodak X-Omat AR). Three weeks later, the film was developed for qualitative analysis of clomazone translocation.

Separation and Identification of Clomazone Metabolites

Seedlings of all four species were grown hydroponically as described earlier. ^{14}C-Clomazone was supplied to the plants through their roots and was added to the nutrient solution at a concentration of 10 μM containing 5.0 μCi/L of nutrient solution. Plants were again harvested at 12, 48, and 96 h after treatment. Harvested plants were divided into roots and shoots plus leaves, weighed, and ground to fine powder with liquid nitrogen. Radiolabeled herbicide and its metabolites were extracted with two aliquots of 50 ml of 80% acetone primarily from shoot and leaf tissues, since most of the root-applied clomazone was found to accumulate in the leaves of all four species examined in this study. The acetone aliquots were combined and concentrated to 1 ml by rotoevaporation at 40°C. A 0.1-ml sample of each extract was then counted by liquid scintillation spectrometry. Unextracted plant samples were oxidized as described earlier and counted by liquid scintillation spectrometry. To analyze plant extracts for clomazone and its metabolites, 50-μl aliquots were spotted on silica-gel thin-layer chromatography (TLC) plates (Whatman Silica Gel 150 A LK5DK, Whatman Inc., Clifton, NJ, USA). The plates were developed in butanol:acetic acid:water (12:3:5, vol/vol/vol) and co-chromatographed with standard radiolabeled clomazone (Rf = 0.95). Developed TLC plates were separated into 1-cm segments from the origin to the solvent front, scraped, and radioactivity was quantitatively determined by liquid scintillation spectrometry.

Statistical Analysis

Three replications of all treatments were made, and each exper-

Table 1. Absorption and distribution of total ^{14}C recovered in various parts of soybeans and *Amaranthus* species at 12, 48, and 96 h after root application of ^{14}C-clomazone.[a]

Species	Time after application (h)	Absorption (% of applied)	Distribution of radioactivity (% of absorbed)		
			Root	Stems	Leaves
Soybean	12	38	20	17	62
	48	27	15	16	68
	96	53	17	17	65
LSD (time within species)		18	NS	NS	NS
A. hybridus	12	24	14	17	70
	48	17	13	15	72
	96	34	15	16	69
LSD (time within species)		NS	NS	NS	NS
A. retroflexus	12	23	22	17	61
	48	43	16	22	62
	96	80	9	24	67
LSD (time within species)		14	NS	NS	NS
A. lividus	12	46	8	19	73
	48	64	10	9	80
	96	83	13	15	72
LSD (time within species)		22	NS	NS	NS
LSD (between species)		23	NS	NS	NS

LSD, Fischer's protected least significant difference; NS, not significant.
[a] Data are means of two experiments with three replications each.

iment was repeated twice in time. Means were separated by Fisher's protected least significance difference (LSD) test at the 5% level.

Results

Clomazone Absorption and Translocation

In general, absorption of clomazone via root application increased over the course of the study in all four species (Table 1). Clomazone absorption by the sensitive weeds *A. lividus* and *A. retroflexus* was greater than that observed with tolerant soybeans and *A. hybridus*. At 96 h after treatment, greater than 80% of the applied ^{14}C-clomazone was absorbed by *A. retroflexus* and *A. lividus*. During the same time period, absorption of ^{14}C-clomazone by soybean and *A. hybridus* was 53 and 34%, respectively (Table 1). Thus, in terms of their efficiency in absorbing root-applied clomazone, the four species can be ranked as follows: *A. lividus* > *A. retroflexus* > soybean > *A. hybridus*.

Of the absorbed radioactivity, 61 to 80% was translocated to the leaves of all four plant species examined following application of ^{14}C-clomazone to the roots (Table 1). The rest of the absorbed radioactivity from root-applied ^{14}C-clomazone remained either in the roots or translocated to the shoots of the four species (Table 1). Autoradiographic analysis of all plant species revealed the same pattern of distribution (acropetal translocation) of the absorbed radioactivity. Autoradiographs in Fig. 1, illustrate the distribution pattern of root-applied ^{14}C-clomazone in the sensitive weeds *A. lividus* (Fig. 1a) and *A. retroflexus* (Fig. 1b) as well as in the tolerant species *A. hybridus* (Fig. 1c). The movement of absorbed radioactivity from roots to shoots and leaves appeared to be independent of the time after treatment with root-applied ^{14}C-clomazone in all four species (Table 1).

Clomazone Metabolism

Because of the greater absorption and more uniform distributon of root-applied clomazone to all four species, studies on the metabolism of this herbicide were conducted by feeding ^{14}C-clomazone to the roots. Approximately 50% of the applied clomazone was metabolized by all four species by 12 h after treatment. The levels of unaltered clomazone and those of clomazone metabolites did not change significantly with time of treatment, suggesting either a steady-state uptake and metabolism or only an initial metabolism of the herbicide. Therefore, only data for the 96-h period are presented in Table 2. TLC analysis of combined shoot and leaf extracts from soybeans and the three *Amaranthus* weeds re-

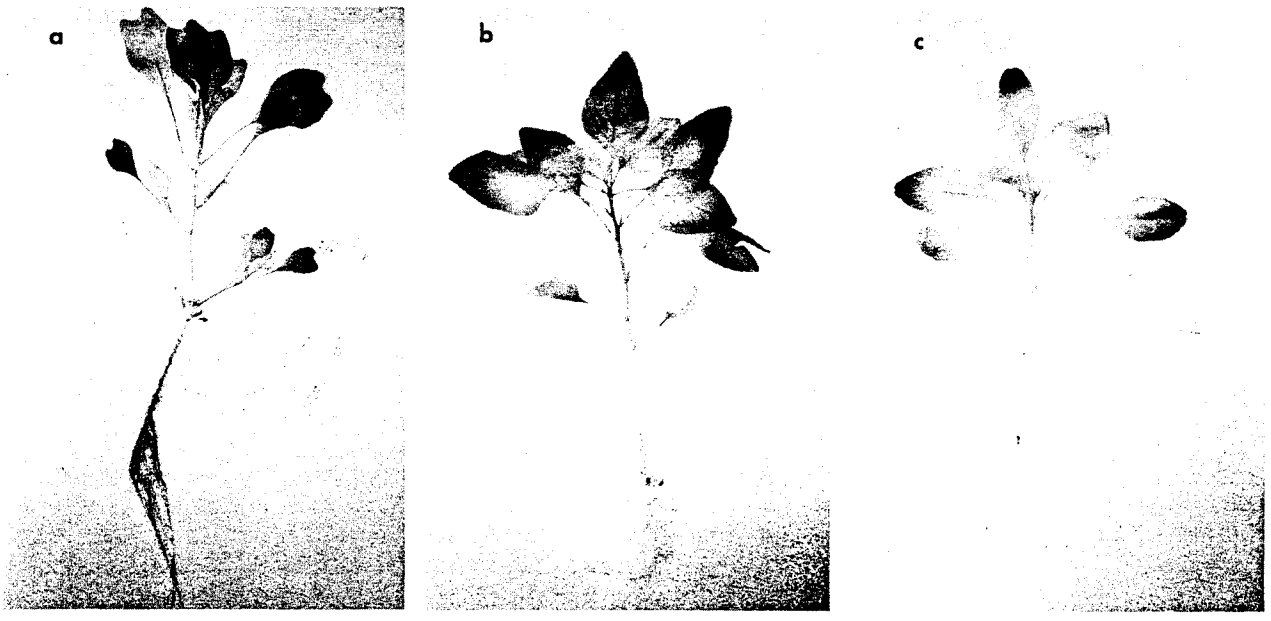

Fig. 1. Autoradiographs showing the distribustion of root-applied ^{14}C-clomazone in (**a**) *Amaranthus lividus*, (**b**) *A. retroflexus*, and (**c**) *A. hybridus*.

Table 2. Metabolism of root-applied clomazone in leaf tissue of soybeans and *Amaranthus* species, 96 h after treatment.

		% of radioactivity spotted[a]			
Metabolite	Rf value[b]	Soybean	*A. hybridus*	*A. retroflexus*	*A. lividus*
Unknown no. 1	0.4	23	21	22	25
Unknown no. 2	0.8	24	29	38	30
Clomazone	0.95	52	50	40	45
LSD (within each species)		15	14	NS	13

LSD, Fisher's protected least significant difference; NS, not significant.
[a] Data are means of three replications repeated in time.
[b] Metabolites were separated by TLC using a solvent system of butanol:acetic acid:water (12:3:5, vol/vol/vol).

vealed the formation of two major metabolites of clomazone. These metabolites had Rf values of 0.4 (unknown no. 1) and 0.8 (unknown no. 2), respectively (Table 2). Unaltered clomazone had an Rf value of 0.95 (Table 2). The levels of unknown no. 1 metabolite of clomazone formed in soybean and the three *Amaranthus* weeds were very similar. Although not statistically significant, the levels of the unknown no. 2 metabolite of clomazone that were formed in the three *Amaranthus* weeds were higher than the levels of this metabolite formed in soybean (Table 2).

Discussion

Data obtained from the root absorption studies and autoradiography indicate that clomazone is mainly a xylem-mobile herbicide moving acropetaly following root application. Clomazone absorption appears to be species-dependent, especially among weeds of the genus *Amaranthus*. *A. retroflexus* and *A. lividus*, which are clomazone-sensitive species, absorbed more clomazone through the roots than clomazone-tolerant soybean and *A. hybridus*. Of the clomazone absorbed in all species, most was translocated to the leaf tissue which is in agreement with data of other researchers (Anonymous 1986; Weston and Barrett 1989).

The identity of the two metabolites of clomazone found in all plant species examined in this study is not presently known. The chlorine present in the phenyl ring of the clomazone molecule may react

with endogenous compounds containing sulfhydryl groups such as glutathione. In fact, the production of a synthetic conjugate of clomazone with reduced glutathione (GSH) under in vitro conditions has been reported by Vencill et al. (1989). The unknown no. 1 metabolite of clomazone detected in extracts from all four plants appeared to have similar chromatographic properties to those of the synthetic GS-clomazone conjugate in the TLC developing system (butanol:acetic acid:water, 12:3:5, vol/vol/vol) used in this and an earlier study (Vencill et al. 1989). Additional studies are needed, however, to conclusively characterize this metabolite as a GS-conjugate of clomazone.

The detection of polar metabolites of clomazone in extracts obtained from both tolerant and susceptible plant species has been reported also by Weston and Barrett (1989) and Norman and Liebl (1989). Weston and Barrett (1989) postulated that the polar metabolites of clomazone formed in tolerant bell peppers, as well as in susceptible tomato, were conjugates of clomazone with sugars such as glucose. Treatment of these metabolites with β-glucosidase which cleaves β-1,4-glucose linkages and hesperidinase which cleaves α-1,6-glucose linkages did not result in any significant release of ^{14}C-clomazone from these suspected glucose conjugates (Weston and Barrett 1989). On the other hand, acid hydrolysis of these metabolites with HCl was very effective in releasing ^{14}C-clomazone. The results of Weston and Barrett (1989) suggest that the polar metabolites of clomazone detected in their study may not necessarily be glycosides of this herbicide. Alternatively, it is possible that clomazone may be forming conjugates with both sugars and glutathione similar to the case of the bleaching herbicide pyridate (Gaillardon et al. 1989, Zohner 1987).

Differential metabolism or differential rate of metabolism of clomazone did not appear to explain the tolerance of soybean and *A. hybridus* or the susceptibility of *A. retroflexus* and *A. lividus* to this herbicide. This conclusion is in agreement with the findings of Weston and Barrett (1989) and Norman and Liebl (1989) who reported that differential metabolism does not seem to play an important role in the selective action of the herbicide clomazone.

Our data showed that tolerant plants such as soybean and *A. hybridus* absorbed less root-applied clomazone than the sensitive species *A. retroflexus* and *A. lividus*. The observed differences in clomazone uptake may be part of the reason why *A. retroflexus* and *A. lividus* are more sensitive to this herbicide. Differences in the distribution of the absorbed radioactivity following root application of ^{14}C-clomazone were not significant enough to account for the differential sensitivity of the three *Amaranthus* weed species to this herbicide.

An alternative hypothesis for explaining the selectivity of the herbicide clomazone was proposed recently by Norman and Liebl (1989). They postulated that clomazone may be actually a proherbicide which is metabolized to an active form by both tolerant and susceptible plants and differences at the site of action (carotenoid biosynthesis) may account for the selectivity of this herbicide. However, Norman and Liebl (1989) did not present any data to support their hypothesis. Further research is needed to substantiate this hypothesis and conclusively elucidate the exact basis or bases for the observed selectivity of the herbicide clomazone.

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References

Anonymous (1986) Command herbicide—Technical information. Princeton, New Jersey

Crafts AS, Yamaguchi S (1964) The autoradiography of plant material. California Agricultural Experiment Station Circular 35

Duke SO, Paul RN (1986) Effects of dimethazone (FMC-57020) on chloroplast development. II. Pigment synthesis and photosynthetic function in cowpea (*Vigna unguiculata* L.) primary leaves. Pestic Biochem Physiol 25:11–18

Duke SO, Kenyon WH, Paul RN (1985) Clomazone effects on chloroplast development in pitted morningglory (*Ipomoea lacunosa*) cotyledons. Weed Sci. 33:786–794

Gaillardon P, Guichaoua JC, Gasquez J, Scalla R (1989) Absorption, translocation and metabolism of pyridate in a tolerant crop (*Zea mays*) and two susceptible weeds (*Polygonum lapathiofolium* L. and *Chenopodium album* L.). Weed Res 29:45–51

Hagood ES (1986) Economic losses due to weeds—Virginia. Research Report Southern Weed Science Society 39:135

Harville AM, Bradley TR, Stevens CE (1981) Atlas of the Virginia flora. Virginia Botanical Association, Farmville, VA

Hatzios KK, Penner D (1982) Metabolism of herbicides in higher plants. Burgess Publishing Company, Minneapolis, MN

Hoagland DR, Arnon DI (1950) The water culture method for growing plants without soil. California Agricultural Experiment Station Circular 347

Liebl RA, Norman MA (1989) Responses of corn, soybean, smooth pigweed, *Amaranthus hybridus* L., and velvetleaf, *Abutilon theophrasti* Medik., to clomazone. Abstr Weed Sci Soc Am 29:88

Norman MA, Liebl RA (1989) Uptake and metabolism of clomazone in soybean (tolerant) and cotton (susceptible) photomixotrophic cell suspensions. Abstr Weed Sci Soc Am 29:69

Sandmann G, Böger P (1986) Interference of dimethazone with the formation of terpenoid compounds. Z Naturforsch 41c:729–732

Sandmann G, Böger P (1987) Interconversion of prenyl pyrophosphates and subsequent reactions in the presence of FMC-57020. Z Naturforsch 42c:803–807

Shimabukuro RH (1985) Detoxification of herbicides. In: Duke SO (ed) Herbicide physiology, Vol. II. CRC Press, Boca Raton, Florida, pp 216–236

Vencill WK, Hatzios KK, Wilson HP (1990) Interactions of the bleaching herbicide clomazone with reduced glutathione and other thiols. Z Naturforsch (in press)

Wallinder CJ, Choban RG, Estores RA (1986) Weed control in conventional soybeans with FMC-57020. Proc Northeast Weed Sci Soc 40:77

Weston LA, Barrett M (1989) Differential tolerance of tomato (*Lycopersicon esculentum*) and bell pepper (*Capsicum annum*) to clomazone. Weed Sci 37:285–289

Zohner A (1987) Mode of crop tolerance to pyridate in corn and peanuts. Proc Brit Crop Prot Conf—Weeds 10 B-2, pp 1083–1090