Edgington, L. V., *Pestic. Chem., Proc. Int. Congr. Pestic. Chem., 2nd* **5**, 379 (1972).
Fahmy, M. A. H., Fukuto, T. R., Metcalf, R. L., Holmstead, R. L., *J. Agr. Food Chem.* **21**, 585 (1973).
Frey-Wyssling, A., "Submicroscopic Morphology of Protoplasm and Its Derivatives," Elsevier, New York, N. Y., 1948, p 225.
Fujita, T., Iwasa, J., Hansch, C., *J. Amer. Chem. Soc.* **86**, 5175 (1964).
Gunther, F. A., Westlake, W. E., Jaglan, P. S., *CSIR (Counc. Sci. Ind. Res. S. Afr.), Res. Rev.* **20**, 488, 513, 524 (1968).
Hall, H. K., *J. Amer. Chem. Soc.* **79**, 5441 (1957).
Hansch, C., Fujita, T., *J. Amer. Chem. Soc.* **86**, 1616 (1964).
Hansch, C., Maloney, P. P., Fujita, T., Geiger, C. F., Streich, M. J., *J. Amer. Chem. Soc.* **85**, 2817 (1963).
Leary, J. B., *J. Ass. Offic. Anal. Chem.* **54** (6), 1396 (1971).
Mel'nikov, N. N., Zen'kevich, A. G., *J. Gen. Chem. USSR* **25**, 793 (1955).
Metcalf, R. L., *Agrochimica* **2**, 105 (1967).
Metcalf, R. L., Reynolds, H. T., Fukuto, T. R., Collins, C., *J. Econ. Entomol.* **57**, 531 (1964).
Miller, D. M., *World Rev. Pest Contr.* **10**, 60 (1971).
Quistad, G. B., Fukuto, T. R., Metcalf, R. L., *J. Agr. Food Chem.* **18**, 189 (1970).
Shephard, M. C., *Pestic. Chem., Proc. Int. Congr. Pestic. Chem., 2nd* **5**, 383 (1972).
Taft, R. W., in "Steric Effects in Organic Chemistry," Newman, M. S., Ed., Wiley, New York, N. Y., 1956, p 556.
Wain, R. L., Carter, G. A., "Fungicides," An Advanced Treatise, Vol. I, Torgeson, D. C., Ed., Academic Press, New York, N. Y., 1967, p 561.

Received for review September 7, 1973. Accepted December 3, 1973. This investigation was supported by Research Grant No. 73-261 from Cotton Incorporated, Raleigh, N. C., and with Federal funds from the Environmental Protection Agency under Grant No. R801837. The contents do not necessarily reflect the views and policies of the Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

Uptake and Metabolism of Dichlobenil by Emersed Aquatic Plants

Harish C. Sikka,* Robert S. Lynch, and Margaret Lindenberger

[^{14}C]Nitrile-labeled 2,6-dichlorobenzonitrile (dichlobenil) was readily absorbed by the roots of the emersed aquatic plants, alligator weed (*Alternanthera philoxeroides*) and parrot feather (*Myriophyllum brasiliense*) and was translocated to the shoots. The concentration of the ^{14}C was greater in parrot feather than in alligator weed 24 hr after treatment. The roots of the alligator weed contained a considerably greater amount of ^{14}C than the shoots, whereas in parrot feather, a major portion of the absorbed ^{14}C was present in the shoot. [^{14}C]Dichlobenil leaked from the roots of alligator weed but not from those of parrot feather. Alligator weed was not able to metabolize dichlobenil; on the contrary, parrot feather transformed the herbicide into several metabolites. The major metabolite resulting from the transformation was 3-hydroxy-2,6-dichlorobenzonitrile; small amounts of 2,6-dichlorobenzamide and 2,6-dichlorobenzoic acid were also present. A significant amount of radioactivity (14–18% of ^{14}C in the shoot) was present as highly polar, unknown metabolites. The tolerance of parrot feather to dichlobenil appears to be related to its ability to metabolize the herbicide.

The herbicide dichlobenil (2,6-dichlorobenzonitrile) is recommended for the control of many aquatic weeds. The utility of this herbicide for killing a variety of aquatic plants was demonstrated by a number of investigators (Frank *et al.*, 1963; Lawrence *et al.*, 1963; Walker, 1964). The metabolic fate of dichlobenil in the aquatic environment is of obvious concern since the herbicide metabolites may be more persistent and more toxic than the parent molecule to the aquatic fauna and flora. Metabolism by plants is one of the important factors which determine the fate of an herbicide in the aquatic environment.

Presently, the information available on the fate of dichlobenil in aquatic plants is very limited; however, a few workers have studied the uptake and metabolism of this herbicide in terrestrial plants. Massini (1961) reported that dichlobenil was readily absorbed by roots of bean (*Phaseolus vulgaris*) seedlings and was translocated throughout the plant *via* the xylem, but more slowly than water due to affinity of the herbicide for plant tissue. Pate and Funderburk (1966) and Verloop and Nimmo (1969) reported that dichlobenil was absorbed only slightly by bean and alligator weed (*Alternanthera philoxeroides*) when applied as a foliar spray but it was readily absorbed by the root. Verloop and Nimmo (1969, 1970) found that bean, wheat (*Tritium vulgare*), and rice (*Oryza sativa*) plants absorbed the herbicide through the roots and translocated it fairly rapidly to the shoots. They observed that an appreciable amount of dichlobenil was lost from the leaves due to evaporation. Price and Putnam (1969) found that dichlobenil readily entered the corn roots (*Zea mays*), but was not actively held within the cells. About 71% of the absorbed herbicide was excreted from the roots into the nutrient solution in 24 hr.

Pate and Funderburk (1966) described 2,6-dichlorobenzoic acid as a metabolite of dichlobenil in bean and alligator weed. However, according to the results of Verloop and Nimmo (1969, 1970), hydrolysis of dichlobenil is a very minor metabolic process in bean and wheat seedlings, since very low amounts of 2,6-dichlorobenzamide and 2,6-dichlorobenzoic acid were found. They reported that hydroxylation, followed by conjugation, was the main metabolic process by which dichlobenil was converted in the plants.

The present investigation was conducted to study the fate of [^{14}C]dichlobenil in two species of emersed aquatic plants, parrot feather (*Myriophyllum brasiliense*) and alligator weed, which differ markedly in their tolerance to the herbicide.

MATERIALS AND METHODS

Chemicals. [^{14}C]Dichlobenil labeled in the nitrile carbon, with a specific activity of 28.8 μCi/mg, was supplied by N. V. Philips-Duphar, Weesp, The Netherlands. Nonradioactive samples of dichlobenil, 3-hydroxy-2,6-dichlorobenzonitrile, 4-hydroxy-2,6-dichlorobenzonitrile, and 3-hydroxy-2,6-dichlorobenzamide were supplied by Thomp-

Life Sciences Division, Syracuse University Research Corporation, Syracuse, New York 13210.

son-Hayward Chemical Co., Kansas City, Mo., and Philips-Duphar. 2,6-Dichlorobenzamide and 2,6-dichlorobenzoic acid were purchased from Aldrich Chemical Co., Milwaukee, Wis.

Culture of Plants. Shoot cuttings of alligator weed and parrot feather were placed in water for 1 week to allow roots to develop. The plants were then transferred to half-strength Hoagland and Arnons' (1950) No. 2 nutrient solution and allowed to grow for 7–10 days before treatment with the herbicide. The plants were grown and treated in a growth chamber where the environmental conditions were a 14-hr day at 85°F and a 10-hr night at 75°F. The light intensity was approximately 2000 ft-candles provided by a mixture of incandescent and fluorescent lamps. The plants were 20–25 cm long at the time of treatment. The phytotoxicity of dichlobenil was determined by treating the alligator weed and parrot feather plants (four plants per jar) in jars containing several concentrations of dichlobenil in 250 ml of nutrient solution and examining the plant for visible injury symptoms.

Uptake of [^{14}C]Dichlobenil. The plants were transferred to jars containing 0.5 ppm of [^{14}C]dichlobenil in nutrient solution. Due to the volatility of the herbicide, all the experiments were conducted without aeration. At various time intervals after treatment, two plants were removed from the [^{14}C]dichlobenil solution and the roots were thoroughly rinsed with distilled water and blotted free of excess water. The alligator weed plants were sectioned into roots and stems and the fresh weight of each fraction was determined. The plant tissue was chopped into small pieces and homogenized in a Virtis blender with absolute methanol. The homogenate was shaken 30 min and centrifuged, and the extract was decanted. The plant residue was then extracted twice with 80% methanol in the same manner. The three extracts were combined; the amount of radioactivity in the pooled extract was measured by adding 1-ml aliquots to 15 ml of liquid scintillation solution containing 2,5-diphenyloxazole, 1,4-bis[2-(5-phenyloxazolyl)]benzene, dioxane, naphthalene, toluene, and ethanol and counting in a liquid scintillation counter. The amount of ^{14}C remaining in the extracted plant material was determined by subjecting it to wet combustion (Smith *et al.*, 1964). The $^{14}CO_2$ evolved from the combusted residue was trapped in a CO_2-trapping solution consisting of a monoethanolamine and 2-methoxyethanol (1:2, v/v) (Metcalf *et al.*, 1967). Aliquots of trapping solution were added to the scintillation fluid and counted for radioactivity.

Efflux of Dichlobenil. The plants were allowed to absorb [^{14}C]dichlobenil from the nutrient solution containing 0.5 ppm of the herbicide. After 24 hr, they were removed from the solution and the roots were thoroughly rinsed with distilled water. The plants were then transferred to a fresh nutrient solution without the herbicide and the efflux of ^{14}C-labeled material was measured by counting the radioactivity in the nutrient solution. For analysis of radioactivity in the effluent, the nutrient solution was extracted with benzene and the benzene extract was chromatographed on thin-layer silica gel plates in the solvent systems described below.

Metabolism of [^{14}C]Dichlobenil. In these studies, alligator weed plants were treated with 0.5 ppm of [^{14}C]dichlobenil, whereas parrot feather plants were treated with 2 ppm. The plants were harvested at various times after treatment and extracted with methanol as described above. Dichlobenil was separated from its metabolites in the extract by steam distillation at pH 9. The metabolites were then fractionated by extraction of the residual solution with ether at different pH values, as described by Verloop and Nimmo (1969). In this fractionation procedure, neutral compounds were extracted at pH 11; free phenols were extracted at pH 4; free extractable conjugated phenols were obtained by extraction at pH 4 after hydrolysis with 2 *N* HCl at 100° for 1 hr; ether-extractable acids were separated by extraction at pH 0.5. The amount of radioactivity in each fraction was determined by liquid scintillation counting. The metabolites in the different fractions were separated by thin-layer chromatography on silica gel plates. The plates were developed over a distance of 15 cm in the following solvent systems: chloroform–ethanol–acetic acid (89:10:1) (system I) and benzene–methanol (9:1) (system II) (Verloop and Nimmo, 1969). The pH 4 fraction was also chromatographed on thin-layer plates (Merck Polyamide 11F-254 on aluminum) in a solvent mixture consisting of benzene–methanol (4:1) to obtain a better resolution of 3-hydroxy- and 4-hydroxydichlorobenzonitrile. It was not possible to separate these two compounds by thin-layer chromatography on silica gel plates in solvent systems I or II. The polyamide chromatograms were allowed to develop for 3 hr after the solvent had reached the edge of the chromatogram. Radioactive compounds were detected by scanning the chromatograms on a Nuclear-Chicago actigraph; nonradioactive compounds were detected by ultraviolet light absorption. Authentic compounds were cochromatographed for comparison with unknown metabolites.

The identity of the metabolites was determined by comparing their R_f values with those of known compounds. The quantity of each resolved ^{14}C-labeled metabolite was determined by scraping the radioactive zone off the plate into the liquid scintillation fluid containing 4% thixotropic gel powder and counting it for ^{14}C.

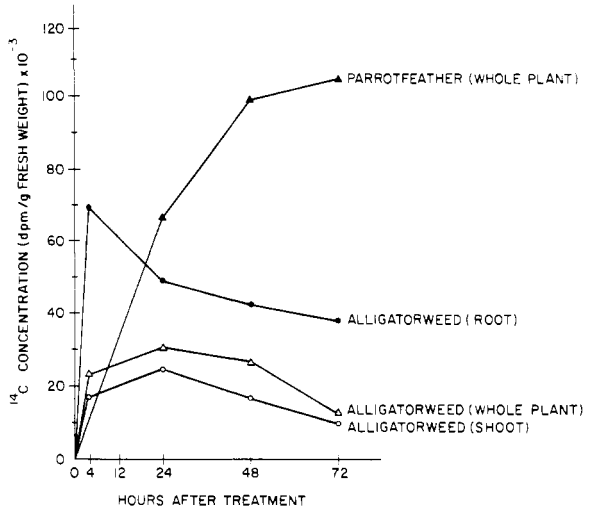

Figure 1. Absorption of [^{14}C]dichlobenil by alligator weed and parrot feather.

RESULTS

Parrot feather was markedly more resistant to dichlobenil than alligator weed. Visible injury symptoms were apparent in alligator weed within 24 hr of treatment with 1 ppm of dichlobenil. On the other hand, parrot feather showed no visible injury after 5 days of treatment with 2 ppm of the herbicide.

Absorption of [^{14}C]Dichlobenil by Alligator Weed and Parrot Feather. [^{14}C]Dichlobenil was absorbed readily by the roots of both alligator weed and parrot feather. Figure 1 shows the uptake of dichlobenil by the two plants from a solution containing 0.5 ppm of the ^{14}C-labeled herbicide. In alligator weed, a rapid absorption of dichlobenil occurred during the first 4 hr after treatment. The plant continued to absorb the herbicide up to 24 hr; thereafter, the amount of ^{14}C in the plant continued to decrease. The highest concentration of radioactivity in the roots was attained at 4 hr and then continuously decreased until 72 hr. The concentration of radioactivity in the shoot continued to increase for the first 24 hr, but decreased over the

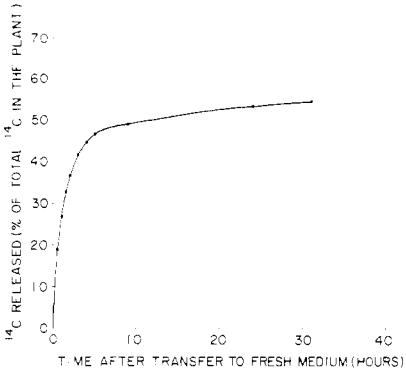

Figure 2. Efflux of [^{14}C]dichlobenil from alligator weed.

next 48 hr. The decrease in ^{14}C concentration in the roots during the 4–24-hr period was accompanied by an increase in the concentration of ^{14}C in the shoots during the same period. This decline can be attributed in part to the translocation of ^{14}C from the roots to the shoots. It was observed that about 50% of the ^{14}C was lost from the nutrient solution without plants within 24 hr. A continued decrease in the concentration of ^{14}C both in the roots and shoots after 24 hr indicates that in the presence of limiting amounts of [^{14}C]dichlobenil, there was a net loss of ^{14}C from the shoot and/or root due to volatilization and efflux into the nutrient solution. Verloop and Nimmo (1969) and Price and Putnam (1969) have demonstrated the evaporation of dichlobenil from the shoots of bean and corn plants after root uptake. Price and Putnam (1969) also showed that dichlobenil was lost from the roots of corn plants preloaded with the herbicide.

Parrot feather continued to absorb dichlobenil during the experimental period (72 hr). A rapid uptake of the herbicide occurred during the first 24 hr; thereafter, the rate of absorption decreased. This increase may be due to a decreasing concentration of dichlobenil in the medium. In contrast to alligator weed, the concentration of ^{14}C in parrot feather did not decrease during the experimental period, indicating that either ^{14}C was not lost from the plant or that parrot feather continued to absorb [^{14}C]dichlobenil at a rate sufficient to mask any loss of ^{14}C from the plant. The radioactivity in the root and shoot portions of parrot feather was not determined separately on account of a relatively small amount of root tissue in this experiment.

Distribution of Radioactivity in the Plant. Alligator weed and parrot feather showed a striking difference in the relative distribution of radioactivity in shoots and roots. The alligator weed roots contained 63% of the total ^{14}C in the plant as compared to 37% in the shoots 24 hr after treatment with [^{14}C]dichlobenil. In parrot feather, a major portion of the absorbed radioactivity was present in the shoot. In this plant, the roots and shoots contained 2 and 98%, respectively, of the total radioactivity in the plant after the same period of time.

Efflux of [^{14}C]Dichlobenil or Its Metabolites from the Plant. It has been shown that [^{14}C]dichlobenil diffuses from the roots of corn seedlings into the nutrient solution (Price and Putnam, 1969). It was, therefore, of interest to determine if a similar efflux of dichlobenil occurs from the roots of alligator weed and parrot feather. About 48% of the initial radioactivity present in alligator weed which had been previously exposed to [^{14}C]dichlobenil was released into the nutrient solution within 5 hr (Figure 2). Thin-layer chromatographic analysis of the nutrient solution showed that all the ^{14}C released from the plant was present as dichlobenil. On the contrary, no efflux of ^{14}C was observed from the roots of parrot feather. The findings indicate that dichlobenil is not actively held within the root cells of alligator weed, or that it alters the cell permeability, permitting the leakage of intracellular dichlobenil.

Table I. R_f Values of [^{14}C]Dichlobenil and Its Metabolites in Parrot Feather

Fraction	^{14}C-Labeled compd in plant extract	R_f value for solvent system[a] I	II
Steam distillate	2,6-Dichlorobenzonitrile	0.67	0.63
Non-steam-distillable			
Ether extract, pH 11	2,6-Dichlorobenzamide	0.45	0.30
Ether extract, pH 4	3-Hydroxy-2,6-dichlorobenzonitrile	0.63	0.37
	3-Hydroxy-2,6-dichlorobenzamide	0.36	0.17
	Unknown	0.1	0.00
Ether extract, pH 4, after hydrolysis with 2 *N* HCl	3-Hydroxy-2,6-dichlorobenzonitrile	0.58	0.42
	3-Hydroxy-2,6-dichlorobenzamide	0.38	0.15
	Unknown	0.03	0.02
Ether extract, pH 0.5	2,6-Dichlorobenzoic acid	0.26	0.16
	Unknown	0.06	0.02
Non-ether-extractable	Unknown	0.00	0.00

[a] Solvent system (v/v): (I) chloroform–ethanol–acetic acid (89:10:1); (II) benzene–methanol (9:1).

Metabolism of [^{14}C]Dichlobenil. Both the roots and shoots of alligator weed were analyzed for [^{14}C]dichlobenil and its metabolites, whereas in the case of parrot feather only the shoots were analyzed since the roots contained a very small amount of radioactivity. Extraction with 80% methanol removed more than 95% of the radioactivity from the shoots and between 80 and 85% from the roots. When the shoot and root extracts of alligator weed treated with [^{14}C]dichlobenil for 24 hr were steam distilled, practically all the radioactivity was present in the steam distillate fraction. This fraction cochromatographed with authentic dichlobenil. The results indicate that alligator weed was not capable of metabolizing the herbicide. In contrast to alligator weed, the ^{14}C in the extracts of parrot feather treated with the herbicide for 24 hr was present in the form of dichlobenil and several ^{14}C-labeled metabolites. The R_f values of the [^{14}C]dichlobenil and its metabolites detected in the various fractions of the shoot extracts are shown in Table I. The amounts of [^{14}C]dichlobenil and its metabolites present in the shoot 24 and 120 hr after treatment are shown in Table II. The major ^{14}C-labeled component in the shoot at 24 and 120 hr was 3-hydroxy-2,6-dichlorobenzonitrile. In addition, small amounts of radioactivity were found in the following metabolites: 2,6-dichlorobenzamide, 3-hydroxy-2,6-dichlorobenzamide, 2,6-dichlorobenzoic acid, and extractable conjugates of 3-hydroxy-2,6-dichlorobenzonitrile and 3-hydroxy-2,6-dichlorobenzamide. The phenol and acid fractions also contained small amounts of unknown ^{14}C-labeled compounds. A significant amount of radioactivity (14–18% of the ^{14}C in the shoot) was present as non-ether-extractable compounds which remained in the aqueous fraction after various ether extractions. This material did not move from the origin on the silica gel plates in solvent systems I or II.

It is apparent that, with time, there was a decrease in the quantity of [^{14}C]dichlobenil. This decrease was accompanied by an increase in the amount of metabolites suggesting that dichlobenil was being transformed by the plant. However, the increase in the radioactivity in various metabolites was not large enough to account for the

Table II. Distribution of [^{14}C]Dichlobenil and Its Metabolites in Parrot Feather Shoot

Compound	24 hr dpm/g fresh wt	24 hr % ^{14}C in shoot	120 hr dpm/g fresh wt	120 hr % ^{14}C in shoot
2,6-Dichlorobenzonitrile	161,727	31.7	4,915	1.2
2,6-Dichlorobenzamide	15,243	3.0	7,688	1.8
3-Hydroxy-2,6-dichlorobenzonitrile	193,000	37.8	234,599	55.1
3-Hydroxy-2,6-dichlorobenzamide	5,040	1.0	7,709	1.8
3-Hydroxy-2,6-dichlorobenzonitrile (directly extractable conjugates)	14,797	2.9	19,306	4.5
3-Hydroxy-2,6-dichlorobenzamide (directly extractable conjugates)	10,531	2.1	11,718	2.8
2,6-Dichlorobenzoic acid	4,607	0.9	16,025	3.8
Unknown (pH 4 fraction)	11,970	2.3	14,389	3.4
Unknown (pH 4 fraction, after hydrolysis)	7,835	1.5	14,299	3.4
Unknown (pH 0.5 fraction)	2,168	0.42	1,780	0.41
Non-ether-extractable compounds (aqueous phase)	73,165	14.3	76,884	18.1
Residue	10,198	2.0	16,088	3.8

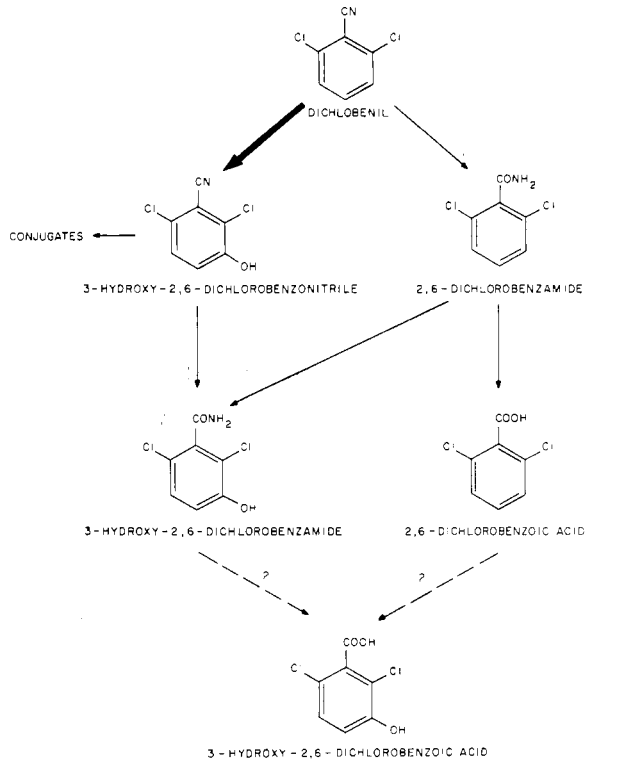

Figure 3. Proposed scheme for the metabolic transformation of dichlobenil in parrot feather. The heavy arrow indicates the major pathway.

decrease in the quantity of [^{14}C]dichlobenil. This would suggest that part of the [^{14}C]dichlobenil was lost from the plant. It was also noticed that in contrast to other ^{14}C-labeled metabolites, the amount of 2,6-dichlorobenzamide decreased with time.

DISCUSSION

These findings show that dichlobenil is absorbed by the roots of alligator weed and parrot feather and is translocated to the shoots. This is in agreement with the previous reports about the uptake and movement of dichlobenil in bean, wheat, rice, and corn plants (Massini, 1961; Pate and Funderburk, 1966; Price and Putnam, 1969; Verloop and Nimmo, 1969, 1970). Though dichlobenil was readily absorbed by the roots of both plants, ^{14}C from dichlobenil tended to accumulate more in alligator weed roots than in parrot feather roots, indicating that [^{14}C]dichlobenil and/or its metabolites are more mobile in parrot feather. Since parrot feather absorbed more [^{14}C]dichlobenil than alligator weed, the difference in susceptibility of the two species is not due to a difference in their capacity to absorb the herbicide. Alligator weed appeared unable to metabolize dichlobenil. These results do not agree with those of Pate and Funderburk (1966), who reported 2,6-dichlorobenzoic acid as a metabolite of dichlobenil in alligator weed. In contrast to alligator weed, parrot feather was quite effective in metabolizing the herbicide. Thus, it seems that the tolerance of parrot feather to dichlobenil results primarily from its ability to transform the herbicide to relatively nonphytotoxic metabolites. Differential metabolism appears to be a major factor contributing to the difference in susceptibility between parrot feather and alligator weed.

The major metabolite of dichlobenil in parrot feather was 3-hydroxy-2,6-dichlorobenzonitrile, indicating that hydroxylation is the major process by which the herbicide is transformed in this plant. The possible products of hydrolysis such as 2,6-dichlorobenzamide and 2,6-dichlorobenzoic acid were also detected but in small quantities, suggesting that hydrolysis of the nitrile group of the herbicide is a minor metabolic process in parrot feather. The fact that the amount of 2,6-dichlorobenzamide decreased with time indicates that this metabolite was further transformed. A likely possibility is the hydrolysis or hydroxylation of dichlorobenzamide to produce dichlorobenzoic acid or hydroxydichlorobenzamide, respectively. A small amount of 3-hydroxy-2,6-dichlorobenzamide was also detected. It is assumed that this compound was formed from the metabolites 3-hydroxy-2,6-dichlorobenzonitrile or 2,6-dichlorobenzamide. The two hydroxy metabolites were also present as conjugates with plant constituents. On the basis of the above findings, the pathway shown in Figure 3 is proposed for the metabolic transformation of dichlobenil in parrot feather.

With some exceptions, the pathway for the metabolism of dichlobenil in parrot feather appears to be similar to that operative in two terrestrial plants, bean and wheat as reported by Verloop and Nimmo (1969, 1970). They found that the hydroxylation of dichlobenil is the major process by which the herbicide is metabolized in the two plants. However, in bean and wheat plants treated with dichlobenil, 3-hydroxy-2,6-dichlorobenzonitrile was present mainly as conjugate(s) of plant constituents, whereas in parrot feather this metabolite was present primarily in an unconjugated form. A small amount of 3-hydroxy-2,6-dichlorobenzamide was found in parrot feather but not in bean and wheat plants. Another difference between the metabolism of dichlobenil in bean and parrot feather is the formation of highly polar metabolites in the latter. These metabolites were possibly polyhydroxy derivatives of dichlobenil and/or their conjugates. In parrot feather, these metabolites were present in the aqueous fraction remaining after various ether extractions, but were absent in the extracts of bean plants.

LITERATURE CITED

Frank, P. A., Hodgson, R. H., Comes, R. D., *Weeds* **11**, 124 (1963).

Hoagland, D. R., Arnon, D. I., *Calif. Agr. Exp. Sta. Circ.* **No. 347** (1950).

Lawrence, J. M., Beasley, P. G., Funderburk, H. H., *Proc. So. Weed Conf.* **10**, 368 (1963).
Massini, P., *Weed Res.* **1**, 142 (1961).
Metcalf, R. L., Osman, M. F., Fukuto, T. R., *J. Econ. Entomol.* **60**, 445 (1967).
Pate, D. A., Funderburk, H. H., Symposium on Use of Isotopes in Weed Research, International Atomic Energy Agency, Vienna, 1966, pp 17–25.
Price, H. C., Putnam, A. R., *J. Arg. Food Chem.* **17**, 135 (1969).
Smith, G. N., Ludwig, P. D., Wright, K. C., Bauriedel, W. R., *J. Agr. Food Chem.* **12**, 172 (1964).
Verloop, A., Nimmo, W. B., *Weed Res.* **9**, 357 (1969).
Verloop, A., Nimmo, W. B., *Weed Res.* **10**, 59 (1970).
Walker, C. R., *Weeds* **12**, 267 (1964).

Received for review September 10, 1973. Accepted December 10, 1973. Presented at the Meeting of the Weed Science Society of America, Atlanta, Ga., Feb 6–8, 1973. This investigation was supported by Contract DAC31-71-C-0026 from the Office of the Chief of Engineers.

Fate of 3,3-Dimethyl-1-(methylthio)-2-butanone *O*-(Methylcarbamoyl)oxime (Diamond Shamrock DS-15647) in Cotton Plants and Soil

Chandler J. Whitten* and Don L. Bull

In field-grown cotton plants, the carbamate pesticide Diamond Shamrock DS-15647 (3,3-dimethyl-1-(methylthio)-2-butanone *O*-(methylcarbamoyl)oxime) was rapidly oxidized to its toxic sulfoxide derivative, which was further oxidized, but more slowly, to the more toxic sulfone form. Degradation of the toxic forms occurred primarily by conversion to unidentified water-soluble products. In soil, the chemical changes of DS-15647 were similar to those found in plants, but they proceeded at slower rates. Cotton seeds that had been surface treated with DS-15647 and planted in the greenhouse absorbed 20% of the dose after 1 day. The sulfoxide and sulfone derivatives were the primary products recovered from plants grown from the treated seeds. In tests with several species of insects, DS-15647 applied topically was more effective than the sulfoxide or sulfone derivatives. The sulfone form was the most potent anticholinesterase agent.

Diamond Shamrock DS-15647 (3,3-dimethyl-1-(methylthio)-2-butanone *O*-(methylcarbamoyl)oxime), a new carbamate pesticide being developed by Diamond Shamrock Corp., Cleveland, Ohio, has shown potential systemic and contact insecticidal properties against certain phytophagous pests. For example, granular formulations of DS-15647 applied in the seed furrow at the time cotton is planted have demonstrated good control of thrips (*Frankliniella* spp.), the cotton aphid (*Aphis gossypii* Glover), the cotton fleahopper (*Pseudatomescelis seriatus* (Reuter)), the serpentine leafminer (*Liriomyza brassicae* (Riley)), and spider mites (*Tetranychus* spp.) (Davis and Cowan, 1974).

The fate of DS-15647 in biological systems has not been determined. However, the metabolism of a structurally similar carbamate, aldicarb [Temik; 2-methyl-2-(methylthio)propionaldehyde *O*-(methylcarbomyl)oxime], has been extensively studied in cotton plants (Bartley *et al.*, 1970; Bull, 1968; Coppedge *et al.*, 1967; Metcalf *et al.*, 1966), in insects (Bull *et al.*, 1967a; Metcalf *et al.*, 1966), in potatoes (Andrawes *et al.*, 1971b), and in soil (Andrawes *et al.*, 1971a; Bull, 1968; Bull *et al.*, 1970; Coppedge *et al.*, 1967). These investigations revealed that aldicarb is oxidized to toxic sulfinyl and sulfonyl derivatives that are degraded by hydrolysis to oximes or by conversion to other nontoxic metabolites. The present paper reports the results of similar studies designed to provide a general understanding of the fate of DS-15647 in cotton plants and soil.

EXPERIMENTAL SECTION

Chemicals. Quantities of ^{35}S-labeled DS-15647 and its sulfonyl derivative DS-20238 (3,3-dimethyl-1-(methylsulfonyl)-2-butanone *O*-(methylcarbamoyl)oxime) (initial specific activities of 18 and 2.2 mCi/mmol, respectively) were supplied by Diamond Shamrock Corp., Cleveland, Ohio. The labeled compounds were greater than 99% pure, as shown by thin-layer chromatography (tlc) and autoradiography. Nonradioactive chemicals used included DS-15647, DS-17839 (3,3-dimethyl-1-(methylsulfinyl)-2-butanone *O*-(methylcarbamoyl)oxime), DS-20238, DS-15619 (3,3-dimethyl-1-(methylthio)-2-butanone oxime), DS-20243 (3,3-dimethyl-1-(methylsulfinyl)-2-butanone oxime), and DS-20242 (3,3-dimethyl-1-(methylsulfonyl)-2-butanone oxime).

DS-15647 and its metabolites were resolved by tlc on glass plates coated (0.25-mm thick) with silica gel G by using solvent mixtures of: chloroform, ethyl acetate, and ethyl ether (6:2.5:2, v/v); chloroform, ethyl acetate, and butyl ether (6:2:2, v/v); or chloroform, ethyl acetate, butyl ether, and dioxane (4:2:2:2, v/v). Identification of metabolites was based on cochromatography of radioactive compounds with the authentic standards that were located colorimetrically by exposure to iodine vapors.

Radioassays of extracts or radioactive areas from chromatograms were made by liquid scintillation at ambient temperature. Data were corrected for radioactive decay and quenching.

TEST PROCEDURES AND RESULTS

Toxicity Studies. The relative toxicities of the three biologically active compounds (DS-15647 and its sulfoxide and sulfone) were investigated both *in vivo* and *in vitro*. The *in vivo* toxicity of the compounds to certain insect species associated with cotton was compared by determining the LD_{50} values of topical applications (serial dilutions in acetone solutions) to adult boll weevils (*Anthonomus grandis* Boheman), adult convergent lady beetles (*Hippodamia convergens* Guerin-Meneville), 2nd stage larvae of the common green lacewing (*Chrysopa carnea* Stephens), and 3rd stage larvae of the tobacco budworm

Cotton Insects Research Laboratory, Agricultural Research Service, U. S. Department of Agriculture, College Station, Texas 77840.