Metabolism of Dimethoate in Cotton Leaves

JOSEPH HACSKAYLO
and D. L. BULL

Crops Research Division and Entomology Research Division, United States Department of Agriculture in Cooperation with the Texas Agricultural Experiment Station, College Station, Texas

The metabolism of the systemic insecticide dimethoate was studied in excised cotton leaves. Its halflife was found to be 1.8 days through 4 half lives, but increased slightly thereafter. Eleven metabolites were resolved by paper chromatography, and eight of these were identified. The metabolite found in largest amount was the carboxy derivative. The oxygen analog remained relatively constant but consistently less than 6% of the total metabolites recovered. The proposed metabolic degradation of dimethoate after its introduction directly into the leaves of cotton is similar to that found in mammals but dissimilar to that reported when the insecticide was applied as a foliar spray. Its metabolism in leaves deficient in N, P, and Fe also is reported.

DIMETHOATE [*O,O*-dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorodithioate] is a unique compound which has both plant (*1, 13, 19*) and animal (*7, 14*) systemic insecticidal properties. It is a desirable compound to use for investigational purposes since it is a white solid at room temperature and is soluble in water as well as in many organic solvents (*1*). It has the further advantage of being easily isolated and determined chromatographically.

Detailed studies on the metabolism of dimethoate in the boll worm [*Heliothis zea* (Boddie)] and boll weevils (*Anthonomus grandis* Boheman) (*2*) and in mammals (*3, 4, 11*) have been completed. The absorption, translocation, and some aspects of the metabolism of this insecticide in plants have been reported (*6, 10, 13, 16, 18*). Santi and de Petri-Tonelli (*17*) isolated the oxygen analog [*O,O*,-dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorothiolate] of dimethoate from broadbean plants after root absorption of the insecticide. These investigators did not report the isolation of any of its other metabolites. Dauterman *et al.* (*5*) performed a detailed study on the degradation of dimethoate when the insecticide was applied as a spray to several plants including cotton. They also proposed a pathway for its degradation in plants based on their findings.

Primarily, the investigations herein reported were undertaken to establish the metabolic fate of dimethoate when the insecticide was introduced directly into leaves of cotton. Also, observations in this laboratory indicated that cotton plants containing the insecticide but grown in a medium low in phosphorus remained toxic to aphids longer than plants cultured in a substrate containing adequate nutrients. Therefore, another aspect of this investigation was to establish whether leaves from plants deficient in a particular essential element would metabolize the insecticide differently than leaves from plants grown in complete nutrients. Since the oxygen analog is the only metabolite of dimethoate known to be toxic to animals, it was of prime importance; the other metabolites, however, were studied also.

Materials and Methods

Cottonseed of the variety Deltapine 15 were germinated in flats of sand in the greenhouse. Three days after emergence, the seedlings were transferred to a modified Hoagland's nutrient solution (*8*) and to nutrient solutions having singular omissions of N and P. To obtain cotton leaves deficient in Fe, control plants were transferred at 4 weeks of age to solutions with Fe omitted. Only the leaves of these plants formed subsequently developed Fe chlorosis.

When the plants were 8 weeks old, the third and fourth leaves from the terminal were removed by cutting the petioles under water. The basal ends of the petioles were inserted into separate small vials containing 100 μg. of P^{32}-labeled dimethoate dissolved in 200 μl. of water. After the solutions in the vials were absorbed, each leaf was transferred to a 125-ml. Erlenmeyer flask containing water. The leaves were then placed under fluorescent lights in the laboratory and exposed to a 12-hour photoperiod. The light intensity was 1800 foot-candles and the ambient temperature 76° to 89° F.

Extraction. Leaves from each series were harvested in duplicate 1, 3, 6, and 14 days after treatment with the insecticide. Each leaf was macerated in a tissue grinder with 34 ml. of a 95:5 acetone:water solution (v./v.). The tissue grinder was placed in an ice bath to prevent heating during grinding. The tissue homogenate was cooled in a deep freeze for 4 hours and centrifuged for 10 minutes at 2000 r.p.m. The supernatant was decanted into a small flask and evaporated under a jet of air in the hood.

Chromatography. The paper chromatographic procedure used to resolve dimethoate and its metabolites was similar to that reported by Chamberlain *et al.* (*3*). Briefly, portions of the extract were spotted on an 11- × 14-inch sheet of Whatman No. 1 filter paper. The paper was shaped to fit a glass jar 18 inches tall and 6 inches in diameter. The solvent system was a 40:9:1 mixture of acetonitrile–water–ammonium hydroxide (v./v.). After the solvent had ascended to about 30 cm., the chromatograph was removed, dried, and exposed to x-ray film. Five days later, the x-ray film was developed. The chromatograph was matched with the radioautograph and each radioactive spot circled and cut out. The amounts of radioactivity associated with each metabolite were determined by counting each spot in a thin-window gas flow counter. Duplicate chromatographs were run on each leaf extract.

Radioactive Compound. The P^{32}-labeled dimethoate, which was obtained from Volk Radiochemical Co., Skokie, Ill., had an original specific activity of 4200 c.p.m. per microgram. The compound was only about 95% pure; therefore, it was further purified by dissolving it in chloroform and extracting out the impurities with 10% aqueous sodium carbonate and then with water.

Table I. Common Names, R_f Values, and Scientific Names of Dimethoate and Its Metabolites Recovered from Cotton Leaves

(40:9:1 acetonitrile:H_2O:NH_4OH, paper chromatograph)

Metabolite No.	Common Name	R_f	Scientific Name
1	Phosphoric acid	0.00	Phosphoric acid
2	Unknown A	0.03	
3	Unknown B	0.08	
4	Desmethyl thiocarboxy derivative	0.11	*O*-Methyl *O*-hydrogen *S*-carboxymethyl phosphorodithioate
5	Dimethyl phosphate	0.18	*O,O*-Dimethyl phosphoric acid
6	Thioate	0.34	*O,O*-Dimethyl phosphorothioic acid
7	Dithioate	0.47	*O,O*-Dimethyl phosphordithioic
8	Thiocarboxy	0.51	*O,O*-Dimethyl *S*-carboxymethyl phosphorothioate
9	Unknown C	0.58	
10	Oxygen analog	0.77	*O,O*-Dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorothiolate
11	Dimethoate	0.91	*O,O*-Dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorodithioate

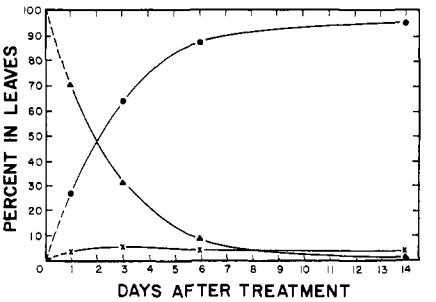

Figure 1. Dimethoate, nontoxic metabolites, and oxygen analog in cotton leaves treated by excised-leaf techniques

(●, nontoxic metabolites; ▲, dimethoate; x, oxygen analog)

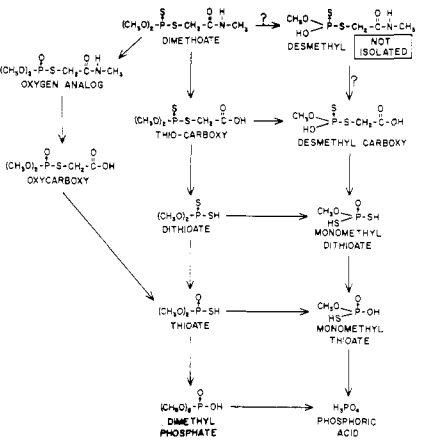

Figure 2. Proposed metabolic pathway for dimethoate in cotton leaves after treatment by excised-leaf technique

The final product was 99% pure as established by paper chromatography and had a specific activity of 3930 c.p.m. per microgram.

Results

Identification of Compounds. Eleven radioactive sites were found consistently following chromatography of the extracts from the leaves treated with P^{32}-labeled dimethoate. Eight of these compounds were identified by matching their R_f values with known materials, whereas three remained unidentified (Table I). The three unknown materials occurred in relatively small amounts in the leaf extracts when compared with the total radioactivity found. Since it is apparent that the R_f values of the metabolites of dimethoate increase as their polarities decrease, tentative identification of unknowns A and B can be as monomethyl phosphorothioate and monomethyl phosphorodithioate, respectively. Also, it can be speculated that unknown C is the oxycarboxy derivative.

Recovery of Metabolites. The amounts of each radioactive metabolite of dimethoate found in the leaves of cotton 1, 3, 6, and 14 days after treatment are listed in Table II. These data show that leaves deficient in N, P, or Fe retained an over-all ability to metabolize this insecticide. The leaves deficient in Fe metabolized less dimethoate than those from control plants 1 and 6 days after treatment; however, this reduction was not evident at the 3-day harvest. Reliable data were not obtained for the Fe-deficient leaves at 14 days because of their poor vigor. The leaves deficient in N and P metabolized dimethoate as rapidly as the control leaves at 1 day, but it was apparent that the rate of metabolism in the leaves from these two treatments was slower than the controls at the three subsequent harvests.

The oxygen analog, the only known toxic metabolite of dimethoate, remained consistently less than 6% in all leaves regardless of treatment. The leaves deficient in Fe had the lowest oxygen analog content when compared with the leaves from other treatments. At all harvest times and in all leaves, the carboxy derivative accumulated in greater quantities than the other metabolites. The dithioate, thioate, and dimethyl phosphate were found in the next largest amounts. The remaining metabolites were present in relatively small quantities.

Figure 1 shows the relative amounts of dimethoate, the oxygen analog, and nontoxic metabolites remaining with time in control leaves. After 14 days, less than 5% of the total radioactivity in the extract was present as the toxic materials. The halflife of dimethoate in the leaves was found to be 1.8 days through 4 halflives but lengthened slightly thereafter.

Discussion

The excised-leaf technique is used in many laboratories throughout the world and is excellent for studying the metabolism of a compound by plants. Metcalf *et al.* (*15*) used this procedure to investigate the effects of temperature on the metabolism of the systemic insecticide Di-Syston in leaves of various plants. By this method, it is possible to administer a given quantity of material to a leaf and follow its breakdown with little fear of excretion as is the case in animals.

In the present investigation, the metabolite of dimethoate accumulating in greatest amount is the carboxy derivative. Dauterman *et al.* (*5*) were unable to recover this intermediate from leaves of cotton when dimethoate was applied as a foliar spray. On the other hand, these same authors were able to recover large quantities (69.2%) as the desmethyl derivative from the water-soluble fraction, whereas this metabolite was not detected in the present investigation. Although a known standard of the desmethyl derivative was not available for this study, Chamberlain *et al.* (*3*) stated its R_f value to be 0.25 when acetonitrile:H_2O (80:20 v./v.) was used to develop the paper chromatograph. By using their system to resolve the plant extracts obtained in the current study, a spot with a comparable R_f value could not be obtained. A small quantity of the desmethyl carboxy derivative was detected and was probably preceded by the production of the desmethyl and/or the carboxy derivative.

The leaves deficient in iron consistently had less oxygen analog present than any of the other treatments, and this reduction was probably associated with reduced cytochrome oxidase and peroxidase activity. Knaak *et al.* (*12*) recently presented evidence which suggests that peroxidases in plants play an important role in the metabolism of parathion and related phosphorothionates. The rapid increase in the carboxy derivative in leaves from this particular

Table II. Dimethoate and Its Metabolites in Excised Leaves Following Treatment with the Insecticide by the Excised-Leaf Technique at Indicated Days and Nutrient Level (all values as percent)

Metabolite No.[a]	Nutrient Level: Complete, Days				Minus N, Days				Minus P, Days				Minus Fe, Days		
	1	3	6	14	1	3	6	14	1	3	6	14	1	3	6
1	0.6	2.0	1.4	2.2	tr	1.7	1.7	1.6	0.8	2.2	1.9	2.5	tr	1.3	0.9
2	0.6	0.8	0.8	1.7	tr	0.8	1.4	0.8	tr	0.5	1.6	1.7	tr	0.9	0.5
3	tr	0.5	2.7	4.9	0.8	0.4	3.2	2.3	0.7	2.0	2.7	4.4	tr	2.4	1.3
4	0.9	2.0	1.5	2.8	0.8	2.5	1.1	1.0	0.7	1.2	3.1	2.1	tr	1.0	0.7
5	2.5	5.8	7.5	10.9	2.2	4.5	6.6	6.8	2.1	4.2	6.6	8.2	0.9	6.0	5.5
6	1.9	7.1	6.2	12.0	2.5	4.5	6.2	8.0	2.4	6.9	7.2	9.0	1.4	5.7	3.9
7	3.5	5.5	10.6	10.9	6.0	4.9	9.1	5.2	3.9	4.9	10.9	9.3	1.4	3.3	6.2
8	15.0	38.0	53.4	46.8	11.7	26.1	47.2	48.3	10.5	25.6	44.2	47.4	10.5	45.0	58.0
9	1.8	2.2	3.2	3.3	0.5	1.7	3.4	2.9	1.1	1.8	3.3	2.3	tr	1.7	2.7
10	2.9	4.6	3.9	2.6	2.7	3.8	4.3	5.5	3.6	3.9	5.4	2.9	0.5	2.3	2.1
11	70.0	31.0	8.6	1.9	72.2	48.0	15.5	17.0	74.4	46.1	13.0	10.3	85.0	30.3	17.2

[a] See Table I for identification of metabolites.

treatment, as well as in the others, strongly indicates that the first major step in the enzymatic breakdown of dimethoate is through the splitting of the C—N bond. Only a minor portion of the insecticide passes through the oxygen analog intermediate. A proposed pathway for the metabolic degradation of dimethoate in leaves of cotton is shown in Figure 2. This pathway is very similar to that proposed for sheep (*3*), rats, and cows (*4*), but very unlike the one proposed for plants when the insecticide was applied as a foliar spray (*5*).

One can evaluate the significance of the various metabolic pathways outlined in Figure 2 by assessing the relative importance of the enzyme systems involved in the degradation of dimethoate and its metabolites. The most significant is the amidase which splits the C—N bond of dimethoate or its oxygen analog. Next is the esterase which splits the two-carbon fragment at the S—C linkage from the phosphate moiety. The enzymes responsible for converting the thiono-function to the corresponding oxo-function and the one cleaving the *O*-methyl esters are relatively inactive. The latter two systems are very important in foliar applications where they are not competing with the amidase.

The metabolism of dimethoate per se in the leaves of plants deficient in N, P, and Fe proceeded at an over-all reduced rate when compared with the controls. Hacskaylo and Lindquist (*9*) previously reported that only minor differences were found when the metabolism of dimethoate in healthy cotton leaves was compared with that of leaves deficient in a number of essential elements. However, the deficiency symptoms in the above work were not as acute as those in the present experiment. Hacskaylo and Lindquist also reported that after root absorption of the insecticide, the intermediates recovered from intact plants were the same as those recovered from excised leaves, but the former degraded the insecticide three times as fast.

In evaluating the toxic components, the oxygen analog must be considered, even though it occurred in amounts less than 6% of the total recoverable metabolites. The data presented herein and those of Dauterman *et al.* (*5*) show that the metabolism of dimethoate by the cotton plant after foliar application is very unlike that found when the insecticide was fed directly into the leaves. Therefore, the mode of application of the insecticide in evaluating residual problems must be considered also.

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