Benomyl was persistent in the soil and was detected in all elms treated from 1970 to 1974. The amount of benomyl in roots was greater than the amount detected in leaves for every year except 1974, although 1973 treatments were similar (Table III). The amount of benomyl detected in the roots was greatest the year of application. Thereafter, a fairly constant amount of accumulation was detected, averaging 0.4 μg/ml. Leaf assays made more than 1 year following treatment indicated accumulated levels of benomyl not significantly different from untreated trees. Chloroform-extraction procedures detected benomyl for five growing seasons in root samples and for at least two growing seasons in leaf samples.

Although the side effects of the injection of benomyl into the soil are unknown, literature is available which states that the practice is effective in disease control (Smalley, 1971; Stipes, 1973). At the rate of application required for efficacy, benomyl or a benomyl derivative can be detected by bioassay in leaves, wood, and bark of treated elms (Biehn and Dimond, 1971; Hock et al., 1970; Zaronsky and Stipes, 1969). Inhibition of spore germination on seeded agar plates may not be sufficiently sensitive to detect the fungicide (Smalley, 1971; Hock and Schreiber, 1971), the principal limitation of the seeded plate bioassay being the sensitivity of the test fungus. More sensitive bioassay methods have been used successfully (Smalley et al., 1973). These bioassays will detect benomyl at concentrations as low as 0.01 μg/ml (Black, 1975).

The amount of benomyl required for efficacy against Dutch elm disease has not been established. In in vitro tests a concentration of 0.5 μg/ml of benomyl stopped growth of *C. ulmi*, while at 0.1 μg/ml growth was only slightly inhibited (Edgington et al., 1971; Hart, 1972). Neely (1973) noted, using the same field trees as in this test, that the 36 g/m^2 rate resulted in complete control of Dutch elm disease, while the 9 g/m^2 rate was only partially successful. His results, combined with this study, would show that detected levels of accumulation of not less than 0.5 μg/ml in the leaves would indicate sufficient benomyl uptake for total Dutch elm disease control. Levels of accumulation less than 0.5 μg/ml were not consistently successful in preventing *C. ulmi* colonization.

Sufficient benomyl accumulation to afford protection is rapidly reached. In the greenhouse levels of accumulation in the leaves of 0.5 μg/ml were reached only 7 days after treatment. In field tests, such levels were reached within 30 days following treatment, at the same rate.

The soil injection of benomyl will result in a detectable systematic accumulation of benomyl within the elm. Benomyl, so applied, will remain available to the tree in amounts that can control Dutch elm disease beyond the year of application. Levels of accumulation of benomyl as low as 0.1 μg/ml can be readily detected using the simple, inexpensive chloroform-extraction assay of leaves outlined herein. Such an assay, combined with the proper assay fungus, allows for a sensitive assay of whole-tree benomyl content, using only a small amount of tissue without disturbing the landscape.

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Toxicity, Penetration, and Metabolism of Chlordimeform and Its *N*-Demethyl Metabolite in Cabbage Looper Larvae

Cheryl S. Crecelius and Charles O. Knowles*

The insecticide chlordimeform and its *N*-demethyl metabolite, *N'*-(4-chloro-*o*-tolyl)-*N*-methylformamidine or demethylchlordimeform, were more toxic to third instar cabbage looper larvae than to fifth instar larvae, with the parent formamidine being the more toxic in each case. When both larval instars were treated topically with the same amount of chlordimeform-^{14}C, penetration was slower in third instar larvae than in fifth instar larvae. There were no apparent qualitative differences in the chlordimeform metabolites between the two larval instars but quantitative differences were evident. Third instar larvae converted chlordimeform to demethylchlordimeform and to polar metabolites slower than did fifth instar larvae. Metabolism studies with demethylchlordimeform-^{14}C revealed a similar trend between the two instars. The slower penetration and metabolism of chlordimeform in third instar cabbage looper larvae as compared to fifth instar larvae may explain, at least in part, the differential toxicity.

Chlordimeform has an interesting spectrum of insecticidal activity, being toxic primarily to eggs and early instar larvae of some lepidoptera. For example, with the cabbage looper, *Trichoplusia ni* (Hübner), an economically important pest, susceptibility to chlordimeform decreases with increasing larval instars (Kuhr, 1974), and the basis for this phenomenon is obscure. In many instances selective toxicity can be explained on the basis of differences

Department of Entomology, University of Missouri, Columbia, Missouri 65201.

in penetration and/or metabolism. Therefore, the purpose of this study was to investigate the toxicity, penetration, and metabolism of radiolabeled chlordimeform and its toxic *N*-demethyl metabolite, *N'*-(4-chloro-*o*-tolyl)-*N*-methylformamidine or demethylchlordimeform, in third and fifth instar cabbage looper larvae.

MATERIALS AND METHODS

Insects and Compounds. Third and fifth instar cabbage loopers were provided by the Biological Control of Insects Research Laboratory, Columbia, Mo. They were received in cardboard cups containing the standard looper diet.

The following nonradioactive compounds were used in these studies: chlordimeform, *N'*-(4-chloro-*o*-tolyl)-*N*-methylformamidine or demethylchlordimeform, *N'*-(4-chloro-*o*-tolyl)formamidine or didemethylchlordimeform, 4'-chloro-*o*-formotoluidide, and 4-chloro-*o*-toluidine (Benezet and Knowles, 1976). Radioactive compounds included chlordimeform-*tolyl*-^{14}C (specific activity 4.58 mCi/mmol) provided by CIBA-GEIGY Corp., Greensboro, N.C., and demethylchlordimeform-*tolyl*-^{14}C (specific activity 10 mCi/mmol) synthesized in our laboratory (Benezet and Knowles, 1976). The radiochemical purity of chlordimeform and demethylchlordimeform was greater than 99.0 and 90.0%, respectively.

Chromatography and Radioisotope Methodology. TLC was used for separation of chlordimeform-^{14}C, demethylchlordimeform-^{14}C, and their organosoluble metabolites recovered from cabbage loopers. The adsorbent was a 500-μm layer of silica gel GF_{254}, and the chromatogram was developed with a benzene–diethylamine (95:5) solvent system (Sen Gupta and Knowles, 1969). Average R_f values were: 0.64 for chlordimeform, 0.32 for demethylchlordimeform, 0.10 for didemethylchlordimeform, 0.14 for 4'-chloro-*o*-formotoluidide, and 0.47 for 4-chloro-*o*-toluidine. Radioautographs were prepared with no-screen x-ray film (Eastman Kodak Co., Rochester, N.Y.).

The radiocarbon content in samples recovered from metabolism studies was measured with a Beckmann Model LS-100C liquid scintillation counter (Beckmann Instruments, Inc., Fullerton, Calif.). Samples for radioassay were prepared as described by Sen Gupta and Knowles (1970).

Toxicity Studies. Third instar cabbage looper larvae were treated on the dorsum of the abdomen with 0.5 μl of an acetone solution containing the desired amount of formamidine. Fifth instar larvae were treated similarly but with 1 μl of solution. Each treated larva was placed in a small test tube (12 × 75 mm) which was subsequently plugged with cotton. The cabbage loopers were kept for 48 h at 28 °C and mortality was recorded with the aid of a binocular microscope. At least three replicates of 20 loopers per replicate were tested at each concentration. Appropriate controls were treated with acetone.

The influence of piperonyl butoxide on formamidine toxicity also was studied. Loopers were treated topically with 2.5 μg of piperonyl butoxide dissolved in 0.5 μl of acetone 30 min prior to treatment with chlordimeform or demethylchlordimeform.

Toxicity data are reported as percentage mortality at specific concentrations of formamidines, and in some cases as LD_{50} values derived from plots of those parameters.

Metabolism Studies. For each experiment 160 cabbage loopers were treated topically on the dorsum of the abdomen with chlordimeform-^{14}C or demethylchlordimeform-^{14}C. Each insect received 5000 cpm of chlordimeform-^{14}C (0.9 μg) or demethylchlordimeform-^{14}C (0.04 μg) in 0.5 μl of acetone. Each treated larva was placed in a test tube (12 × 75 mm) capped with Parafilm "M" (American Can Company, Neenah, Wis.). Insects treated in this way were kept for periods of 0, 0.5, 1, 3, 6, 12, 24, and 48 h prior to analysis.

At the designated time interval 20 cabbage loopers were transferred from their respective test tube to a ground glass homogenizing tube and were rinsed three times with 5-ml aliquots of acetone. The three rinses were combined in a scintillation vial and evaporated to near dryness (insect rinse). The test tubes were rinsed twice with 0.5-ml aliquots of acetone, and the rinses were combined in a scintillation vial and evaporated to dryness (container rinse). The radioactivity in the scintillation vials containing the insect rinse and the container rinse was measured.

Following the acetone rinse, the cabbage loopers were homogenized in 5 ml of acetone, and the homogenate was transferred to a centrifuge tube. After centrifugation at low speed the supernatant was decanted, and the precipitate was homogenized again in 5 ml of acetone. This homogenate was centrifuged, and the supernatant was decanted. The acetone extraction was repeated once more. The three acetone extracts were combined, dried over anhydrous sodium sulfate, and transferred to a 100-ml boiling flask. The acetone was evaporated almost to dryness on a rotary evaporator.

For third instar loopers, the concentrated extract (organic extract) was diluted to a volume of 3 ml, and a 0.1-ml aliquot was radioassayed. The remaining material was concentrated to a volume of 0.1 ml and subjected to TLC, radioautography, and radioassay for identification and quantification of the radioactive components.

Fifth instar loopers contained substantial amounts of water which was extracted into the acetone. Therefore, the concentrated extract was transferred to a separatory funnel and was partitioned between 15 ml of chloroform and 10 ml of water. The water fraction was extracted three times with 15-ml aliquots of chloroform. A 0.1-ml aliquot was taken from the 10-ml water fraction and radioassayed. The chloroform extracts were combined, added to a boiling flask, and evaporated almost to dryness on the rotary evaporator. The concentrate (organic extract) was diluted to 3 ml and a 0.1-ml aliquot was radioassayed. The remaining material was concentrated to a volume of 0.5 ml. Due to the high lipid content in fifth instar loopers, this concentrate was not easily spotted and resolution of the spots was not good, so the concentrate was streaked on the TLC plate. It was then subjected to TLC, radioautography, and radioassay.

The insect residue remaining after acetone extraction was extracted twice with 3-ml aliquots of water. The aqueous extracts were combined, and a 0.1-ml aliquot was radioassayed. For third instar loopers this constituted the aqueous extract. For fifth instar loopers the radioactivity in this extract was combined with the radioactivity in the water fraction from the water–chloroform partition to comprise the aqueous extract.

The particulate looper residue (insect residue) remaining after the extractions was dried at 28 °C, and the total radioactivity in the insect residue was determined by oxygen flask combustion and subsequent radioassay of the trapped $^{14}CO_2$ (Sen Gupta and Knowles, 1969).

The influence of piperonyl butoxide on the metabolism of chlordimeform-^{14}C was studied following the procedure described above. Cabbage loopers were treated topically with 10 μg of piperonyl butoxide dissolved in 0.5 μl of acetone 30 min prior to treatment with the formamidine.

Table I. Toxicity of Chlordimeform and Demethylchlordimeform Alone and in the Presence of Piperonyl Butoxide to Third and Fifth Instar Cabbage Looper Larvae[a]

Treatment	Formamidine alone[b]	Formamidine + piperonyl butoxide[c]	
Chlordimeform			
Third instar larvae	15, 750	2.5:	20, 22
		20.0:	55, 56
Fifth instar larvae	175, 1346	125.0:	30, 28
		250.0:	69, 65
Demethylchlordimeform			
Third instar larvae	70, 3500	20.0:	13, 55
		120.0:	80, 94
Fifth instar larvae	>600, >4615	200.0:	15, 30

[a] Average weight of larvae was 20 mg for third instar and 130 mg for fifth instar. Mortality was recorded at 48-h posttreatment. [b] LD_{50} expressed as micrograms/larva and micrograms per gram, respectively. [c] Formamidine–piperonyl butoxide toxicity data expressed as follows: formamidine dose in micrograms/larva: percent mortality from formamidine alone, percent mortality from formamidine plus piperonyl butoxide. Larvae were treated topically with piperonyl butoxide (2.5 μg/larva) 30 min prior to topical application of formamidine. There was no mortality from piperonyl butoxide alone.

RESULTS

Toxicity of Chlordimeform and Demethylchlordimeform. The toxicity of chlordimeform and demethylchlordimeform to third and fifth instar cabbage looper larvae is given in Table I. When applied topically both formamidines were more toxic to third instar larvae than to fifth instar larvae, and chlordimeform was more toxic than demethylchlordimeform.

Table I also gives the influence of piperonyl butoxide on the toxicity of chlordimeform and its *N*-demethyl analogue to third and fifth instar cabbage looper larvae. Piperonyl butoxide applied topically at a level of 2.5 μg per larva 30 min prior to treatment with the formamidines had no discernible effect on the toxicity of chlordimeform to third and fifth instar larvae. However, in the case of demethylchlordimeform synergism was evident with both larval instars. For example, mortality of third instar larvae treated with 20 μg of demethylchlordimeform per larva was 13.0% after 48 h. In the presence of piperonyl butoxide mortality was increased to 55.0%.

Metabolism of Chlordimeform. The distribution and metabolism of chlordimeform-^{14}C following topical application to third and fifth instar cabbage loopers at a concentration of 0.9 μg per larva are given in Table II. The external radioactive material (container and insect rinses) can be used as an index of penetration during the initial posttreatment intervals. However, at later time intervals it cannot be used as such because of the rapid excretion of chlordimeform-^{14}C equivalents. Thus, for third instar larvae the external radioactive materials decreased from 98.0% at 0 time to a low of 26.4% at 3 h; for fifth instar larvae the external radioactive material decreased from 91.7% at 0 time to a minimum of 12.4% at 1 h. Therefore, penetration of chlordimeform-^{14}C was more rapid with fifth instar as compared to third instar larvae.

Piperonyl butoxide applied topically at a concentration of 10 μg per larva 30 min prior to topical application with chlordimeform-^{14}C retarded penetration of radioactive material in both instars. For example, with piperonyl butoxide the concentration of external radioactive material was 53.6% at 3 h for third instar larvae (not shown) and 27.6% at 1 h for fifth instar larvae (Table II).

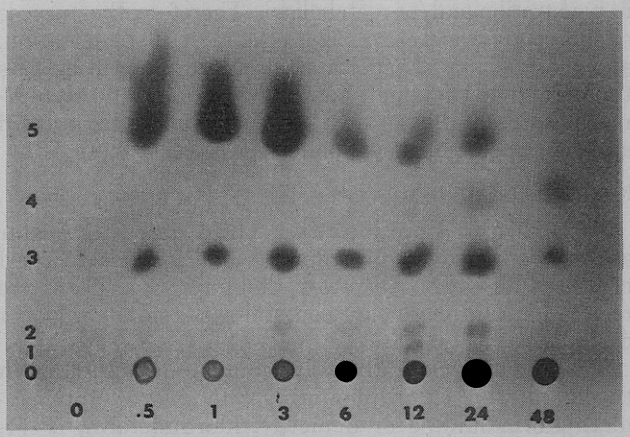

Figure 1. Radioautograph of organosoluble chlordimeform-^{14}C equivalents isolated from third instar cabbage looper larvae. Legend: Horizontal axis gives hours after treatment. Vertical axis: 0 = TLC origin (R_f 0.00); 1 = didemethylchlordimeform (R_f 0.10); 2 = 4′-chloro-*o*-formotoluidide (R_f 0.14); 3 = demethylchlordimeform (R_f 0.32); 4 = 4-chloro-*o*-toluidine (R_f 0.47); and 5 = chlordimeform (R_f 0.64).

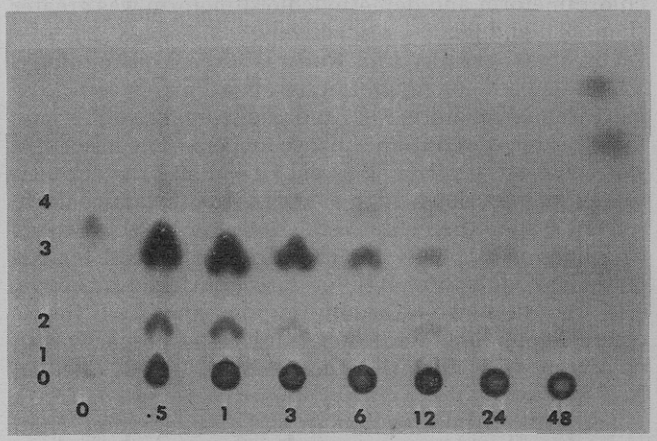

Figure 2. Radioautograph of organosoluble demethylchlordimeform-^{14}C equivalents isolated from third instar cabbage looper larvae. See Figure 1 for legend.

The distribution and nature of the internal radioactive material from chlordimeform-^{14}C-treated third and fifth instar larvae also are given in Table II, and Figure 1 shows the results obtained when the organosoluble fraction from third instar larvae was subjected to TLC. In third instar larvae chlordimeform increased from 1.2% at 0 time to a peak of 49.5% at 3 h and decreased to only 0.5% by 48 h. With fifth instar larvae chlordimeform increased from 3.9% at 0 time, reached a maximum level of 58.0% at 0.5 h, and declined to 5.2% at 48 h. Demethylchlordimeform was a major metabolite in both larval instars (Table II). It reached a peak of 12.9% at 3 h in third instar larvae and 15.6% at 1 h in fifth instar larvae. Didemethylchlordimeform, 4′-chloro-*o*-formotoluidide, and 4-chloro-*o*-toluidine were minor chlordimeform metabolites in cabbage looper larvae (Table II). There were appreciable amounts of polar radioactive material in both larval instars (Table II). These materials reached a peak of 18.6% at 24 h for third instar and 36.7% at 3 h for fifth instar larvae. Levels of radioactivity in the insect residue were low and with one exception (3.1%) never exceeded 1.7% (Table II).

Pretreatment of cabbage looper larvae with piperonyl butoxide had a dramatic effect on the ratio of the internal radioactive material (Table II). Generally, there were higher levels of chlordimeform in both third and fifth

Table II. Penetration and Metabolism of Chlordimeform-^{14}C in Third and Fifth Instar Cabbage Looper Larvae Alone and in the Presence of Piperonyl Butoxide[a]

Distribution and nature of radioactive material	% recovered radioactive material at indicated h after treatment													
	0		0.5		1.0		3.0		12.0		24.0		48.0	
	Third	Fifth	Third	Fifth	Third	Fifth	Third	Fifth	Third	Fifth	Third	Fifth	Third	Fifth
External radioactive material														
Container rinse			15.7	3.5	14.9 (18.1)	1.9 (7.8)	10.4	9.6	34.3 (26.6)	38.9 (33.8)	29.9	42.5	40.1	30.5
Insect rinse	98.0	91.7	40.6	10.9	27.3 (30.7)	10.5 (19.8)	16.0	19.0	27.5 (24.6)	19.5 (12.5)	35.5	21.7	38.6	22.1
Internal radioactive material														
Chlordimeform	1.2	3.9	37.2	58.0	46.8 (46.7)	45.3 (63.4)	49.5	20.8	7.9 (33.0)	5.6 (23.2)	6.2	6.1	0.5	5.2
Demethylchlordimeform	<0.1	<0.1	3.1	7.7	5.6 (0.8)	15.6 (1.8)	12.9	9.3	11.3 (6.0)	4.6 (2.3)	7.1	2.9	1.0	2.2
Didemethylchlordimeform		<0.1		0.4		0.5 (0.3)		0.5		0.3 (0.1)		0.1		<0.1
4'-Chloro-o-formotoluidide	<0.1	0.2	0.6	0.9	0.9 (0.8)	1.7 (1.2)	1.9	1.2	2.0 (1.3)	0.5 (0.8)	1.1	0.5	0.4	1.2
4-Chloro-o-toluidine	<0.1	<0.1	0.4	0.8	0.6 (0.7)	1.3 (0.7)	1.5	0.7	0.9 (0.9)	0.6 (0.7)	0.9	0.9	0.8	2.1
Aqueous extract + TLC origin	0.6	3.5	2.2	16.1	3.7 (2.2)	21.5 (4.8)	7.5	36.7	15.8 (7.4)	26.9 (25.9)	18.6	24.5	17.3	35.6
Insect residue	0.2	0.7	0.2	1.7	0.2 (0.1)	1.7 (0.2)	0.3	2.2	0.3 (0.2)	3.1 (0.7)	0.7	0.8	1.3	1.1
Applied radioactivity recovered, %	93.5	92.7	90.6	99.4	93.5 (95.6)	99.0 (90.2)	94.5	91.3	90.4 (86.7)	90.5 (98.1)	90.0	93.5	94.2	98.9

[a] Figures are means of at least three determinations. The piperonyl butoxide data, shown for 1.0 and 12 h only, are given in parentheses; larvae were treated topically with piperonyl butoxide (10.0 μg/larva) 30 min prior to topical application of the formamidine.

Table III. Penetration and Metabolism of Demethylchlordimeform-^{14}C in Third and Fifth Instar Cabbage Looper Larvae[a]

Distribution and nature of radioactive material	% recovered radioactive material at indicated h after treatment													
	0		0.5		1.0		3.0		12.0		24.0		48.0	
	Third	Fifth	Third	Fifth	Third	Fifth	Third	Fifth	Third	Fifth	Third	Fifth	Third	Fifth
External radioactive material														
Container rinse			22.6	6.1	23.0	5.3	27.0	12.8	27.1	24.0	20.7	33.7	32.4	31.0
Insect rinse	95.7	92.8	31.5	17.1	30.0	13.8	35.2	7.7	26.4	13.4	31.8	26.2	38.3	29.1
Internal radioactive material														
Demethylchlordimeform	3.0	5.0	28.1	50.3	25.4	31.6	12.4	22.2	2.1	5.9	1.1	2.3	0.8	1.5
Didemethylchlordimeform	<0.1	<0.1	1.5	0.7	1.5	0.7	1.3	1.2	0.6	0.4	0.5	0.3	0.3	0.4
4'-Chloro-o-formotoluidide	0.3	0.5	4.3	6.2	3.7	5.6	2.0	2.6	0.5	0.7	0.4	0.7	0.4	1.0
4-Chloro-o-toluidine	<0.1	<0.1	2.9	0.2	2.9	0.4	2.3	0.3	1.6	2.1	1.0	1.3	0.4	3.8
Aqueous extract + TLC origin	1.0	1.4	8.9	19.1	13.2	42.1	19.0	52.5	40.7	52.4	43.4	34.3	26.1	31.9
Insect residue	<0.1	0.3	0.2	0.3	0.3	0.5	0.8	0.7	1.0	1.1	1.1	1.2	1.3	1.3
Applied radioactivity recovered, %	94.8	96.4	92.0	89.4	88.5	97.1	91.6	96.0	91.3	94.6	95.2	91.9	91.5	97.3

[a] Figures are means of at least three determinations.

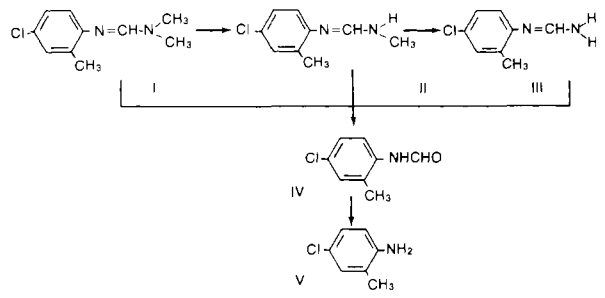

Figure 3. Proposed metabolic fate for chlordimeform and demethylchlordimeform in cabbage looper larvae. Legend: I = chlordimeform; II = demethylchlordimeform; III = didemethylchlordimeform; IV = 4′-chloro-*o*-formotoluidide; and V = 4-chloro-*o*-toluidine.

instar larvae in the presence of piperonyl butoxide than in those larvae treated with chlordimeform alone. Also, there were generally lower levels of demethylchlordimeform and of polar metabolites (aqueous extract + TLC origin) (Table II).

Metabolism of Demethylchlordimeform. Table III presents the distribution and metabolism of demethylchlordimeform-^{14}C following topical application to cabbage loopers at a concentration of 0.04 μg per larva. For third instar larvae the external radioactive material decreased from 95.7% at 0 time to 54.1% at 0.5 h; with fifth instar larvae the level of radioactivity decreased from 92.8 to 23.2% for the same time interval. Based on the levels of radioactivity in the container and insect rinses it appeared that demethylchlordimeform penetrated faster in fifth instar larvae than in third instar larvae.

The distribution and nature of the internal radioactive material from demethylchlordimeform-^{14}C-treated cabbage looper larvae also are given in Table III, and Figure 2 shows the results obtained when the organosoluble fraction from third instar larvae was subjected to TLC. In third instar larvae demethylchlordimeform increased from 3.0% at 0 time to a maximum of 28.1% at 0.5 h and decreased to 0.8% at 48 h. For fifth instar larvae demethylchlordimeform increased from 5.0% at 0 time to a peak of 50.3% at 0.5 h and decreased to 1.5% by 48 h. The major metabolite identified from both larval instars was 4′-chloro-*o*-formotoluidide. Didemethylchlordimeform and 4-chloro-*o*-toluidine were minor metabolites. There were appreciable amounts of polar radioactive materials (aqueous extract + TLC origin); for third instar larvae the peak level was 43.4% at 24 h, and for fifth instar larvae the peak level was 52.5% at 3 h. Levels of radioactivity in the insect residue were low and did not exceed 1.3% (Table III).

DISCUSSION

Chlordimeform was more toxic to third instar cabbage looper larvae than to fifth instar larvae. At the LD_{50} level fifth instar larvae tolerated 11.7 times as much chlordimeform as did third instar larvae; on a weight basis the tolerance was 1.8 times. This discussion will be devoted primarily to a consideration of the role that differential penetration and/or metabolism might have played in this phenomenon.

Qualitatively there were no discernible differences in the metabolic paths of chlordimeform in third and fifth instar cabbage looper larvae (Figure 3). Chlordimeform (I) was N-demethylated to demethylchlordimeform (II) and didemethylchlordimeform (III). These formamidines were subsequently converted to 4′-chloro-*o*-formotoluidide (IV) and 4-chloro-o-toluidine (V) as well as to other unidentified polar compounds. Also, there were no discernible qualitative differences in the metabolic fate of demethylchlordimeform in third and fifth instar cabbage looper larvae. Demethylchlordimeform (II) was metabolized to didemethylchlordimeform (III) and subsequently to 4′-chloro-*o*-formotoluidide (IV) and 4-chloro-*o*-toluidine (V) (Figure 3).

However, quantitative differences in chlordimeform metabolism between third and fifth instar cabbage looper larvae were evident, and this was especially noticeable when a comparison was made between levels of chlordimeform and demethylchlordimeform in the two larval instars. Chlordimeform and demethylchlordimeform were selected for this comparison because they were both toxic to cabbage looper larvae, and their levels inside the larvae could be critical in terms of selectivity. From the data presented in Table II it is evident that the third instar larvae contained higher levels of chlordimeform plus demethylchlordimeform for longer periods of time than did fifth instar larvae. Third instar larvae also contained much lower levels of polar metabolites than did fifth instar larvae.

Penetration of chlordimeform was rapid in the case of both larval instars. However, it was faster in fifth instar larvae than in third instar larvae.

Therefore, between the two larval instars there existed differences both in chlordimeform penetration and metabolism. It seemed that the difference in penetration was a consequence of the difference in metabolism. Thus, chlordimeform penetration was dependent upon the removal of the internal chlordimeform by metabolism. It appeared from this study that fifth instar cabbage looper larvae were more resistant than third instar larvae to the toxic action of chlordimeform at least in part because they were more efficient in degrading it to polar nontoxic metabolites. This contention was supported by the results of experiments with piperonyl butoxide. Piperonyl butoxide is a known inhibitor of microsomal mixed function oxidases, and chlordimeform is metabolized by this enzyme system (Ahmad and Knowles, 1971). The piperonyl butoxide was applied topically to larvae 30 min prior to chlordimeform to preclude a physical interference in the penetration of the toxicant and to allow sufficient time to inhibit, at least partially, the mixed function oxidase system. In these experiments there was a decrease in chlordimeform penetration, an increase in internal levels of chlordimeform, a decrease in demethylchlordimeform, and a decrease in water-soluble metabolites.

In view of these effects by piperonyl butoxide on chlordimeform metabolism and subsequently on penetration it is interesting that there was no discernible influence on toxicity either to third or fifth instar larvae. This was due possibly to differences in the levels of piperonyl butoxide used in the toxicity and metabolism studies. For toxicity studies a piperonyl butoxide level of 2.5 μg per larva was used, and for metabolism studies the level of piperonyl butoxide was 10 μg per larva. Thus, there was four times more piperonyl butoxide used in the metabolism experiments.

The piperonyl butoxide did slightly synergize the toxicity of demethylchlordimeform to third and fifth instar looper larvae. This probably resulted from interference by piperonyl butoxide with the very rapid degradation of demethylchlordimeform by cabbage looper larvae.

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Ovicidal Activity and Its Relation to Chemical Structure for the Two-Spotted Spider Mite (*Tetranychus urticae* Koch) in a New Class of Miticides Containing the Cyclopropyl Group

Clive A. Henrick,* W. Edward Willy, Gerardus B. Staal, and George F. Ludvik

Several series of cyclopropyl-containing compounds have been studied and some relationships between chemical structure and direct contact ovicidal activity were found by bioassay on the two-spotted spider mite, *Tetranychus urticae* Koch. Ovicidal activity appears to be the predominant response observed on mites. The data suggest that the ovicidal potency of the compounds is primarily related to the presence of the cyclopropyl group in the molecule, or to the ability of the compounds to be biosynthetically converted in vivo to some toxicant (or toxicants) which probably contains a cyclopropyl ring. General and specific requirements for activity in this class of compounds are presented.

Mites (Acarina) occur throughout the world and can be serious pests of plants, animals, and man (cf. Krantz, 1971). Of the phytophagous mites, members of the Tetranychidae are among the most destructive to agricultural crops. In our work, which was directed toward the chemical control of agriculturally important mites, we used the two-spotted spider mite (*Tetranychus urticae* Koch) and studied (Staal et al., 1975) a new class of cyclopropyl-containing miticides (Henrick and Staal, 1974, 1975a–e; Nelson and Show, 1975; Hurkova and Matolin, 1975) whose action, unlike many present commercial miticides (cf. Billings, 1974; Kenaga and End, 1974), is predominantly ovicidal. Certain members of this class of compounds appear promising for use as commercial miticides judging from the results of large scale field tests. Previously we have discussed the general biological properties of these compounds (Staal et al., 1975) and here we wish to describe some detailed relationships between structure and direct contact ovicidal activity selected from our extensive investigation of this class of compounds.

BIOASSAY PROCEDURE

Assay. All direct ovicidal assays described in this paper were carried out with *Tetranychus urticae* Koch. Our colony, a nonresistant strain, was obtained from Dr. W. W. Allen, University of California, Berkeley, and has been reared in our laboratories on lima bean plants for several years without exposure to chemicals. For the bioassay, compounds were dissolved in acetone. Since many of the compounds in this study were insoluble in water and were solids at room temperature we were unable to find a standard emulsifier base which gave consistent emulsification over a wide range of compounds. Rather than using different emulsifier compositions for different groups of compounds and thus introducing another element of uncertainty, the compounds were all dispensed in acetone solutions after we were satisfied that the differences in activity between a water formulation and an acetone dilution were insignificant in this type of assay. Acetone dipping does kill the plant tissue of the leaf disks, but this did not appear to effect the emergence of the eggs, provided that the leaf disks were allowed to dry out completely. With this technique we were able to ensure that only intrinsic differences in activity, not influenced by differences in dispersion and adjuvants, were studied. Separate solutions with concentrations ranging from 10^{-3} to 10^{-7} g/ml were prepared by successive tenfold dilutions of 1 mg/ml stock solution of compound. Generally, an initial assay series was run using three dilutions (10^{-3}, 10^{-4}, and 10^{-5} g/ml). In some cases where compounds exhibited high ovicidal activity, a more extensive series of fractional dilutions was subsequently applied in order to obtain more precise dose–response curves and LC_{50} values from probit analysis (cf. Finney, 1971).

Approximately 35 eggs, 0–24 h of age, were obtained on the upper surface of a 1.0-cm diameter lima bean primary leaf disk held on moist glass wool, from 24-h oviposition of six female mites. The females were then removed and the egg infested disks were dipped for 2 s into the acetone assay solutions (three disks were treated at each dose level). After dipping the disks were held face down on a paper towel for 1 s and then turned over until the acetone had evaporated. Each disk was then glued (Elmer's Glue-All) individually to a section of a 50-mm disposable plastic petri dish to prevent crumpling while drying out. The eggs were then incubated, after counting, at 25 °C and 68–72% relative humidity for 6 days. The number of unhatched eggs was then recorded and the mortality was calculated using Abbott's correction (Abbott, 1925) for any spontaneous failure to emerge observed in the controls (three disks).

LC_{50} Values. These were obtained by interpolation from lines derived from semilog plots of concentration (definition: 1% concentration = 1 g/100 ml) vs. percent response. The activity range of interest for the LC_{50} was

Chemistry and Biology Research Departments, Zoecon Corporation, Palo Alto, California 94304.