SP2100 column. The retention time of the two detectable components of Et_3 was identical with the retention time of the methyl esters of 2,4,5-T-Glu and 2,4,5-T-Asp. Figures 3 and 4 show the mass spectra of the methyl esters of the two components of Et_3 and compare these spectra with that of the synthetic compounds. As is evident, the major fragmentation ions of the isolated compounds are congruent with that of 2,4,5-T-Glu and 2,4,5-T-Asp. Collectively these data indicate that the major metabolites of 2,4,5-T in 4-week-old soybean callus tissue cultures incubated with 2,4,5-T for 6 days are 2,4,5-T-Glu (major) and 2,4,5-T-Asp (minor).

A major portion (ca. 73%) of water-soluble metabolites was recovered in the diethyl ether fraction upon Emulsin treatment (Figure 1). This indicates the presence of aglycons which were released by enzymatic hydrolysis (β-glucosidase) (Hamilton et al., 1971). Although the compounds found in the water fraction are generally referred to as glycosides, they might also contain conjugates of sugar esters since Emulsin is a crude preparation of different hydrolytic enzymes. For example, 2,4-D-Glu is hydrolyzed to 2,4-D and glutamic acid with Emulsin (Feung et al., 1973). The hydrolyzate contained only trace amounts of 2,4,5-T, so the carboxylic glycoside was not present in any significant extent as has been found for 2,4-D (Feung, et al., 1975).

Ring hydroxylation has been thought to be a means of detoxification of 2,4-D and a number of hydroxylated derivatives of 2,4-D have been identified. With 2,4,5-T only 4OH-2,5-D has been reported as a minor metabolite in bean (Hamilton et al., 1971). If hydroxylation is a method of reducing biological activity then the relatively small amounts of water-soluble metabolites in soybean callus tissue indicate that detoxification through hydroxylation takes place at a much slower rate for 2,4,5-T than for 2,4-D. On the other hand, the biological activity of 2,4,5-T and its amino acid conjugates, which are present in significant amounts, are considerably less biologically active than 2,4-D or its amino acid conjugates (Arjmand et al., 1978b).

These data indicate that 2,4,5-T is metabolized by soybean cotyledon callus tissue to ether- and water-soluble metabolites in a similar manner to that of 2,4-D. Only a small portion of the ^{14}C label is found in the callus tissue residue; therefore, little 2,4,5-T is associated with macromolecular fractions. Also, the high recovery of radioactivity precludes appreciable decarboxylation in the tissue. The main portion of the ^{14}C label was recovered in the ether fraction which contained at least eight different compounds of which 2,4,5-T-Glu and 2,4,5-T-Asp consisted of 81–82% of the metabolites of this fraction. No attempt was made to identify any of the other minor metabolites.

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Metabolism of *cis*- and *trans*-[^{14}C]Permethrin by the Tobacco Budworm and the Bollworm

Walter S. Bigley and Frederick W. Plapp, Jr.*

The fate of ^{14}C-acid- and -alcohol-labeled cis and trans isomers of permethrin (3-phenoxybenzyl 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate) was studied after topical treatment of larvae of the tobacco budworm, *Heliothis virescens* (F.), and the bollworm, *Heliothis zea* (Boddie). All permethrin preparations were very stable in *Heliothis* larvae under in vivo conditions, but older larvae detoxified the insecticide more rapidly than younger larvae. Permethrin metabolism was both hydrolytic and oxidative and the trans isomer was metabolized more rapidly than the cis isomer. Metabolism was more rapid in the tobacco budworm than in the bollworm, a factor probably responsible for the twofold greater tolerance of the former to permethrin.

The tobacco budworm, *Heliothis virescens* (F.), and the bollworm, *Heliothis zea* (Boddie), are major pests of cotton in the United States, Mexico, and Central America. The tobacco budworm is resistant to essentially all insecticides registered for use on cotton (Adkisson, 1968; Nemec and Adkisson, 1969; Plapp, 1971, 1972). Studies with resistant tobacco budworms indicate resistance is due primarily to

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increases in detoxification mechanisms involving aliesterases, mixed-function oxidases, and conjugating enzymes (Whitten and Bull, 1970; Bull and Whitten, 1972; Williamson and Schecter, 1970; Plapp, 1973). The bollworm is resistant to the organochlorine insecticides (Adkisson, 1964), but, in contrast to the tobacco budworm, has shown no field resistance to organophosphates or carbamates. Resistance, therefore, is a major problem with both species, but is more serious with the tobacco budworm.

The recent development of highly insecticidal photostable pyrethroids is an important advance for insect control. The pyrethroid insecticide permethrin possesses properties of photostability and high activity (Elliott et al., 1976) which make it a prime candidate for use in controlling the tobacco budworm and bollworm in cotton. Permethrin is an ester of 3-phenoxybenzyl alcohol (Elliott et al., 1973a). Its isomers are very potent insecticides and persist longer than many related compounds (Elliott et al., 1973b, 1974; Burt et al., 1974). Gaughan et al. (1977) showed that the cis and trans isomers of permethrin are readily metabolized by ester cleavage in rats and that the ester linkage of *cis*-permethrin is more stable than that of *trans*-permethrin. They also found that oxidation occurs but is secondary in importance to ester cleavage as a detoxification mechanism.

This paper deals with the metabolism of *cis*- and *trans*-[^{14}C]permethrin in larvae of laboratory-reared bollworms and tobacco budworms. This knowledge is needed to explain why permethrin is an effective insecticide for the control of *Heliothis* spp. and to provide base line data should resistance problems occur later.

The effect on permethrin metabolism of acephate and chlordimeform, two insecticides known to be synergistic with permethrin (Plapp, 1977), was also measured. Acephate is a good substrate for esteratic (hydrolytic) detoxifying enzymes (Suksayretrup and Plapp, 1977) and chlordimeform is a good substrate for microsomal oxidases (Knowles and Sen Gupta, 1970).

MATERIALS AND RESULTS

Chemicals. Samples of cis (c) and trans (t) isomers of permethrin labeled with ^{14}C in the alcohol and acid moieties were obtained from FMC corporation, Middleport, N.Y. Specific activities were 57.0 mCi/mmol for the ^{14}C alcohol isomers and 54.8 mCi/mmol for the ^{14}C acid isomers. Thin-layer chromatography demonstrated the labeled materials were more than 98% pure after chromatographic cleanup. Technical grade acephate and chlordimeform used in synergist studies were supplied by the manufacturers. Structures of test compounds are shown in Figure 1.

Insects. Insects were third to sixth instar larvae of the tobacco budworm and early fifth instar larvae of the bollworm, reared in the laboratory on a standard diet (Vanderzant et al., 1962) and maintained on a 14:10 light–dark cycle at 27 °C and 60% relative humidity

Bioassays. The toxicities of permethrin and its cis and trans isomers were determined to third instar larvae of the tobacco budworm and the bollworm. The test method, previously described in detail (Plapp, 1971), consisted of exposing larvae for 96 h to films of insecticide in 20-mL glass vials. Twelve–twenty larvae were tested at each of a series of doses causing partial mortality. LC_{50}, LC_{90}, and slope values of the dosage–mortality lines were determined by a probit analysis program on a Hewlett-Packard computer, Model 9810-A.

In Vivo Assays. Groups of five to ten tobacco budworm larvae were treated topically with the desired

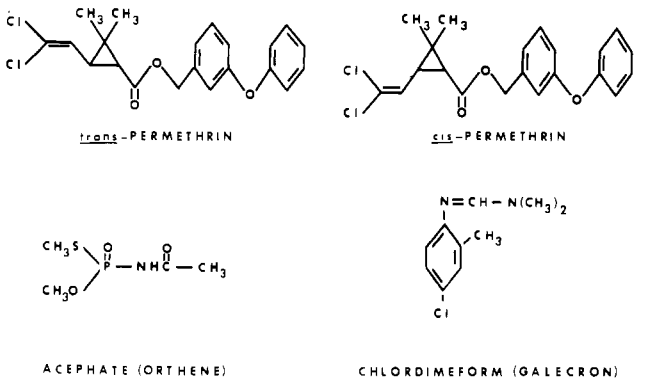

Figure 1. Structures of *cis*- and *trans*-permethrin and the insecticidal synergists, acephate and chlordimeform.

sublethal concentrations of the ^{14}C isomers of permethrin in 1 μL of acetone. When acephate and chlordimeform were used, they were applied topically to larvae 2–4 h before treatment with the permethrins. The tests were replicated two–five times. After treatment, insects were held individually in scintillation vials without food or water.

Larvae were sacrificed at 8, 16, or 24 h after treatment by freezing. They were rinsed with 5–10 mL of acetone (surface fraction) and homogenized with methanol in glass tissue homogenizers. All homogenization procedures were carried out on ice. Homogenates were centrifuged for 10 min at 2000*g*. The supernatants were removed, and the pellets were resuspended and recentrifuged twice in 2 mL of methanol. The supernatants for each group were pooled (internal fraction) and placed in a freezer overnight to allow further precipitation of proteins. Pellets after centrifugation were air-dried (residue).

Fecal pellets and test containers were soaked 24 h in 3 mL of methanol and homogenized and centrifuged at 2000*g* for 10 min. The pellet was resuspended twice in 1 mL of methanol and recentrifuged. Supernatants and container rinses were pooled (excreted fraction).

Aliquots of all extracts (0.2–0.5 mL) were quantitated by counting in a liquid scintillation counter.

Organosoluble and water-soluble radioactivity were separated by partitioning the total methanol extracts, after concentration, three times with water and chloroform or ether. Combined organic solvents were then extracted again with water. In some tests radioactivity remaining in the water was extracted with ethyl acetate. Conjugated metabolites were freed by acid (1 N HCl) hydrolysis in sealed vials for 10 min in a boiling water bath, or by treating first with 1 N NaOH for 10 min in a boiling water bath followed by acidification to pH 2.0 with HCl. Base- and acid-hydrolyzed fractions were extracted three times with 2 mL of ether. Ether extracts were pooled, dried over sodium sulfate, and concentrated with a stream of nitrogen. The known [^{14}C]permethrin isomers were also carried through the base–acid hydrolysis and extraction procedures and the products characterized by TLC.

Thin-Layer Chromatography. Concentrates of extracts from surface, internal, and excreted fractions, and their organosoluble acid or base–acid hydrolyzed conjugates were characterized by TLC. Brinkman Silcel Mix 25 or Analtech Silica Gel 60 with fluorescent indicator (F-254), 0.25-mm layer thickness precoated glass plates were used (EM Laboratories Inc., Elmsford, N.Y.). Solvent systems consisted of benzene–carbon tetrachloride–acetic acid (50:50:1) (BCA) and benzene (saturated with formic acid by shaking 10 mL of benzene with 1 mL of 88%

Table I. Thin-Layer Chromatographic Characteristics of *trans*- and *cis*-Permethrins and Their Metabolites

	R_f			
	BEF[a]		BCA[b]	
compound	cis	trans	cis	trans
permethrin	0.96	0.96	0.87	0.78
esterase metabolites				
Cl_2CA	0.82	0.78	0.27	0.26
PB-alcohol	0.69	0.69	0.24	0.24
secondary metabolites[c]				
c- or *t*-HO-Cl_2CAs*	0.57	0.54		
	0.46	0.41	0.17	0.15
	0.43	0.35		
c-HO-Cl_2CA lactone*	0.72	0.70		
2′-HO-PB-acid	0.57	0.57		
2′-HO-PB-alcohol	0.53	0.53		
4′-HO-PB-acid	0.5	0.5	0.06	0.06
4′-HO-PB-alcohol	0.34	0.34	0.03	0.03
PB-acid	0.81	0.81	0.25	0.25
oxidase metabolites				
2′-HO-permethrin	0.91	0.91		
4′-HO-permethrin	0.74	0.74		
c- or *t*-HO-permethrin*	0.62	0.61	0.22	0.22
unknowns	0.14	0.14		
	0.25			
	0.64	0.64		

[a] BEF solvent system: benzene (saturated with formic acid by shaking 10 mL of benzene with 1 mL of 88% formic acid)-ether (10:3). [b] BCA solvent system: benzene-carbon tetrachloride-acetic acid (50:50:1). [c] Metabolites followed by asterisk are tentative identifications of compounds for which no authentic standards were available.

formic acid–ether (10:3) (BEF). All extracts were characterized by both systems.

Developed chromatographs were viewed under UV light and exposed to X-ray film for location of standards and radioactive spots. Amounts of insecticide and metabolites were determined by scraping appropriate areas from TLC plates and counting in 5–10 mL of scintillation fluid. R_f values for all metabolites are presented in Table I.

The nomenclature used to designate the hydroxylated permethrin metabolites is characterized by 4′-HO, *c*-HO, *c*-permethrin, which represents the *cis*-permethrin isomer hydroxylated at the 4′ position of the alcohol moiety and at the methyl group of the *gem*-dimethyl moiety which is cis to the carboxyl group. The hydrolysis products from the acid moieties of *trans*- and *cis*-permethrin are *t*-Cl_2CA and *c*-Cl_2CA (2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acids), respectively, and PB-alc (phenoxybenzyl alcohol). Cl_2CA and its hydroxylated derivative and lactone are shown in Figure 2. Derivatives of PB-alc and phenoxybenzoic acid (PB-acid) included compounds hydroxylated at the 2′, 4′ and possibly the 6′ positions (Figure 3). The nomenclature used in this paper to describe the permethrin metabolites is similar to that reported by Gaughan et al. (1977).

The zones on TLC plates thought to represent the HO-Cl_2CA and lactone metabolites are tentative identifications. For furher characterization, these spots were eluted from the plates, hydrolyzed with base, and extracted with ether, and then acidified and extracted with ether. In all cases the radioactivity was recovered in the acid–ether extracts, demonstrating they were ester-cleaved acid moiety metabolites.

Authentic standards of the permethrin isomers and several permethrin metabolites were cochromatographed with the appropriate extracts. Standards supplied by FMC included *cis*- and *trans*-permethrin, PB-alc, PB-acid, 2′- and 4′-HO-PB-alc and the corresponding hydroxylated

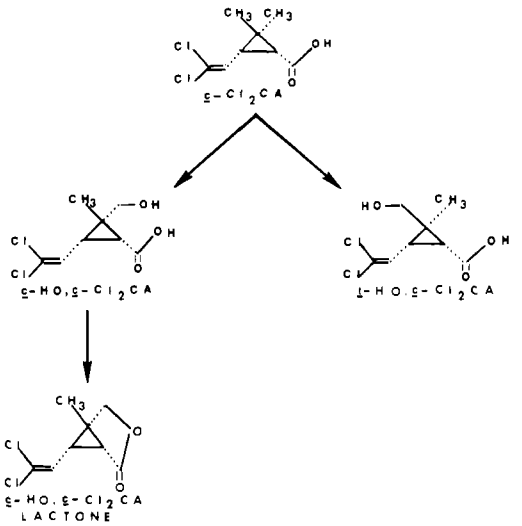

Figure 2. Structures of possible ester metabolites from *cis*-[*acid*-^{14}C]permethrin.

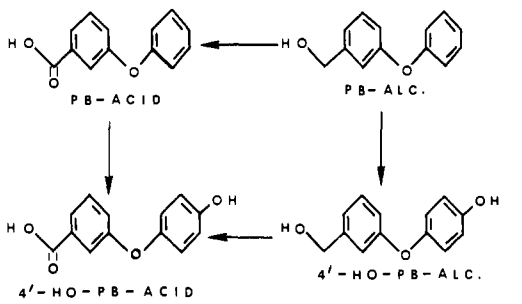

Figure 3. Structures of possible *cis*- and *trans*-[*alcohol*-^{14}C]-permethrin metabolites. Hydroxylation may also occur at the 2′ and 6′ position of the phenoxybenzyl moiety.

PB-acid derivatives, 2′- and 4′-O-methyl-(±)-*c*,*t*-permethrin and (±)-*c*,*t*-Cl_2CA.

General Recoveries of Radioactivity. Extraction efficiency was greater than 98% for all permethrin isomers as determined by treating larvae and larval homogenates with known amounts of ^{14}C-labeled permethrin isomers and assaying as previously described. Nonextractable radioactivity from third and fifth instar larvae accounted for less than 0.4% of the applied dose, and residues of sixth instar larvae contained no more than 10% of the applied radioactivity. Total recovery in all experiments was never less than 95% of the applied dose.

RESULTS

Bioassays. The toxicities of permethrin (60:40 trans–cis isomers) and its cis and trans isomers to the tobacco budworm and the bollworm are shown in Table II. The data indicate permethrin is a highly effective insecticide against these pests. It is about twice as toxic to the bollworm and about ten times as toxic to the budworm as methyl parathion, the standard insecticide used for control of these insects on cotton (Plapp, 1971, 1972). The twofold difference in response to permethrin between species may reflect a natural difference in susceptibility to the insecticide or more likely, cross-resistance to permethrin present in the test tobacco budworm strain, a strain known to be resistant to methyl parathion. No susceptible strains of the tobacco budworm were available for testing.

cis-Permethrin was about 2.5 times as toxic as permethrin itself to both species. Since commercial permethrin is 40% cis isomer, the data suggest permethrin toxicity is due largely to this isomer. *trans*-Permethrin

Table II. Toxicities of Permethrin (60:40 Trans-Cis Isomers) and Its Cis and Trans Isomers to Third Instar Larvae of the Tobacco Budworm and the Bollworm

	96-h toxicity in μg of insecticide/vial				slope of *d–m* line	
	LC_{50}		LC_{90}			
insecticide	budworm	bollworm	budworm	bollworm	budworm	bollworm
permethrin	4.58 (3.71–5.61)	2.88 (2.15–3.72)	17.43 (12.98–26.76)	12.26 (8.28–26.77)	0.96	0.88
cis-permethrin	2.03 (1.69–2.40)	1.14 (0.65–1.57)	5.52 (4.43–7.47)	4.91 (3.54–8.68)	1.28	0.88
trans-permethrin	11.37 (8.86–14.18)	3.12 (2.62–3.67)	36.41 (27.03–58.52)	7.92 (6.37–10.88)	1.10	1.38

Table III. Characterization of Organosoluble Radioactivity in Fifth Instar Bollworm Larvae 24-h after Topical Application of Permethrin Isomers

				% recovered organosoluble radioactivity				
					metabolites			
isomer	fraction	dose, ppm	%[a] applied dose	permethrin	esterase	oxidase	secondary	origin[b]
trans acid	surface	0.11	7.3	79.6	5.8	0.0	8.6	6.0
	internal		61.9	67.8	9.3	0.0	8.9	14.0
	excreted		30.6	40.5	15.2	0.0	22.3	22.0
cis acid	surface	0.11	12.3	94.5	0.0	0.0	3.0	2.5
	internal		68.9	88.4	1.8	0.9	1.6	7.3
	excreted		18.6	78.8	5.2	0.0	5.4	10.6
trans alcohol	surface	0.22	15.5	87.7	0.7	0.0	2.2	9.4
	internal		50.6	78.0	0.6	0.0	9.5	11.9
	excreted		33.7	84.7	0.4	0.0	4.3	10.6
cis alcohol	surface	0.11	15.5	98.1	0.0	0.0	0.9	1.0
	internal		61.3	80.7	1.3	0.0	11.2	6.8
	excreted		22.9	80.2	2.1	0.0	9.2	8.5

[a] Remainder is unextracted residue ^{14}C which equaled 0.2 to 0.3%. [b] Origin radioactivity also contains minor amounts of unknown metabolites.

was much less toxic than *cis*-permethrin, particularly to the tobacco budworm. The greater tolerance of the budworm to this isomer suggests permethrin tolerance may be due to differences between species in ability to metabolize this isomer.

Metabolism. Preliminary Tests. In preliminary tests with third instar tobacco budworm larvae, metabolism of the four radioactive preparations was studied by TLC analysis of extracts of insects sampled 16 h after topical treatment with sublethal concentrations. The result (data not shown) indicated both acid- and alcohol-labeled trans preparations were metabolized more extensively than the corresponding cis preparations.

Similar experiments were performed with sixth instar *H. virescens* larvae sampled 8 h after topical treatment with the radioactive permethrin preparations. Again, the trans preparations were metabolized more extensively than the cis preparations, and the overall metabolism of both compounds was greater than in third instar larvae.

Metabolism. Bollworm Larvae. Fifth instar larvae of the bollworm were treated topically with the four radioactive permethrin preparations and sampled 24 h later. In these tests, efforts were made to characterize conjugated metabolites as well as those directly extractable with ether. Results of analyses of internal and excreted radioactivity are summarized in Tables III and IV and Figure 4.

Measurements of distribution of the chemicals showed similar rates of uptake for all treatments (Table III). Retention of absorbed radioactivity was lower and excretion more rapid for *trans*-permethrin than for *cis*-permethrin. Most of the radioactivity from all treatments was present as unmetabolized insecticide.

The trans acid preparation was metabolized more extensively than the cis acid and the proportion of secondary metabolites was higher with the trans acid. Both alcohol preparations were metabolized at similar rates, but the trans alcohol was used at twice the dose of the cis alcohol. This was done to compare metabolism of *cis*- and

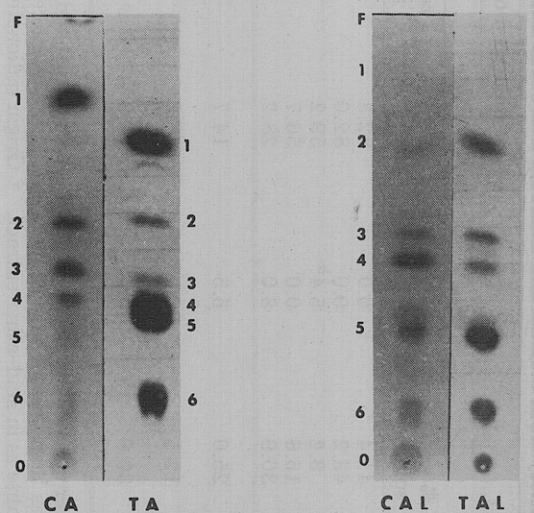

Figure 4. Radioautographs of the acid–ether extractable permethrin metabolites after base–acid hydrolysis of water-soluble conjugates from fifth instar bollworm larvae. CA = *cis*-[*acid*-^{14}C]permethrin internal fraction; TA = *trans*-[*acid*-^{14}C]permethrin excreted fractions; CAL = *cis*-[*alcohol*-^{14}C]permethrin internal fraction; TAL = *trans*-[*alcohol*-^{14}C]permethrin excreted fraction. Compounds from CA and TA excreted extracts were characterized as; 1, *c*- and *t*-Cl_2CA; 2, lactones (tentative identification); 3, 4, and 5 hydroxylated Cl_2CA metabolites (tentative identifications); 6, unknown. Compounds from CAL and TAL excreted extracts were characterized as; 1, PB-acid; 2, PB-alc; 3, 2′-HO-PB-alc; 4, 4′-HO-PB-acid; 5, 4′-HO-PB-alc; 6, unknown; 0, origin; F, front. Solvent = BEF.

trans-permethrin at physiologically similar doses, since *cis*-permethrin was twice as toxic to the bollworm as *trans*-permethrin.

Metabolites remaining in the water fractions after ether extraction were subjected to base (NaOH) treatment and ether extraction followed by acid (HCl) treatment and ether extraction to hydrolyze conjugates. PB-alcohol was

Table IV. Characterization of Conjugated Radioactivity from Internal and Excreted Bollworm Extracts 24-h after Topical Treatment with Permethrin Isomers[a]

isomer	fraction	% water soluble	% of recovered organosoluble radioactivity: fractionation: base ether	acid ether	remaining in water	extract	ether-soluble metabolites: esterase	secondary	origin[b]
trans acid	internal	41.4	0.0	82.3	17.7	acid	17.8	75.3	6.9
	excreted	43.2	0.0	82.0	18.0	acid	20.5	62.7	16.8
cis acid	internal	8.2	5.4[b]	59.2	35.4	acid	60.5	37.5	2.0
	excreted	15.6	0.0	56.7	43.3	acid	46.5	39.1	14.4
trans alcohol	internal	30.9	8.0	28.2	63.8	base	90.4	0.0	9.6
						acid	1.2	91.3	7.5
	excreted	25.0	5.5	14.7	79.8	base	92.5	0.0	7.5
						acid	7.4	74.0	18.6
cis alcohol	internal	12.5	24.7	28.9	46.4	base	94.6	0.0	5.4
						acid	13.2	82.4	4.4
	excreted	30.8	40.4	11.9	47.7	base	100.0	0.0	0.0
						acid	15.0	82.9	2.1

[a] Radioactivity remaining in the water fraction after ether–water partitioning of total internal and excreted fraction was hydrolyzed in base (1 N NAOH) and extracted with ether. The remaining water-soluble radioactivity was then treated with acid (1 N HCl) and extracted with ether. The organosoluble radioactivity after base and acid treatment was characterized by TLC. [b] Same primary metabolite as in acid–ether extract. [c] Origin radioactivity also contains minor amounts of unknown metabolites.

Figure 5. Organosoluble excreted metabolites from fifth instar tobacco budworm (top) and bollworm (bottom) larvae 24 h after topical treatment with [^{14}C]permethrin isomers alone and pretreated (4 h) with acephate (A) or chlordimeform (C). CA and TA represent cis and trans ^{14}C-acid-labeled permethrin, respectively; CAL and TAL represent cis and trans alcohol-labeled permethrin, respectively. *cis*- and *trans*-permethrin compounds separated were: CA(+A+C) and TA(+A+C) 1, Cl_2CA; 2, lactones (tentative identification); 3, *c*- or *t*-HO-permethrin (tentative identification); 4, 5, and 6, hydroxylated Cl_2CA metabolites (tentative identification); 7, unknown; F, *cis*- or *trans*-permethrin; 0, origin. CAL(+A+C) 1, PB-acid; 2, PB-alc; 3, unknown; 4, 2′-HO-PB-acid; 5, 2′-HO-PB-alc; 6, 4′-HO-PB-acid; 7, 4′-HO-PB-alc; 8, unknown; F, *cis*-permethrin; 0, origin. Budworm, TAL(+A+C) 1, PB-acid; 2′, 4′-HO-Permethrin; 2, PB-alc; 3, unknown; 4, 2′-HO-PB-alc; 5, 4′-HO-PB-acid; 6, 4′-HO-PB-alc; 7, unknown. Bollworm, TAL(+A+C) 1, PB-acid; 2′, 4′-HO-permethrin; 2, PB-alc; 3, unknown; 4, 2′-HO-PB-acid; 5, 2′-HO-PB-alc; 6, 4′-HO-PB-acid; 7, 4′-HO-PB-alc.

the major soluble in ether after base treatment. Metabolites soluble in ether after acidification included Cl_2CA and its unknowns which were probably hydroxylated derivatives from the ^{14}C-acid-labeled permethrins and hydroxylated PB-alc and PB acid derivatives from the ^{14}C-alcohol-labeled permethrins. Data are summarized in Table IV and Figure 4.

Eight to 43% of the radioactivity in the various fractions remained in the water after ether extraction. The amounts were greater with *trans*-permethrin.

Eighty percent of the radioactivity from insects treated with *trans*-[*acid*-^{14}C]permethrin was extractable by ether from the acid treatment; with cis ^{14}C-acid treatments only 60% was ether extractable. In insects treated with the ^{14}C-alcohol-labeled preparations, 20 to 54% was recovered in ether from base and acid fractions. More ^{14}C was recovered in the base–ether extracts (PB-alc) from *cis*-[*alcohol*-^{14}C]permethrin, while more was recovered in the acid–ether extracts (secondary PB-alc and PB-acid metabolites) from *trans*-[*alcohol*-^{14}C]permethrin. This means hydrolysis was relatively more important in *cis*-permethrin metabolism and oxidation was relatively more important in *trans*-permethrin metabolism. The radioactivity remaining in the water after base–acid hydrolysis and ether extraction was not further characterized.

The TLC comparison of acid–ether metabolites (Figure 4) clearly shows the more extensive metabolism of the

Table V. Characterization of Organosoluble Radioactivity Excreted by Fifth Instar Tobacco Budworm and Bollworm Larvae 24-h after Topical Treatment with [^{14}C]Permethrin Isomers (Dose, 0.13 ppm)

isomer	species	pretreatment (4 h)[a]	% applied dose excreted	% of recovered organosoluble radioactivity				
				permethrin	metabolites: esterase	oxidase	secondary	origin[b]
cis acid	tobacco	none	15.9	22.9	5.7	6.0	53.3	12.1
	budworm	acephate	16.0	67.5	5.5	4.1	9.2	13.7
		chlordimeform	11.5	65.0	0.9	3.3	17.4	13.4
	bollworm	none	18.3	38.0	4.6	1.2	31.0	25.2
		acephate	19.4	71.8	3.0	1.0	11.5	12.7
		chlordimeform	25.0	80.8	2.5	2.0	6.4	8.3
trans acid	tobacco	none	27.6	6.0	10.7	0.2	55.5	27.6
	budworm	acephate	17.9	28.1	35.3	1.1	16.6	18.9
		chlordimeform	13.0	56.2	29.2	0.0	6.9	7.7
	bollworm	none	19.6	26.9	6.1	1.0	40.3	25.7
		acephate	37.7	49.9	9.5	2.5	21.4	16.7
		chlordimeform	23.5	45.3	23.0	1.0	22.1	8.6
cis alcohol	tobacco	none	22.2	45.6	6.4	3.1	27.3	17.6
	budworm	acephate	20.2	82.9	1.8	2.9	5.8	6.6
		chlordimeform	22.1	93.4	0.8	0.5	2.1	3.2
	bollworm	none	19.4	21.5	8.1	0.3	22.7	47.4
		acephate	28.1	68.7	4.2	1.6	6.6	18.9
		chlordimeform	21.9	63.5	6.8	0.3	8.4	21.0
trans alcohol	tobacco	none	32.3	13.2	3.4	0.0	22.6	60.8
	budworm	acephate	25.4	53.3	5.3	5.1	11.9	24.4
		chlordimeform	21.4	40.4	2.4	0.0	9.7	47.5
	bollworm	none	22.0	25.3	3.5	4.0	22.1	45.1
		acephate	44.3	56.1	2.0	3.7	11.8	25.4
		chlordimeform	25.7	43.8	2.5	0.0	18.7	35.0

[a] Acephate 10 ppm, chlordimeform 100 ppm. [b] Origin radioactivity also contains minor amounts of unknown metabolites.

trans-permethrin preparations. Cl_2CA, spot no. 1 on the CA and TA chromatographs, was a major metabolite of both *cis*- and *trans*-permethrin. CA 2 and TA 2 probably represent the lactones derived from *c*-HO-*t*- or *c*-Cl_2CA. CA and TA 3, 4, and 5 probably represent hydroxylated derivatives of Cl_2CA (Robinson, 1977). The majority of the metabolites from the alcohol-labeled permethrins were hydroxylated derivatives of PB-alcohol and PB-acid. Larger percentages of these hydroxylated metabolites were present in the *trans*-permethrin extracts.

To demonstrate the validity of the procedure used to recover conjugated metabolites, the known [^{14}C]permethrin preparations were subjected to the same base–acid hydrolysis and extraction procedures as the water extracts from the bollworms. Ether extracts were characterized by TLC. The [*acid*-^{14}C]permethrin metabolites partitioned nearly quantitatively into the acid–ether fraction and the [*alcohol*-^{14}C]permethrin metabolites partitioned quantitatively into the base–ether fraction. In all cases the major metabolites were primary hydrolysis products, the Cl_2CA's from the [*acid*-^{14}C]permethrins and PB-alc from the [*alcohol*-^{14}C]permethrins.

Comparative Metabolism. Bollworm and Tobacco Budworm. A study was made to compare the in vivo metabolism of the permethrin preparations after topical treatment of early fifth instar tobacco budworm and bollworm larvae. Acephate (10 ppm) and chlordimeform (100 ppm) were used as pretreatments to aid in the determination of metabolic differences between the species. The treatment doses of insecticides and synergists were low enough that all treated insects survived throughout the 24-h test period.

Prior to TLC, all internal and excreted aqueous fractions were extracted with both ethyl ether and ethyl acetate, and the organosoluble extracts were pooled. Enough additional ^{14}C was recovered with ethyl acetate so that only minimal amounts of radioactivity remained in the water fractions.

Data on the metabolism of the permethrin preparations by tobacco budworms and bollworms are summarized in Table V and Figure 5. They show that the trans preparations were metabolized more readily than the cis preparations by both species. The tobacco budworm metabolized all preparations more extensively than the bollworm. Synergist pretreatments greatly suppressed permethrin metabolism and usually had less effect in the bollworm than in the tobacco budworm.

Excretion was more rapid with *trans*- than with *cis*-permethrin in the tobacco budworm (Table V). With the bollworm, excretion rates were similar with both isomers. Synergist pretreatments had little effect on excretion rates except that acephate increased the excretion of radioactivity from *trans*-permethrin by the bollworm. In both species, a higher proportion of *cis*- than of *trans*-permethrin was excreted as unmetabolized insecticide.

Distribution of recovered radioactivity in the tobacco budworm (data not shown) ranged from 4 to 17% in surface fractions and from 60 to 80% in internal fractions. In the bollworm, recovered radioactivity ranged from 9 to 20% in surface fractions and from 47 to 67% in internal fractions. There was no consistent differences between isomers or between species.

Pretreatment with the synergists reduced the metabolism of all permethrin preparations and increased the proportion excreted without metabolism. Generally, treatment with the synergists caused an increase in the proportion of esterase metabolites and decreases in the proportion of oxidative metabolites, secondary metabolites, and origin-located radioactivity.

The amounts of radioactivity remaining at the origin of chromatographs increased as metabolism increased in the preparations. This material was not analyzed further. However, previous studies (see Table IV) have demonstrated it consisted mainly of conjugated secondary metabolites.

The data in Figure 5 illustrate the metabolism of the permethrin preparations by the tobacco budworm and the bollworm. Larger amounts of fewer metabolites were present in excreta from insects treated with *trans*-per-

methrin than from those treated with *cis*-permethrin. Overall, the synergist pretreatments reduced metabolism of all permethrin preparations. The figure also indicates that synergist pretreatments had less effect on metabolism of *cis*-permethrin than on metabolism of *trans*-permethrin and less effect on metabolism in the bollworm than with the tobacco budworm.

DISCUSSION

Both hydrolytic and oxidative reactions are involved in permethrin metabolism by the bollworm and the tobacco budworm. The use of synergist pretreatments reduced permethrin metabolism in both species. The effect was greater in the tobacco budworm than in the bollworm and greater with *cis*- than with *trans*-permethrin.

The present study shows that in *Heliothis*, as in rats, *cis*-permethrin is more resistant to metabolism than *trans*-permethrin. Tobacco budworm larvae metabolized both isomers more effectively than did bollworm larvae.

Elliot et al. (1976) and Gaughan et al. (1977) concluded that permethrin is highly biodegradable in mammals. The present study indicates a much greater degree of permethrin stability in *Heliothis*. This differential stability between insect and mammal is an appropriate property for a candidate insecticide.

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Wild Oat Herbicide Studies. 2. Physiological and Chemical Changes in Barley and Wild Oats Treated with Diclofop-methyl Herbicide in Relation to Plant Tolerance

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Diclofop-methyl [methyl 2-[4-(2,4-dichlorophenoxy)phenoxy]propanoate], a selective, postemergence herbicide for the control of annual grassy weeds, was studied, using greenhouse seedlings of moderately resistant barley (*Hordeum vulgare* L.) and susceptible wild oats (*Avena fatua* L.). More physiological and chemical changes were noted in wild oats treated with diclofop-methyl than in barley, including reduced growth of shoots and roots, decreased chlorophyll content and photosynthetic activity, inhibition of photosynthate translocation to the roots, reduction of adenosine 5'-triphosphate (ATP) content and accumulation of sugars in the shoots. These differential changes were closely related to the tolerance of barley and wild oats to diclofop-methyl. The changes associated with herbicidal action are discussed.

Wild oats are extensively distributed on the agricultural lands of the northern temperate regions of the world (Thurston and Phillipson, 1976). Herbicides are widely used to control this pest in crops. Diclofop-methyl was recently registered in Canada and several European countries for the control of wild oats and other annual grassy weeds. This herbicide not only has good selectivity between mono- and dicotyledonous families but also adequate selectivity within monocotyledons (Chow, 1978). The latter selectivity is sufficient to control wild oats in cereals. The differential retention of applied diclofop-methyl on the leaves of four gramineous species was a contributing factor in the selectivity (Todd and Stobbe, 1977). The damaged chloroplasts in wild oats treated with diclofop-methyl were observed (Brezeanu et al., 1976). The metabolism and residues of this herbicide in wheat were reported (Gorbach et al., 1977).

Field observations indicated that a week or so after application of diclofop-methyl the leaves of susceptible species became chlorotic, mottled, then wilted and the plant had very little root development. Thus, we undertook to study the changes of the parameters such as

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