Degradation of *trans*- and *cis*-Permethrin on Cotton and Bean Plants

Loretta C. Gaughan and John E. Casida*

(1*RS*)-*trans*- and (1*RS*)-*cis*-permethrin on cotton leaves in the field and greenhouse are degraded mainly by ester cleavage, more rapidly for the trans than the cis isomer, and conjugation of the liberated acid and alcohol fragments. Minor pathways with each isomer lead to: esters with one hydroxyl substituent at the 2′ or 4′ position of the phenoxy group or the methyl group trans to the ester functionality; an ester with hydroxylation at both the 4′-phenoxy and *trans*-methyl sites; the acid moiety hydroxylated at either methyl group and conjugates of these acids. Some trans/cis isomerization at the cyclopropane takes place on photodecomposition. In treated beans the permethrin isomers undergo similar reactions and also form hydroxy ester conjugates. These products from plants are identical with permethrin metabolites in mammals except for the nature of the conjugating moieties.

3-Phenoxybenzyl 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate (permethrin) is highly effective in controlling many insect pests of cotton and other crops (Elliott, 1977; Elliott et al., 1973), so it is important to define its degradation rate and pathways on plants. (1*R*)-*trans*- and (1*R*)-*cis*-permethrin on bean leaves undergo various reactions including ester cleavage, hydroxylation of the acid and alcohol moieties before or after ester cleavage, and conjugation of the various hydroxy and carboxylic acid derivatives (Ohkawa et al., 1977). Photoisomerization at the cyclopropane, i.e., interconversion of the trans and cis isomers, is also a significant reaction on plants (Ohkawa et al., 1977) and under other environmental conditions (Holmstead et al., 1977, 1978).

The present study and a preliminary report on this investigation (Gaughan et al., 1977b) concern the fate of (1*RS*)-*trans*- and (1*RS*)-*cis*-permethrin applied to leaves on cotton plants under field and greenhouse conditions and applied to leaves or injected into the stem of bean plants in the greenhouse.

MATERIALS AND METHODS

Chemicals. Four labeled preparations were used (radiochemical purity >99%): (1*RS*)-*trans*-permethrin (*t*-per) and (1*RS*)-*cis*-permethrin (*c*-per) labeled in the carboxyl group of the acid moiety ([^{14}C]acid-*t*- and -*c*-per, each 58.2 mCi/mmol); *t*- and *c*-per labeled in the methylene position of the alcohol moiety ([^{14}C]alc-*t*-per and -*c*-per, each 55.9 mCi/mmol) (Gaughan et al., 1977a). The permethrin metabolites and degradation products under consideration are designated by abbreviations as shown in Figure 1. Unlabeled standards corresponding to the nonconjugated derivatives in this figure and related compounds for comparison were available from syntheses described by Unai and Casida (1977).

Chromatography. Thin-layer chromatography (TLC) utilized silica gel 60 F-254 chromatoplates (0.25 mm gel thickness) developed with the following solvent systems: BAW, 1-butanol–glacial acetic acid–water (6:1:1); BC, benzene–carbon tetrachloride (1:1); BE, benzene–ethyl acetate (6:1); BEM, benzene–ethyl acetate–methanol (15:5:1); BFE, benzene (saturated with formic acid)–ether (10:3), two developments; CE, carbon tetrachloride–ether (3:1); CFE, chloroform (saturated with formic acid)–ether (10:3). TLC R_f values for all standard compounds and degradation products (other than the conjugates) in these systems are given by Gaughan et al. (1977a) and Unai and Casida (1977).

Pesticide Chemistry and Toxicology Laboratory, Department of Entomological Sciences, University of California, Berkeley, California 94720.

Treatment and Analysis of Plants. Studies were conducted with cotton (Stoneville 7A variety) under field conditions near Davis, Calif. and in a greenhouse in Berkeley, Calif. and with garden snapbeans (Contender variety) in the greenhouse, in each case using plants 10–12 cm high and with at least four leaves (cotton) and two leaves (bean) at the time of treatment. Plants in the field received 4.5 cm of water/week from an overhead sprinkler. Those in the greenhouse received water and 4-h artificial light after sunset each day. Average temperatures were 34 and 12 °C during the day and night, respectively, under field conditions and 20–21 °C in the greenhouse.

Fully developed leaves (0.3–0.5 g) on cotton and bean plants were individually treated with each [^{14}C]per preparation (~1 μg) using ethanol (30 μL) to uniformly spread the samples and give deposits of ~0.16 μg/cm^2. Other bean plants (2.5–4.0 g above ground weight) were injected into the stem with each [^{14}C]per preparation (~3.8 μg) in ethanol (10 μL).

Cotton and bean leaves and bean plants were analyzed immediately after harvest except in field studies where the leaves were held in glass containers for up to 24 h at −20 °C prior to analysis. The cotton leaves were cut into small pieces and soaked sequentially, without homogenization, in three portions of methanol–chloroform (2:1) with decanting after each extraction as follows: 10 mL for 3–4 h; 10 mL for 40 h; 5-mL rinse. This procedure provides much lower ^{14}C residues in the unextractable portion than the method used in early studies on treated bean leaves involving a single soaking period of 30 min with methanol–chloroform (15 mL). The bean plants were extracted by homogenizing (polytron) in methanol–chloroform (2:1) (30 mL × 2). The combined extracts and rinses were concentrated under N_2 for ^{14}C determinations by liquid scintillation counting of an aliquot of the total extract and of individual compounds separated by two-dimensional TLC (first development with BAW and second with BFE, i.e. BAW × BFE). Radiocarbon in the unextractable residue was analyzed by combustion.

The tabulated results are averages of two experiments with injected beans and treated cotton leaves in the greenhouse and four experiments in all other cases.

Identification of Metabolites and Degradation Products. Permethrin derivatives were tentatively identified by cochromatography with unlabeled standards using the following one- or two-dimensional TLC developments: *t*- and *c*-per–BC; HO-per and $(HO)_2$-per derivatives, BAW × BFE and BE × CE; Cl_2CA and PBalc, BAW × BFE and BFE × BEM; HO-Cl_2CA derivatives, BAW × BFE and BFE × CFE.

Compounds designated as conjugates are those retained at the origin in all TLC solvent systems except BAW. For

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Table I. Radiocarbon Recovery as Permethrin and Its Degradation Products at 3 and 6 Weeks after Topical Application of [^{14}C]Acid- or [^{14}C]Alcohol-*trans*- and *cis*-Permethrin at ~3 ppm to Cotton Leaves

	Radiocarbon recovery at 3 weeks (and 6 weeks), % of indicated isomer under field and greenhouse conditions							
	Field				Greenhouse			
	Acid		Alcohol		Acid		Alcohol	
Compound	Trans	Cis	Trans	Cis	Trans	Cis	Trans	Cis
Permethrin and Hydroxy-Permethrin Derivatives								
Per[a]	10.2 (2.5)	11.4 (7.2)	10.8 (6.1)	23.5 (10.2)	9.6 (5.7)	33.4 (15.6)	13.5 (8.0)	29.6 (17.4)
t-HO-per	0.3 (0.3)	0.2 (0.4)	0.6 (0.2)	0.8 (0.4)	0.4 (0.3)	0.6 (0.4)	0.3 (0.1)	0.7 (0.3)
2′-HO-per	0.3 (0.2)	0.3 (0.2)	0.4 (0.3)	0.5 (0.2)	0.3 (0.2)	0.6 (0.3)	0.3 (0.3)	0.7 (0.5)
4′-HO-per	0.4 (0.2)	0.3 (0.1)	0.7 (0.3)	0.5 (0.5)	0.3 (0.2)	0.8 (0.3)	0.3 (0.1)	0.3 (0.3)
t-HO,4′-HO-per	0.1 (0.0)	0.6 (0.3)	0.6 (0.2)	0.4 (0.1)	0.1 (0.0)	0.6 (0.9)	0.2 (0.3)	0.4 (0.5)
Metabolites of the Acid Moiety								
Cl_2CA	0.0 (0.1)	0.0 (0.0)			0.2 (0.4)	0.0 (0.0)		
Cl_2CA-glyc[b]	12.6 (13.7)	8.5 (6.5)			11.9 (14.1)	7.2 (11.5)		
Cl_2CA-conj[c]	16.5 (11.7)	0.0 (0.0)			18.8 (22.0)	0.0 (0.0)		
HO-Cl_2CA[d]	0.3 (0.1)	0.5 (0.1)			0.4 (0.3)	0.7 (0.9)		
HO-Cl_2CA-conj[e]	2.4 (4.8)	6.6 (4.5)			5.4 (7.2)	7.3 (11.4)		
Unknown-conj[f]	0.0 (0.0)	5.4 (6.2)			0.0 (0.0)	3.2 (6.4)		
Metabolites of the Alcohol Moiety								
PBalc			0.6 (0.3)	0.5 (0.2)			0.2 (0.3)	0.4 (0.5)
PBalc-glyc[b]			16.1 (10.3)	7.2 (6.2)			6.9 (20.8)	4.1 (3.7)
PBalc-conj[c]			19.7 (21.5)	9.6 (12.4)			31.8 (28.1)	6.0 (13.0)
Unknown-conj[f]			0.0 (0.0)	0.0 (0.0)			1.4 (1.6)	1.2 (4.0)
Unknown-conj[f]			0.0 (0.0)	0.0 (0.0)			1.0 (1.5)	1.1 (3.3)
Others								
Organoextractable	1.4 (1.8)	5.1 (6.5)	6.9 (5.2)	2.8 (5.6)	2.7 (3.8)	2.3 (4.3)	1.9 (4.8)	4.6 (9.6)
Unextractable	4.2 (4.4)	5.8 (6.4)	5.2 (5.8)	5.6 (7.7)	2.9 (6.2)	6.3 (9.7)	5.0 (6.8)	6.3 (10.5)
Total								
	48.7 (39.8)	44.7 (38.4)	61.6 (50.2)	51.4 (43.5)	53.0 (60.4)	63.0 (61.7)	62.8 (72.7)	55.4 (63.6)

[a] Isomerized product accounts for 21% of the recovered permethrin in the field and 11% in the greenhouse without significant difference between applied *t*- and *c*-per and 3 and 6 weeks after application. [b] Glyc refers to conjugates from both *t*- and *c*-per characterized as follows: R_f (BAW) 0.45 for Cl_2CA-glyc and 0.37 for PBalc-glyc; essentially quantitative cleavage by glucosidase, cellulase, and HCl. [c] Conj refers to conjugates from both *t*- and *c*-per characterized as follows: R_f (BAW) 0.37 for Cl_2CA-conj and 0.28 for PBalc-conj; 0–30, 0–100, and 100% cleavage by glucosidase, cellulase, and HCl, respectively. [d] Consists of *t*-HO, *c*-HO, and *c*-HO-lactone derivatives of each of *t*- and *c*-Cl_2CA. [e] Conjugates of *c*-HO,*t*-Cl_2CA from *t*-per and of *t*-HO,*c*-Cl_2CA and a smaller amount of *c*-HO,*c*-Cl_2CA from *c*-per are characterized as follows: R_f (BAW) 0.31 for *c*-HO,*t*-Cl_2CA-conj and 0.45 for the *t*-HO,*c*-Cl_2CA-conj and *c*-HO,*c*-Cl_2CA-conj mixture; poorly or not cleaved by glucosidase but readily cleaved by cellulase and HCl. [f] Unknown-conj are products not defined except as follows: from [^{14}C]acid-*c*-per, R_f (BAW) 0.26 and not cleaved by glucosidase, cellulase, or HCl; from [^{14}C]alc-*t*- and -*c*-per, R_f (BAW) 0.44 and 0.54 and cleavage properties not examined.

tentative conjugate identification, the individual products separated by the BAW system were recovered from the silica gel by extraction with methanol and individually treated with glucosidase, cellulase, and HCl for identification of the released ^{14}C compounds by cochromatography as indicated above. Cleavage conditions were as follows: β-glucosidase (3 mg) (from almonds; Sigma Chemical Co., St. Louis, Mo.) in 0.2 M pH 5.0 acetate buffer (1 mL) incubated for 24 h at 37 °C; cellulase (3 mg) (from *Aspergillus niger*; Sigma) in 0.2 M pH 4.5 acetate buffer (1 mL) incubated for 24 h at 47 °C; 6 N HCl (1 mL) maintained for 24 h at 70–74 °C, followed by addition of 1 mL of water; 0.17 N HCl (1.2 mL) incubated for 6 h at 37 °C, followed by addition of 1.3 mL of water, a method used only for acid cleavage of conjugates of HO-per derivatives. The glucosidase cleavage was complete under the specified conditions since no further cleavage took place on recovery of uncleaved conjugates and reincubation with glucosidase. Products liberated on hydrolysis were recovered for TLC cochromatography by addition of solid $(NH_4)_2SO_4$ (0.2 g/mL incubation mixture) and extraction with ether–ethanol (3:1) (5 mL ×2).

RESULTS

Fate of Permethrin on Cotton Leaves under Field and Greenhouse Conditions. About 30% of the ^{14}C is lost within 1 week after application of [^{14}C]per to cotton leaves in the field with minor differences between the isomers and labeling positions, i.e., 26, 41, 23, and 31% for [^{14}C]acid-*t*-per, [^{14}C]acid-*c*-per, [^{14}C]alc-*t*-per, and [^{14}C]-alc-*c*-per, respectively. The ^{14}C loss in subsequent periods (~12% between the first and second weeks, ~7% between the second and third weeks, and ~10% between the third and sixth weeks) is independent of the isomer and labeling site.

Radiolabeled products on or in cotton leaves at 3 and 6 weeks after application of [^{14}C]per under field and greenhouse conditions are given in Table I. *t*-per is lost more rapidly than *c*-per. A portion of the recovered ester has undergone isomerization at the cyclopropane, i.e., trans/cis interconversion. Hydroxy esters (*t*-HO-per; 2′-HO-per; 4′-HO-per; *t*-HO, 4′-HO-per) appear in free but not conjugated form, are in larger amounts from *c*- than *t*-per and may include in each case some isomeric material from photoisomerization prior to metabolic hydroxylation. The major degradation pathway is hydrolysis followed by rapid conjugation of *t*- and *c*-Cl_2CA and PBalc. *t*-Cl_2CA and PBalc appear as two types of conjugates, the minor one a glycoside (designated as glyc) readily cleaved by glucosidase and the major one a conjugate (designated as conj) poorly cleaved by this enzyme. *c*-Cl_2CA is found only as the more easily cleaved conjugate, i.e., *c*-Cl_2CA-glyc. Glucosidase appears to cleave *t*-Cl_2CA-glyc more rapidly than *c*-Cl_2CA-glyc. A small portion of the acid moiety from

Table II. Radiocarbon Recovery as Permethrin and Its Metabolites at 3 and 17 Days after Injection of [^{14}C]Acid- or [^{14}C]Alcohol-*trans*- and *cis*-Permethrin at ~1 ppm into the Stems of Bean Plants

Compound	Radiocarbon recovery at 3 days (and 17 days), % of indicated isomer			
	Acid		Alcohol	
	Trans	Cis	Trans	Cis
Permethrin and Hydroxy-Permethrin Derivatives				
Per	57.0 (55.7)	78.9 (83.7)	78.7 (52.0)	89.5 (76.6)
t- or *c*-HO-per-conj A[a]	1.1 (0.9)	0.0 (0.0)	1.0 (1.0)	0.0 (0.0)
t- or *c*-HO-per-conj B[a]	10.4 (8.5)	2.5 (1.6)	4.5 (2.7)	0.6 (1.6)
Metabolites of the Acid Moiety				
Cl_2CA	1.8 (0.0)	0.8 (0.2)		
Cl_2CA-glyc[b]	3.4 (3.2)	0.0 (0.0)		
HO-Cl_2CA	2.2 (0.7)	0.6 (0.4)		
Unknown-conj[c]	2.5 (1.2)	0.0 (0.0)		
Metabolites of the Alcohol Moiety				
PBalc			4.9 (1.0)	0.5 (0.0)
PBacid			0.6 (0.4)	0.1 (0.5)
PBalc-glyc[b]			4.2 (6.8)	0.3 (2.8)
Others				
Organoextractable	0.6 (0.5)	0.3 (0.3)	0.8 (0.3)	0.2 (0.3)
Unextractable	1.6 (3.6)	1.2 (1.3)	1.8 (3.4)	0.4 (2.4)
Total				
	80.6 (74.3)	84.3 (87.5)	96.5 (67.6)	91.6 (84.2)

[a] Conj refers to two conjugates from both [^{14}C]acid- and [^{14}C]alc-*t*-per and one from both labeled preparations of *c*-per characterized as follows: R_f (BAW) 0.61 for A and 0.47 for B; cleavage in each case with glucosidase or acid to give *t*- or *c*-HO-per and *t*- or *c*-HO-Cl_2CA from [^{14}C]acid preparations and *t*- or *c*-HO-per and PBalc from [^{14}C]alc preparations. [b] Glyc refers to conjugates from *t*-per or from both *t*- and *c*-per characterized as follows: R_f (BAW) 0.34 for both Cl_2CA-glyc and PBalc-glyc; essentially quantitative cleavage by glucosidase and HCl. [c] R_f (BAW) 0.56; cleaved by glucosidase and HCl to a polar, unidentified product.

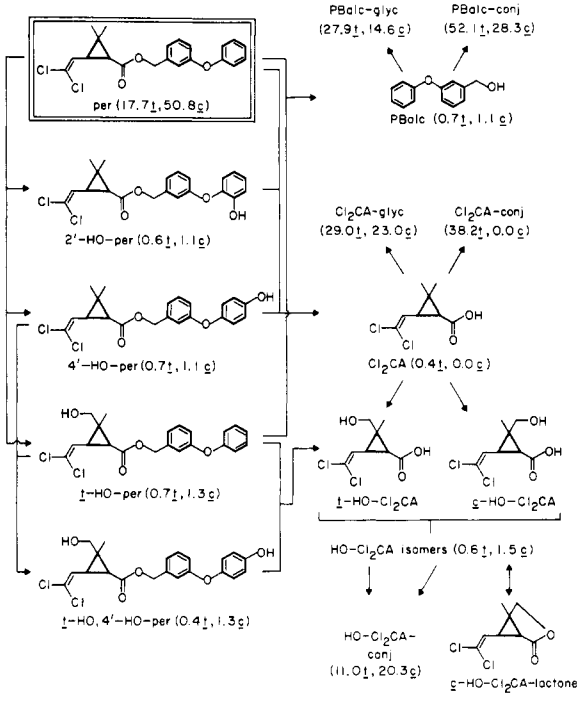

Figure 1. Degradation pathways of *trans*- and *cis*-permethrin on cotton leaves. Numbers in parentheses are percent of each product relative to the total identified products given first for *t*-per and then for *c*-per; these values are averages from field and greenhouse conditions at 3 and 6 weeks after treatment as shown in Table I. Ester products are averages from [^{14}C]acid and [^{14}C]alc preparations, whereas cleavage products are based on either the [^{14}C]acid or [^{14}C]alc preparation, as appropriate. The values total 99.3–100.8% rather than 100.0% due to variations in levels of per and other esters on averaging results with two labeled preparations

both *t*- and *c*-per is detected as HO-Cl_2CA derivatives with hydroxylation at either one of the methyl groups. Most of the HO-Cl_2CA derivatives are present as conjugates not readily cleaved by glucosidase. Products released on cleavage of these conjugates with cellulase and HCl are hydroxylated only on the methyl group cis to the carboxyl with *t*-per and on either methyl group with *c*-per; cleavage of *c*-HO,*c*-Cl_2CA-conj yields *c*-HO,*c*-Cl_2CA-lactone.

An unknown conjugate from the acid moiety of *c*-per either fails to cleave under the hydrolysis conditions used or decomposes on cleavage to products retained at the origin in BFE. Two unknown conjugates from the alcohol moiety of both *t*- and *c*-per appear under greenhouse but not field conditions. None of the conjugates derived from either isomer or labeling position reacts with diazomethane to change its chromatographic properties (BAW), so they are not likely to be amino acid conjugates. Other organoextractable products remain at or near the origin on TLC (BAW). Unextractable products are in small amounts under both field and greenhouse conditions.

Fate of Permethrin on Bean Leaves or in Bean Plants under Greenhouse Conditions. Radiocarbon from the [^{14}C]per isomers is almost completely retained on or in the treated leaves for up to three weeks under greenhouse conditions (Figure 2). Both isomers give similar rates of loss and similar amounts of isomerized per, degradation products and ^{14}C not extracted. The ~25% of ^{14}C in the "not extracted" fraction reflects the suboptimal extraction procedure used in this early study (see Materials and Methods section); the improved extraction procedure (used routinely for cotton) gives much lower residues, i.e., 4–5% unextracted ^{14}C even 5 weeks after treatment of bean leaves.

On injection into the stem of bean plants the permethrin isomers undergo little movement (radioautography of treated plants), and *t*-per is metabolized to a greater extent than *c*-per (Table II). Two pathways are utilized almost equally in metabolism of both *t*- and *c*-per. The first involves hydroxylation at a methyl group and derivatization of the hydroxy ester as two conjugates (for *t*-per)

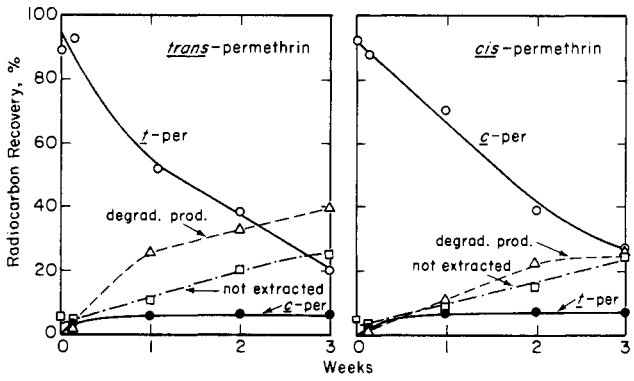

Figure 2. Radiocarbon recovery as permethrin and its degradation products after topical application of [^{14}C]acid- or [^{14}C]-alcohol-*trans*- and *cis*-permethrin at 2–3 ppm to bean leaves under greenhouse conditions. Results are the average of the [^{14}C]acid and [^{14}C]alc data since they gave similar results.

or one conjugate (for *c*-per). The other is initiated by hydrolysis and the cleavage products are then conjugated to give Cl_2CA-glyc and PBalc-glyc. Minor pathways are methyl hydroxylation of Cl_2CA and oxidation of PBalc to PBacid.

DISCUSSION

Products derived from the permethrin isomers on or in cotton leaves are shown in Figure 1 along with their amounts relative to the total identified products. The ester group of *t*-per is cleaved more readily than that of *c*-per based on a ~2.8-fold difference in recoveries of the applied isomers, their isomerized products, and their mono- and dihydroxy derivatives. This isomer difference is not expected on photochemical ester cleavage (Holmstead et al., 1978) or volatilization and therefore results in the most part from metabolism. The liberated acid moiety is rapidly conjugated either before or after hydroxylation of one methyl group. With *t*-per there is considerable specificity for hydroxylation of the methyl group trans to the carboxyl but this specificity is not as prominent with *c*-per. Major conjugates of *t*- and *c*-Cl_2CA are readily cleaved by glucosidase and additional conjugates more refractory to cleavage are found with *t*-Cl_2CA and the HO-Cl_2CA isomers but not with *c*-Cl_2CA. No explanation is currently available for differences in the types of conjugates with the Cl_2CA isomers. The HO-Cl_2CA conjugates are formed in larger amounts from *c*-per than from *t*-per as expected based on the greater resistance of the ester group of the cis isomer to hydrolysis thereby allowing more time for oxidation prior to ester cleavage. The liberated alcohol appears mainly as two conjugates differing in chromatographic properties and ease of cleavage by glucosidase. The ratio of these PBalc conjugates is similar from *t*- and *c*-per on considering the amount of each isomer that has undergone degradation.

In comparative greenhouse studies not detailed here, it was found that the permethrin isomers are degraded about twofold more rapidly after application to bean as compared to cotton foliage (Acala SJ2 variety), in each case the trans isomer decomposing slightly faster than the cis isomer. A large proportion of the metabolites are hydroxy ester conjugates in injected bean plants but not in topically treated cotton leaves. Hydroxylation occurs to a greater extent in the acid than in the alcohol moiety with both cotton and bean plants.

The permethrin isomers are relatively stable compounds on treated plants as expected from their excellent residual activity in insect control. They are photodecomposed and metabolized largely by reactions that cleave the ester group. The types of products are generally the same in plants and mammals except for the nature of the conjugating moieties (Gaughan et al., 1977a, 1978).

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