Figure 8. Hydrolytic cleavage gives rise to 2,6-dichlorobenzoic acid, 2,6-dichlorobenzamide, and 2-amino-5-(4-bromophenyl)-6-methylpyrazine. Hydrolytic cleavage has also been shown to be an important detoxification pathway in the metabolism of diflubenzuron (Ivie et al., 1980).

LITERATURE CITED

Abdel-Monem, A. H.; Cameron, E. A.; Mumma. R. O. *J. Econ. Entomol.* **1980**, *73*, 22-25.
Abdel Monem, A. H.; Mumma, R. O. *J. Agric. Food Chem.* **1981**, *29*, 75-78.
Bligh, E. G.; Dyer, W. J. *Can. J. Biochem. Physiol.* **1959**, *37*, 911-917.
Flint, H. M.; Smith, R. L.; Noble, J. M.; Shaw, D.; DeMilo, A. B.; Khalil, E. *J. Econ. Entomol.* **1978**, *71*, 616-619.
Hamilton, R. H.; Hurter, J. Hall, J. K.; Ercegovich, D. C. *J. Agric. Food Chem.* **1971**, *19*, 480-483.
Ivie, G. W.; Bull, D. L.; Veech, J. A. *J. Agric. Food Chem.* **1980**, *28*, 330-337.
Lilly Research Laboratory, Indianapolis, IN, 1977, Technical Report on EL-494, 1-4.
Mansanger, E. R.; Still, G. G.; Frear, D. S. *Pestic. Biochem. Physiol.* **1979**, *12*, 172-182.
Miller, C. O. In "Moderne Methoden der Pflanzenanalyze"; Linskens, H. F.; Tracey, M. V., Eds.; Springer-Verlag: Berlin, 1963; Vol. 6, p 194.
Mumma, R. O.; Hamilton, R. H. *ACS Symp. Ser.* **1979**, *No. 98*, 35-76.
Retnakaran, A. *Can. Entomol.* **1979**, *111*, 847-850.
Still, G. G.; Leopold, R. A. *Pestic. Biochem. Physiol.* **1978**, *9*, 304-312.

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Interactions of Pyrethroid Insecticides and Toxaphene in Cotton

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Effects of toxaphene on pyrethroid insecticide residues on cotton foliage were determined by spraying aqueous emulsions, extracting leaves with methylene chloride, separating the pyrethroid from toxaphene by preparative thin-layer chromatography, and quantitating by gas chromatography. Fenvalerate residues were increased up to 2-fold due to enhanced persistence in the presence of toxaphene. Permethrin residues were reduced by toxaphene, the effect being greater in 1*RS*,trans isomers after several applications. Toxaphene synergized fenvalerate and permethrin 2-fold in *Spodoptera frugiperda*; susceptibility of *Heliothis virescens* was increased 1.5-fold to both pyrethroids.

Combinations of insecticides for cotton insect control have had a successful history which includes the use of DDT/toxaphene, EPN/methyl parathion, and toxaphene/methyl parathion. While the first and second examples acted as insecticidally synergistic mixtures (Brown, 1971), addition of toxaphene was found to increase the deposit and residual persistence of methyl parathion sprays in cotton (Ware et al., 1979). Since photostable pyrethroid insecticides have replaced methyl parathion for protecting cotton, there is interest in possible combinations of insecticides with pyrethroids.

This report examines toxaphene/pyrethroid mixtures and describes the effects of toxaphene on the residual concentrations of fenvalerate and permethrin on cotton leaves. The combinations were tested further for toxicological interactions in the cotton pests *Heliothis virescens* (Fabricius), the tobacco budworm, and *Spodoptera frugiperda* (J. E. Smith), the fall armyworm, and these results are also reported.

MATERIALS AND METHODS

Applications of Insecticides. Combinations of toxaphene/permethrin and toxaphene/fenvalerate and each pyrethroid alone were sprayed on cotton of the Coker 304 variety in Sumter, SC, in 1978. Each treatment was applied in quadruplicate to randomized 141-m^2 plots of cotton by using a conventional high-clearance sprayer calibrated to deliver 79.5 L/ha. Insecticides were commercial emulsifiable concentrates of permethrin as Pounce, fenvalerate as Pydrin, and toxaphene as formulated by FCX, Inc. Insecticide residue samples consisted of 35 mature leaves gathered from the top portions of plants while walking diagonally across the plots; samples were frozen immediately in sealed plastic bags and later transported to the laboratory under dry ice.

Confirmatory experiments were performed with toxaphene/fenvalerate in 1979 and toxaphene/permethrin in 1980 in Florence, SC, by using plots of Coker 310 cotton which were 324 and 202 m^2, respectively. Application methods were similar to those used in Sumter, and samples were collected and frozen as above.

Determination of Residual Pyrethroid Insecticides. Extraction of pyrethroids from leaves followed a procedure modified from George et al. (1977). Each thawed sample was chopped and a 20-g lot blended for 1 min with pesticide-grade methylene chloride in a Sorvall Omnimixer while cooling with an ice bath. This extract and two subsequent rinses were filtered through granular, anhydrous Na_2SO_4 and concentrated in vacuo at 45 °C.

Preparative thin-layer chromatography (TLC) removed plant lipids and toxaphene from pyrethroids which were further analyzed. A 750-μm layer of silica gel PF-254 (E. Merck, Darmstadt) was spread on glass plates which were air-dried and then activated at 110 °C for 30 min. One-tenth of the extract and the pyrethroid standard were chromatographed to 10 cm in 10% (v/v) ethyl acetate in hexane. Ultraviolet light revealed the standard, and the pyrethroid zone was removed and eluted with 50% (v/v) ethyl acetate in hexane which gave >95% recovery. R_f

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Table I. Residual Concentrations of Pyrethroid Insecticides Applied Alone or with Toxaphene to Cotton in Sumter in 1978[a]

date	(*RS,SR*)-fenvalerate, ppm		(*RR,SS*)-fenvalerate, ppm		(1*RS*)-*cis*-permethrin, ppm		(1*RS*)-*trans*-permethrin, ppm	
	from fenv only	from fenv/tox	from fenv only	from fenv/tox	from perm only	from perm/tox	from perm only	from perm/tox
Aug 1[b]	14.1	16.6	11.7	14.3	7.3	10.8	17.9	18.2
2-7[e]								
8	4.2 (70)[c]	9.5[d] (43)	3.2 (73)	7.4[d] (48)	4.3 (41)	4.4 (59)	6.9 (61)	8.0 (56)
9[b]	14.1	19.8	11.3	16.3	10.2	5.9	18.6	12.7
18	7.1 (49)	11.6 (41)	6.1 (46)	8.9 (45)	5.1 (50)	5.2 (11)	6.5 (65)	6.5 (49)
18[b]	17.1	18.5	13.3	14.6	10.0	9.0	21.2	12.8
24	10.8 (37)	13.6[d] (27)	8.4 (36)	10.7[d] (26)	11.1 (0)	5.4[d] (39)	16.3 (23)	8.4[d] (35)
24[b]	21.9	24.6	18.9	20.2	16.9	12.7	28.3	21.0[d]
Sept 7	6.0 (72)	10.1[d] (59)	4.6 (75)	7.9[d] (61)	6.8 (60)	4.0 (68)	12.2 (57)	6.4[d] (70)
7[b]	11.3	15.8	8.3	11.3	8.6	6.7	19.8	20.8

[a] Applied at 0.224 kg/ha pyrethroid and 2.24 kg/ha toxaphene. [b] Insecticides applied and leaves sampled 1 h later on these dates. [c] Percentage loss from past application in parentheses. [d] $p < 0.05$. [e] See Figure 1.

values were toxaphene 0.67, permethrin 0.52, and fenvalerate 0.40; pyrethroids were separated from five bands of cotton lipids.

Analytical standards of fenvalerate [CAS 51630-58-1, cyano(3-phenoxyphenyl)methyl 4-chloro-α-(1-methylethyl)benzeneacetate] and permethrin [CAS 52645-53-1, (3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate] were provided by manufacturers. Fenvalerate was 58.3% *RS,SR* isomers and permethrin was 29.2% 1*RS*,cis isomers.

Quantitation was accomplished by gas chromatography (GC) using ^{63}Ni-foil electron capture detection and measurement of peak height on a Fisher Recordall chart. All samples were chromatographed in nitrogen on 100–120-mesh Chromosorb WHP coated with 1.5% OV-17 and 1.95% OV-210 prepared by a filter-fluidizer technique and packed in a 1.83-m glass column of 4-mm internal diameter. Toxaphene/pyrethroid samples were sufficiently prepared by TLC since there was no interference with the pyrethroid peaks in gas chromatograms (Figure 1). Twenty samples were chromatographed on 5% SE-30 as a confirmation of identity; retention times of residue samples matched those of standards in both columns. Lesser retained peaks were *RS,SR* isomers for fenvalerate (Holmstead et al., 1978) and 1*RS*,cis isomers for permethrin (Fujie and Fullmer, 1978).

The limit of detection for pyrethroids was 0.5 ng throughout the study. A Micro-Tek Model 2000 GC with detector powered in the pulse mode (rate 270, width 7) linear to 1.0 ng of permethrin and 0.6 ng of fenvalerate was used for 1978 and 1979 samples. A Tracor Model 560 GC with linear electron capture to >500 ng of permethrin was used for 1980 samples.

Data were reported as concentrations on a fresh-weight basis. No corrections were made for losses in analysis or for residues in unsprayed control plots which did not exceed 0.5 ppm in 15 samples in 1979 or 0.4 ppm in 25 samples in 1980; none were collected in 1978. Analysis of variance was used to determine statistical differences.

Tests for Interaction of Insecticidal Activity. Permethrin and fenvalerate and their combinations with toxaphene were tested against laboratory colonies of the tobacco budworm and the fall armyworm which were fed an artificial diet (Ross and Brown, 1982) and tested by the standard susceptibility test method (Brazzel, 1970). Technical-grade samples of insecticides 95% pure provided by the manufacturers were dissolved in glass-distilled acetone. Topical application of 1.0 μL of solution to the mesothorax of the 35-mg larva was performed with an ISCO Model M microapplicator equipped with a 0.25-mL Hamilton syringe and a 27-gauge needle.

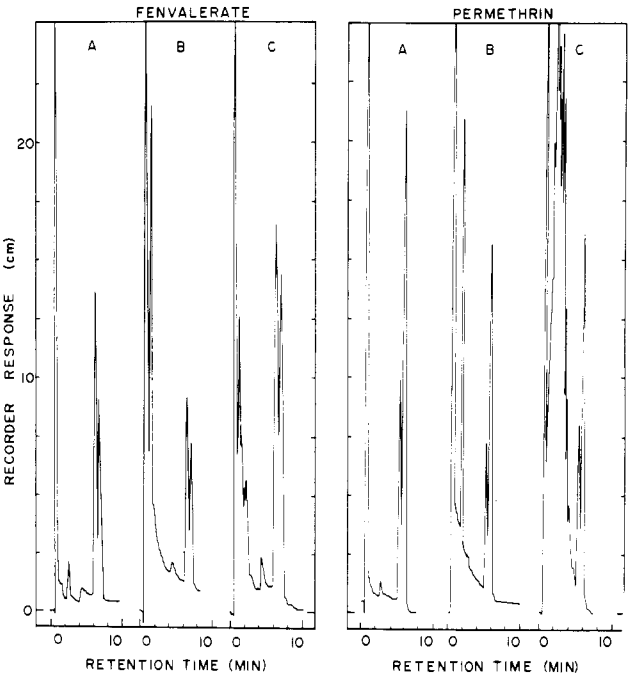

Figure 1. Gas chromatograms of pyrethroids after preparative TLC: (A) analytical standard; (B) extract of pyrethroid-sprayed cotton; (C) extract of pyrethroid/toxaphene-sprayed cotton.

Treated larvae were held at 27 °C, 60% relative humidity, and 14-h photoperiod for 48 h and then scored dead if not responding to a sharpened probe. Median lethal doses were found by computerized probit analysis.

RESULTS

Interactions in Field Sprays. The residual concentration of fenvalerate on cotton leaves was increased when toxaphene was added to the spray mixture in Sumter (Table I). There was a slight increase in fenvalerate deposition with toxaphene in the first application; then from days 1–7 postapplication there was a statistically significant doubling of the residual concentration of fenvalerate when combined with toxaphene (Figure 2). Decay slopes of the *RS,SR* and *RR,SS* enantiomer pairs were −0.034 and −0.035, respectively; these were reduced to −0.016 and −0.020 with toxaphene. Fenvalerate concentrations remained greater in the toxaphene-treated cotton through the remainder of the season which included four additional sprays (Table I). The percentage loss after each spray was always less in the fenvalerate/toxaphene mixture, and there was always a higher concentration of fen-

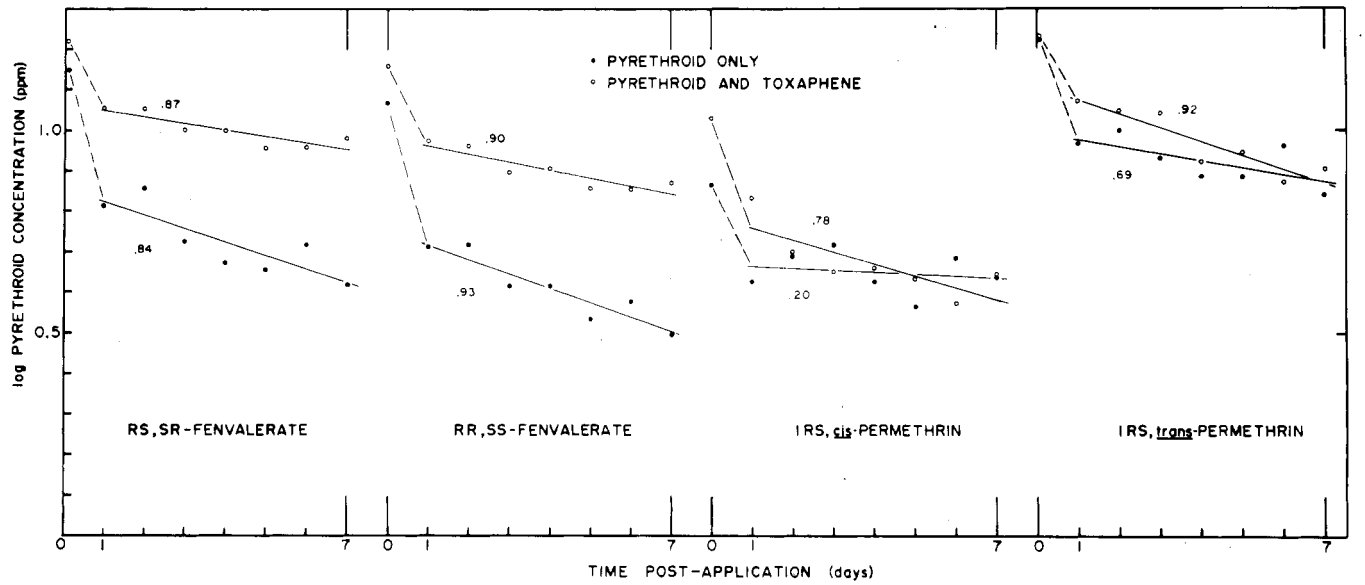

Figure 2. Decay of pyrethroid isomers on cotton foliage and the effects of toxaphene. Solid regression lines from computer analysis of days 1–7 only; correlation coefficients near lines; slopes in the text. Values for fenvalerate on days 1–7 were significantly increased with toxaphene ($p < 0.05$, analysis of variance); values for permethrin were not significantly different with toxaphene.

Table II. Residual Concentration of Fenvalerate Applied Alone or with Toxaphene to Cotton in Florence in 1979[a]

	(*RS,SR*)-fenvalerate, ppm			(*RR,SS*)-fenvalerate, ppm		
date	from fenv only	from fenv/ tox	from 2× fenv	from fenv only	from fenv/ tox	from 2× fenv
July 17[b]	4.4	2.3	4.3	3.4	1.7	3.6
20	0.8	0.6	3.0	0.6	0.5	1.9
Aug 15	5.3	8.9	10.6	4.2	7.3	9.0
15[b]	9.0	10.9	9.9	7.3	9.1	8.5
18	8.3	13.3[c]	7.6	7.0	10.8[c]	6.1
29	4.0	6.0	7.3	3.0	4.5	5.7
29[b]	7.3	13.4[d]	13.7[d]	6.5	11.1[d]	11.5[d]
Sept 1	6.7	13.6[d]	10.0	5.9	11.6[d]	8.2

[a] Applied at 0.056 or 0.112 kg/ha fenvalerate alone and 0.056 kg/ha fenvalerate with 2.24 kg/ha toxaphene. [b] Insecticides applied and leaves sampled 1 h later on these dates; other applications July 23 and 26 and Aug 1, 6, 9, and 20. [c] Differs from 2× fenvalerate, $p < 0.05$. [d] Differs from fenvalerate, $p < 0.05$.

valerate in the presence of toxaphene. In Florence, fenvalerate concentrations were increased again in toxaphene mixtures; doubling of fenvalerate concentration by toxaphene was observed late in the season (Table II).

The residual concentration of permethrin was not increased by toxaphene addition in Sumter; in fact, it was decreased after several applications (Table I). The initial application resulted in a slightly greater deposit of *cis*-permethrin, but this was not statistically significant nor were any values for permethrin isomers over the following week (Figure 2). After two additional sprays and the subsequent decay period, there was less permethrin on the cotton from the toxaphene/permethrin treatment than on the cotton treated only with permethrin (Table I). Very similar results were obtained in Florence where there was nearly always less permethrin in cotton sprayed with the addition of toxaphene (Table III). Again, reduction of the concentration of *trans*-permethrin was greater than that of *cis*-permethrin.

Susceptibility Tests with Insects. Toxaphene was slightly synergistic with pyrethroids (Table IV). Susceptibility of *H. virescens* to both pyrethroids was increased 1.5-fold, while pyrethroid susceptibility of *S. frugiperda* was increased 2-fold by toxaphene. *H. virescens* is a widespread, major pest of cotton while *S. frugiperda* is less important economically and becomes a problem only occasionally in South Carolina.

Table III. Residual Concentration of Permethrin Applied Alone or with Toxaphene to Cotton in Florence in 1980[a]

	(1*RS*)-*cis*-permethrin, ppm		(1*RS*)-*trans*-permethrin, ppm	
date	from perm only	from perm/ tox	from perm only	from perm/ tox
July 28[b]	n.s.[d]	6.5	n.s.	7.2
31	9.7	4.3	14.2	4.1
Aug 5	12.8	8.3	19.5	7.0[c]
5[b]	13.4	14.7	25.7	14.3[c]
8	8.7	6.4	15.3	5.0[c]
12	11.4	3.8[c]	16.5	3.7[c]
12[b]	14.1	12.8	22.1	14.3
15	8.9	8.0	10.8	8.9

[a] Applied at 0.112 kg/ha permethrin and 2.24 kg/ha toxaphene. [b] Insecticides applied and leaves sampled 1 h later on these dates; other applications July 16, 21, 24, and 31. [c] $p < 0.05$. [d] Not sampled.

Table IV. Susceptibility of *H. virescens* and *S. frugiperda* to Pyrethroid/Toxaphene Mixtures[a]

treatment	*n* (doses)	LD_{50}, μg/g	slope	synergist ratio
H. virescens				
fenvalerate	250 (6)	3.06	2.52	
fenvalerate/toxaphene (1:10)	230 (6)	2.00	1.75	1.5
permethrin	220 (6)	2.63	0.90	
permethrin/toxaphene (1:10)	150 (5)	1.80	1.17	1.5
S. frugiperda				
fenvalerate	300 (6)	11.69	1.99	
fenvalerate/toxaphene (1:10)	280 (6)	5.74	2.31	2.0
permethrin	420 (6)	2.69	1.47	
permethrin/toxaphene (1:10)	280 (6)	1.03	1.66	2.6

[a] Toxaphene alone caused no more than 5% mortality at the highest dose.

DISCUSSION

Rates of pyrethroid decay reported here were similar to those previously found with fenvalerate (Holmstead et al., 1978) and with permethrin (Gaughan and Casida, 1978). Disappearance was more rapid initially, especially for the first day postapplication; therefore, regression lines were computed excluding the values for the original deposit. While residue decay kinetics are often biphasic (Matsumura, 1975), the results from Sumter were also subject to a heavy rain of 4.8 cm on Aug 2 which probably increased the initial losses. Normal field conditions prevailed for the next week with no more than 0.1 cm of rainfall on any day; a total of 9.6 cm fell during the experiment.

The increase of fenvalerate persistence by toxaphene was more important than the carrier effect of toxaphene during application. While the initial fenvalerate deposit was increased about 15% with toxaphene, deposition was not increased in four later sprays in Sumter; however, persistence was always greater as seen in the lower percentages of deposits lost with toxaphene (Table I). The mechanism of increased persistence was not investigated.

Toxaphene addition increased deposits and persistence of methyl parathion in cotton in Arizona where molasses had a similar effect (Ware et al., 1980) and in Texas where cedar oil and camphene were slightly less effective than toxaphene (Bigley et al., 1981).

Accumulation of *trans*-permethrin during the season was reduced with toxaphene while there was less effect on *cis*-permethrin. This result could implicate a facilitation of hydrolytic degradation, to which the trans isomers are more susceptible (Gaughan and Casida, 1978), as a possible mechanism to account for the lower permethrin concentrations observed with toxaphene.

Yields of seed cotton with fenvalerate were increased 3.2% in 1978 and 4.0% in 1979 with toxaphene; permethrin yields were decreased 12.4% in 1978 and 7.0% in 1980. Neither increases nor decreases in yields were statistically significant, but it should be noted that the pyrethroids were very effective in controlling insects at the rates used here; thus only slight improvement in yield could be expected from the addition of toxaphene.

The low cost of toxaphene and the expense of pyrethroid insecticides suggest that specific mixtures of these compounds, when compatible and effective, might be economically advantageous. Further investigations of mixtures reported here as well as others with various pyrethroids are needed to explain fully these interactions.

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LITERATURE CITED

Bigley, W. S.; Plapp, F. W.; Hanna, R. L.; Harding, J. A. *Bull. Environ. Contam. Toxicol.* **1981**, *27*, 90.

Brazzel, J. R. *Bull. Entomol. Soc. Am.* **1970**, *16*, 147.

Brown, A. W. A. In "Pesticides in the Environment"; White-Stevens, R., Ed.; Marcel Dekker: New York, 1971; pp 457–553.

Fujie, G. H.; Fullmer, O. H. *J. Agric. Food Chem.* **1978**, *26*, 395.

Gaughan, L. C.; Casida, J. E. *J. Agric. Food Chem.* **1978**, *26*, 525.

George, D. A.; Halfhill, J. E.; McDonough, L. M. *ACS Symp. Ser.* **1977**, *No. 42*, 201.

Holmstead, R. L.; Fullmer, D. G.; Ruzo, L. O. *J. Agric. Food Chem.* **1978**, *26*, 954.

Matsumura, F. "Toxicology of Insecticides"; Plenum Press: New York, 1975; p 306.

Ross, D. C.; Brown, T. M. *J. Agric. Food Chem.* **1982**, *30*, 193.

Ware, G. W.; Estesen, B. J.; Buck, N. A. *Bull. Environ. Contam. Toxicol.* **1979**, *21*, 657.

Ware, G. W.; Watson, T. F.; Estesen, B.; Buck, N. A. *J. Econ. Entomol.* **1980**, *73*, 15.

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