BARD

FINAL REPORT

PROJECT No. I-249-80

Pyrethroid Synergists: Biochemical and Toxicological Aspects

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1985

Pyrethroids
Insecticides- Toxicology
Bemisia tabaci

1363

Standard BARD Cover Page for Scientific Reports

Date December 1985

BARD
P.O. Box 6
Bet Dagan, ISRAEL

BARD Project No. L-249-80

Title of Research Project:

Pyrethroid Synergists: Biochemical and Toxicological Aspects

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Project's starting date: October 1, 1981

Type of Report: *1st Annual*_____ *2nd Annual*_____ *Final* X

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ABSTRACT

The stability of pyrethroids in insects, and thereby their potency, is limited by hydrolytic and oxidative reactions. During the course of this project, studies were conducted to evaluate biochemical and toxicological effects of synergists acting by inhibiting pyrethroid detoxifying enzymes in insects. In three important cotton pests - Trichoplusia ni, Spodoptera littoralis and Bemisia tabaci - esterase inhibitors, and in Tribolium castaneum and Musca domestica vicina - oxidase inhibitors, were efficient synergists to pyrethroids. Apparently, the predominant pathway of pyrethroid detoxification in insects, whether hydrolytic or oxidative, depends largely on the insect species and to some extent on the pyrethroid involved.

Biochemical assays for evaluating pyrethroid detoxifying enzymes have been developed; the inhibition level of these enzymes was used to select efficient synergists to pyrethroids. Pyrethroid esterases of S. littoralis and T. ni gut walls are more active on a per-larva basis than those of the corresponding integuments in hydrolyzing trans-permethrin and cis-cypermethrin. In general, the trans-isomers are hydrolyzed more extensively than the cis-isomers. Esterase inhibitors such as profenofos, monocrotophos, methidathion and acephate synergized considerably the toxicity of various pyrethroids against B. tabaci, T. ni and S. littoralis under both glasshouse and field conditions.

The magnitude of cypermethrin synergism in some insect species by esterase inhibitors was not repeated in mice, perhaps due to differences in the level of oxidases and in the inhibitor specificities of esterases involved in pyrethroid detoxification. These results indicate the possibility of selective synergism towards insects compared with mammals.

Oxidase inhibitors such as piperonyl butoxide, SV-1 and Niagara 16824 synergized the toxicity of various pyrethroids against T. castaneum and M. domestica vicina. Joint application of the insect growth regulator (IGR) compound RO 13-5223 and pyrethroids results in

a dual effect, as expressed by increased inhibition of larval growth due to pyrethroid synergism and a strong reduction in adult emergence due to RO 13-5223's juvenilizing activity.

Another approach, of using mineral oil and pyrethroids, indicates that addition of light-medium range oil increases residue and toxicity of fenpropathrin when applied under high-volume spray conditions against the whitefly B. tabaci. No such effect was observed when assays were carried out under low volume spray conditions.

The mechanism of pyrethroid synergism by various biochemical approaches and the practical implications of the results are discussed in this report.

GENERAL INTRODUCTION

Synthetic pyrethroids are the newest major class of pest control agents. They are modeled on the insecticidal components of pyrethrum flowers, which have been used for more than a century in insect control. Synthetic pyrethroids have been obtained by a series of isosteric modifications of substituents in the natural pyrethrins to improve their overall potency and stability (1).

Pyrethroids combine the favorable properties of exceptional insecticidal potency and relative low acute toxicity to mammals and birds (2). However, control of agricultural insect pests with pyrethroids is usually more expensive than with the current conventional insecticides. It is therefore important to evaluate possible synergists as a means to reduce the required pyrethroid dose and thereby improve cost effectiveness.

The insecticidal activity of pyrethroids is limited by oxidative and hydrolytic detoxification reactions (3) which vary in their relative significance depending on the compound, species, and strain of insects involved. Although early studies emphasized the importance of insect oxidases in detoxifying pyrethroids (4-8), there has been increasing evidence in recent years that esterases also play a major role in pyrethroid detoxification (9-16)*. Pyrethroid esterases have similar properties in three lepidopterous species that have been examined, Trichoplusia ni Hubn. (9,14), Spodoptera eridania Cram. (13) and Spodoptera littoralis (Boisd.) (16). Except for S. eridania, the gut wall esterases are more active than those of the integument on a protein or on a per larva basis. Hydrolysis occurs more rapidly with

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* References 16-18 are papers based on work done within the framework of the current project.

trans-permethrin and trans-cypermethrin than with the corresponding cis-isomers. The slower rate of ester cleavage of cis- compared with trans-isomers parallels their relative insecticidal activities. On the other hand, in Tribolium castaneum (Herbst) larvae, oxidases seem to be more important sites for pyrethroid detoxification than esterases, since oxidase and not esterase inhibitors synergized the toxicity of several synthetic pyrethroids (17). Apparently the predominant pathway of pyrethroid detoxification in an insect, whether oxidative or hydrolytic, depends largely on the insect species and to some extent on the pyrethroid compound involved (18).

This report summarizes our studies during the BARD project on pyrethroid detoxification by insect esterases and oxidases. Furthermore, esterase and oxidase inhibitors are assayed as synergists to pyrethroids in various groups of insects. Biochemical and toxicological assays are carried out under laboratory, glasshouse and field conditions aimed at evaluating the practical importance of pyrethroid synergism for controlling important agricultural pests. Furthermore, a novel approach using mineral oil as a pyrethroid synergist proved to be useful under high-volume spray conditions.

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OBJECTIVES OF ORIGINAL RESEARCH PROPOSAL

This project was planned to provide some of the fundamental biochemical and toxicological background required for the development and expanded use of synergists for pyrethroid insecticides. The hope was that it would contribute to a better understanding of the persistence and toxicity of various pyrethroids and help to overcome that portion of insect resistance resulting from selection for high

activity of detoxifying enzymes. To achieve these goals, studies were needed to isolate and determine the biochemical properties and substrate specificity of the pyrethroid esterases along with their tissue localization in the insect body. Integument and gut esterases are particularly important in pyrethroid hydrolysis after contact application and ingestion, respectively. Both esterases and mixed-function oxidases were examined to evaluate their relative importance in pyrethroid detoxification and the significance of esterase and oxidase inhibitors as pyrethroid synergists. The goal was to find or devise inhibitors acting preferentially to block detoxification in insects but not mammals and thereby preserve the favorable selectivity and safety of pyrethroids. Efficient synergists will reduce the amount and therefore the cost of pyrethroids used in agriculture. They will also minimize the burden of pyrethroid residues in the environment.

BODY OF THE REPORT

The body of the report is divided into three major sections:

A. Pyrethroid Synergism by Esterase Inhibitors
B. Pyrethroid Synergism by Oxidase Inhibitors
C. Effect of Mineral Oil on Pyrethroid Action

Published papers and those in preparation are classified to suit the appropriate sections aiming at presenting major findings and practical importance of the results. At the beginning of each section, a short description summarizing the main assays and results is given.

A. Pyrethroid Synergism by Esterase Inhibitors

The stability of pyrethroids in insects, and thereby their potency, is limited by hydrolytic reactions and oxidation at various substituents depending on the insect species and the particular compound involved (1). In the adult of the whitefly Bemisia tabaci, organophosphorus insecticides such as monocrotophos, methidathion and acephate or their metabolites inhibit pyrethroid esterase activity and synergize considerably the toxicity of cypermethrin under both glasshouse and field conditions (2).

In larvae of both the Egyptian cotton leafworm (Spodoptera littoralis) and the cabbage looper (Trichoplusia ni), the rate of pyrethroid hydrolysis is higher with the trans- than with the cis-isomers, correlating with their relative insecticidal activities (3,4). Under laboratory conditions the toxicity of cis-cypermethrin is synergized by profenofos about 20-fold against T. ni (3) and by profenofos and monocrotophos about three-fold against S. littoralis (4). Phenyl saligenin cyclic phosphonate synergized the toxicity of trans-permethrin more than 60-fold against Chrysopa carnea larvae (5). On the other hand, in larvae of Tribolium castaneum and Musca domestica vicina, oxidases seem to be more important than esterases for pyrethroid detoxification, since oxidase (but not esterase) inhibitors synergize the toxicity of several pyrethroids (6,7).

The first and second papers in this section deal with biochemical and toxicological aspects of pyrethroid synergism in B. tabaci adults and S. littoralis larvae. Assays were carried out to optimize pyrethroid esterase activity isolated from these insects. Inhibitors of the esterase system selected from organophosphorus (OP) insecticides were used as cypermethrin synergists. Assays were carried out under glasshouse and field conditions to evaluate the practical use of synergized cypermethrin for controlling important cotton pests.

Optimization of a synergist for pyrethroids must consider its possible effects on mammalian toxicity. The OP compounds examined were more effective synergists in whiteflies than in mice. Their selectivity may be due in part to their poor activity as inhibitors of the mouse liver pyrethroid esterase activity. Mice also appear to depend on oxidases as well as esterases in cypermethrin detoxification. This limited comparison of whiteflies and mice indicates the possibility of selective synergism in insects compared with mammals.

The third paper in this section evaluates the toxicity of mixtures of several synthetic pyrethroids and OP compounds against S. littoralis larvae under both laboratory and field conditions.

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Cypermethrin synergism by pyrethroid esterase inhibitors in adults of the whitefly Bemisia tabaci.

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ABSTRACT

The optimal assay conditions for pyrethroid esterase activity in the whitefly Bemisia tabaci are 100 µg protein of the postmitochondrial supernatant fraction incubated for 60 min at 37°C with up to 2.5 nmol of trans [^{14}C=O] permethrin or cis [^{14}C=O] cypermethrin in 0.8 ml of 0.1M glycine-NaOH buffer (pH 9.3) containing 0.05% bovine serum albumin. Under optimal enzyme assay conditions trans-permethrin is hydrolyzed faster than its cis-isomer or its α-cyano analogs trans- and cis-cypermethrin and deltamethrin. Organophosphorus insecticides, such as monocrotophos , methidathion and methamidophos (the hydrolytic and active metabolite of acephate) inhibit considerably in vitro the pyrethroid esterase enzymes of the whitefly adults. The I_{50} (concentration needed for 50% enzyme inhibition) of monocrotophos is 9×10^{-7}M, and that of methidathion and methamodophos is 6×10^{-5}M and 10^{-5}M, respectively.

Pyrethroid esterase inhibitors were assayed as possible synergists to cypermethrin, one of the most widely used pyrethroids against insects and whiteflies. Monocrotophos synergizes the toxicity of cypermethrin, under glasshouse conditions, ∿ 36-fold 3 days after treatment, and ∿ 26-fold 10 days after treatment. On the other hand, cypermethrin toxicity under field conditions is synergized by monocrotophos and acephate less efficiently than under glasshouse conditions and the effective period for control is moderately prolonged. The commercial synergized pyrethroid Fenom-S, a mixture of Fenom (cypermethrin with high cis : trans ratio) and methidathion, is more toxic to whitefly adults than Fenom or Cymbush (two non-synergized formulations of cypermethrin) under both glasshouse and field conditions.

Mouse liver esterases hydrolyzing cis-cypermethrin are inhibited by low intraperitoneal doses of profenofos and acephate, but not of monocrotophos and methidathion. The high magnitude of cypermethrin synergism in whiteflies is not repeated in mice, perhaps due to differences in the level of oxidases and in the inhibitor specificities of esterases involved in detoxification.

INTRODUCTION

Evidence continues to accumulate that esterases, in addition to oxidases, play a major role in pyrethroid detoxification in several species of insects (1-7). In larvae of both the Egyptian cotton leafworm (Spodoptera littoralis) and the cabbage looper (Trichoplusia ni), the rate of pyrethroid hydrolysis is higher with the trans- than with the cis-isomers, correlating with their

relative insecticidal activities (1,6). Under laboratory conditions the toxicity of cis-cypermethrin is synergized by profenofos about 20-fold against T. ni (1) and by profenofos and monocrotophos about three-fold against S. littoralis (6). Phenyl saligenin cyclic phosphonate synergized the toxicity of trans-permethrin over 60-fold against Chrysopa carnea larvae (4). On the other hand, in Tribolium castaneum larvae, oxidases seem to be more important than esterases for pyrethroid detoxification, since oxidase (but not esterase) inhibitors synergize the toxicity of several pyrethroids (7). Apparently the predominant pathway of pyrethroid detoxification in insects, whether hydrolytic or oxidative, depends largely on the insect species and to some extent on the pyrethroid compound involved.

The present study considers biochemical and toxicological aspects of cypermethrin synergism by organophosphorus insecticides in adults of the whitefly Bemisia tabaci, a very severe pest of cotton and vegetable crops, which is at present an important target for control by pyrethroids.

MATERIALS AND METHODS

Chemicals. The labelled pyrethroids were obtained and purified as described previously (1,4,6,8). The ^{14}C-acid preparation of cis- and of trans-permethrin was 1RS, that of cis- and of trans-cypermethrin was 1RS,αS, and that of ^{14}C-cis-deltamethrin was 1R,αS. Emulsifiable concentrates of 10% Cymbush (cypermethrin with 60:40 cis:trans ratio, Mahkteshim, Be'er Sheva, Israel), 20% Fenom (cypermethrin with 80:20 cis:trans ratio, Ciba-Geigy, Basel, Switzerland, and 50% Fenom-S (a mixture of 5.4% Fenom and 44.6% methidathion, Ciba-Geigy) were used for toxicological and field control studies.

Organophosphorus insecticides subjected to greenhouse and field assays as candidate pyrethroid synergists were emulsifiable concentrates of 50% profenofos (Ciba-Geigy), 40% monocrotophos (Makhteshim) and 40% methidathion (Ciba-Geigy) and a soluble concentrate of 75% acephate (Chevron, Richmond, California, USA). For enzyme and mouse toxicity assays, technical monocrotophos (97%, Shell Co., London, UK), profenofos (91%, Ciba-Geigy), methidathion (99.6%, Ciba-Geigy), and recrystallized acephate and methamidophos were used.

Whitefly pyrethroid esterase assays. The standard enzyme solution was prepared at 0-3°C by homogenizing 30 mg of whitefly adults (∿1000 adults) in 6 ml H_2O. The post-mitochondrial 12,000 g supernatant (microsome + soluble) fraction obtained after 15 min centrifugation was assayed for pyrethroid esterases activity. The enzyme assay based on a procedure described previously (1,4,6) was optimized in a 0.8 ml reaction mixture consisting of 0.2 ml enzyme solution (equivalent to 1 mg adult weight and approximating 35 adults and 100 µg protein), 0.4 ml 0.1M glycine-NaOH buffer at pH 9.3, and ^{14}C-pyrethroid (up to 2.5 nmol, ∿2,000 cpm) in 0.2 ml 0.2% bovine serum albumin (BSA) solution for 60 min at 37°C. The substrate was solubilized by adding 100 µl ethanolic solution of ^{14}C-pyrethroid to 10 ml 0.2% BSA solution. Enzyme activity was terminated by adding 5 ml scintillation mixture and determined as described previously (1).

Inhibitor solutions were prepared by diluting a stock solution in ethanol with 100-fold water (1,6). The final inhibitor concentration is expressed as that present in 0.2 ml of pre-incubation medium consisting of 0.1 ml enzyme solution and 0.1 ml inhibitor solution. The indicated amount of ethanol had no appreciable effect on enzyme activity . The enzyme reaction

in 0.8 ml final volume at 37°C was initiated after 15 min pre-incubation by adding the buffer and substrate and enzyme activity was determined as described above.

Whitefly synergism assays. Bemisia tabaci were reared on cotton seedlings in a glasshouse at 26±2°C. For glasshouse assays, cotton seedlings were sprayed with an electric sprayer until runoff with the pyrethroid and/or synergist. Six replicates of 12-15 whitefly adults confined in leaf cages were exposed to treated plants at various intervals after application, and kept under glasshouse conditions at 26±2°C for 48-hr mortality determination. For field assays, cotton plants (60-80 cm high) were sprayed with a knapsack sprayer until runoff with the pyrethroid and/or synergist. Three replicates of a 3-meter row were used for each treatment, from which 15 branch samples were cut at various intervals and maintained in water for residual toxicity determination. Twelve to 15 adults were exposed to each branch sample as above and kept under glasshouse conditions for mortality determination. With this procedure 24 hr exposure was used, since leaf wilting was observed after 48 hr. Both glasshouse and field assays were carried out in the summer season of 1983 and 1984 at Bet Dagan.

Mouse synergism and pyrethroid esterase assays. Male Swiss albino mice (18-22 g) were treated intraperitoneally (IP) with the test compounds using methoxytriglycol (MTG) as the carrier vehicle (1 µlg-1 body weight) or as controls with MTG alone. In toxicity studies, the candidate synergist was administered at 3 mg kg^{-1} (monocrotophos), 16 mg kg^{-1} (profenofos, methamidophos and acephate) or 150 mg kg^{-1} (piperonyl butoxide) 1 hr before (1R,αS)-cis-cypermethrin for mortality determination 24 hr later.

LD_{50} values are estimates based on studies with 74 mice (pyrethroid only) or 21-34 mice (each pyrethroid-synergist combination). For assays of liver pyrethroid esterases, the mice were treated with the organophosphorus compound at 0.25 to 16 mg kg^{-1} or with MTG alone and sacrificed after 1 hr by cervical dislocation. The preparation of liver pyrethroid esterase and assay of its activity were modified from earlier studies (2,9,10). The liver was homogenized at 25% (w/v) in 0.1 M sodium phosphate buffer, pH 7.4, and centrifuged at 15,000 g for 15 min. The supernatant was diluted 50-fold with water and used as the enzyme solution. The standard enzyme assay consisted of 0.8 ml 0.1M sodium phosphate buffer, pH 7.4, 0.8 ml enzyme solution (representing 4 mg liver or 100 µg protein) and ^{14}C-*cis*-cypermethrin (~ 2.5 nmol and ~ 2000 cpm) in 0.4 ml 0.2% BSA. The mixture was incubated for 15 min at 37°C. Enzyme activity was terminated by adding 10 ml liquid scintillation mixture and determined as described previously (2).

RESULTS

Whitefly pyrethroid esterase. *trans*-Permethrin hydrolysis by the post-mitochondrial supernatant fraction was optimized with respect to pH reaction time and enzyme concentration (Fig. 1). Hydrolysis approximated linearity for up to 60 min and 100 µg protein, the standard conditions selected. The pH optimum was 9.2-9.4 in the glycine-NaOH buffer. Esterase activity increased with reaction temperature, with an optimum at 49°C and a possible inflection in the curve at 37°C (Fig. 2); the latter temperature was adopted since it approximates the environmental temperature of the whitefly

in the summer season. With these assay conditions, trans-permethrin was hydrolyzed faster than its cis isomer or its α-cyano analogs trans- and cis-cypermethrin and deltamethrin (Table 1). trans-Permethrin was therefore used as the standard substrate.

Monocrotophos, methidathion, acephate and methamidophos (the hydrolytic and active metabolite of acephate) were selected among the organophosphorus insecticides as possible in vitro inhibitors for pyrethroid esterase activity in B. tabaci (Fig. 3). Monocrotophos was the strongest pyrethroid esterase inhibitor; 50% of the enzyme activity was inhibited by 9×10^{-7}M monocrotophos, 10^{-5}M methamidophos and 6×10^{-5}M methidathion. Total enzyme inhibition was obtained with all the three compounds. Acephate was not an inhibitor of pyrethroid esterase activity in vitro.

Whitefly synergism. Organophosphorus compounds assayed as inhibitors of pyrethroid esterase activity were selected as possible synergists to cypermethrin formulations, which are used intensively for the control of whiteflies and other insects. Monocrotophos, profenofos and acephate were efficient synergists in glasshouse assays against B. tabaci adults (Figs. 4-6). Addition of monocrotophos to a cypermethrin formulation (Cymbush) at a ratio of 1:1 a.i., and of profenofos at a ratio of 1:4 a.i., synergized strongly the toxicity of cypermethrin and prolonged its activity. The 50% residual lethal time (RLT_{50}) value of 0.004% cypermethrin was ∿ 3 days vs. ∿ 16 days with the fixture (Fig. 4). According to probit-log concentration curves, monocrotophos synergized the toxicity of cypermethrin about 36- and 26-fold, at 3 and 10 days after

treatment, respectively (Fig. 5). Synergism by acephate under similar experimental conditions was 5- and 9-fold at 1 and 6 days after treatment, respectively (Fig. 6).

On cotton under field conditions, the RLT_{50} value was moderately prolonged by equal amounts of either acephate or monocrotophos, i.e, the RLT_{50} of 0.02% cypermethrin was $\leq$ 1 day vs. $\sim$5 days with the mixture (Table 2). The cypermethrin synergism obtained under these conditions was much less pronounced than under glasshouse conditions.

Methidathion was an effective cypermethrin synergist under both glasshouse and field conditions when used as a commercial mixture of one part cypermethrin to eight parts methidathion (Fenom S). The amount of methidathion present in Fenom-S in these assays, when tested alone, had no appreciable effect on B. tabaci adults. At an equivalent pyrethroid concentration Fenom-S was much more toxic than Fenom (Fig. 7), with 20-fold synergism at the LC_{50} level (Fig. 8). In a comparison under field conditions of three commercial formulations each with 0.02% cypermethrin, the synergized cypermethrin (Fenom S) was considerably more effective than the non-synergized cypermethrin (Cymbush or Fenom) (Fig. 9).

Mouse synergism and pyrethroid esterase. A preliminary study was made to determine the degree to which the organophosphorus compounds increase the susceptibility of mice to cypermethrin. The IP LD_{50} of (1R,αS)-cis-cypermethrin (7.6 mg kg^{-1}) was only slightly reduced (4.9-5.4 mg kg^{-1}) in mice treated IP 1 hr earlier with profenofos, methidathion or acephate at 16 mg kg^{-1}. A higher degree of synergism occurred with either monocrotophos (3 mg kg^{-1}) or piperonly butoxide (150 mg kg^{-1}), which lowered the cypermethrin LD_{50} to 3.2-3.5 mg kg^{-1}.

Profenofos and acephate, administered IP, inhibited the liver esterases hydrolyzing cis-cypermethrin by 50% at 2-3 mg kg^{-1} (Fig. 10). In contrast methidathion and monocrotophos had no appreciable effect at 4 or 16 mg kg^{-1}, respectively.

DISCUSSION

Both hydrolytic and oxidative processes are involved in the metabolism of permethrin, cypermethrin and deltamethrin, so the optimal synergist will depend on the predominant pathway of pyrethroid detoxification in the insect of interest (5,11-14). The effectiveness of esterase inhibitors as synergists with B. tabaci enables us to add it to the list of species for which esterases are the limiting factor in pyrethroid toxicity, i.e., T. ni (1), S. littoralis, (6), and C. carnea (4), in contrast to T. castaneum and Musca domestica vicina, for which oxidase inhibitors are the most effective synergists, (7,11).

Organophosphorus insecticides inhibiting pyrethroid esterase activity in B. tabaci, synergize, to varying extents, the toxicity of cypermethrin under both glasshouse and field conditions. Monocrotophos, the most potent inhibitor of the whitefly pyrethroid esterase activity ($I_{50}=9\times10^{-7}M$) among the test compounds, synergizes most effectively the toxicity of cypermethrin under standardized glasshouse conditions. Methidathion and acephate acting as cypermethrin synergists, undergo probably in vivo activation: methidathion to its oxygen analog and acephate - on hydrolysis - to methamidophos. Whereas acephate has no activity on pyrethroid esterase activity, methamidophos shows a considerable inhibitory effect.

Cypermethrin synergism, under glasshouse conditions, is over 20-fold by monocrotophos or methidathion and up to 9-fold by acephate. Pyrethroid toxicity and synergism under field conditions is less pronounced than under glasshouse conditions. This probably reflects higher photodegradation of the synergist and/or the pyrethroid due to abundant UV light.

Optimization of a synergist for pyrethroids must consider its possible effects on mammalian toxicity. The compounds examined here are more effective synergists in whiteflies than in mice. The selectivity of monocrotophos and methidathion may be due in part to their poor activity as inhibitors of the mouse liver pyrethroid esterase(s). Mice also appear to depend on oxidases as well as esterases in cypermethrin detoxification. This limited comparison of whiteflies and mice indicates the possibility ofselective synergism in insects compared with mammals.

ACKNOWLEDGMENTS

This paper constitutes contribution No. 1324-E, 1985 series, from the Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel. The research was supported by grant No. I-249-81 from the United States-Israel Binational Agricultural Research and Development Fund (BARD), and by grant P01 ES00049 from the United States National Institute of Environmental Health Sciences. The field assays were supported in part by the Cotton Council of Israel and CTS Chemical Company, Tel Aviv. The authors thank Ciba-Geigy, Basel; Chevron, California; and Makhteshim, Be'er Sheva, Israel for supplying most of the test chemicals.

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TABLE 1

Hydrolysis of Permethrin and Cypermethrin Isomers and Deltamethrin by Pyrethroid Esterase(s) from Whitefly Adults

Pyrethroid	Hydrolysis, %[a]		Ratio
	trans	cis	trans/cis
Permethrin	45.7±0.8 f	36.6±1.2 e	1.45
Cypermethrin	9.6±1.0 b	14.0±0.2 c	0.69
Deltamethrin		20.7±1.0 d	

[a]Hydrolysis in 60 min under standard conditions. Data are averages of 8 replicates with their SE values. Means followed by different letters are significantly different at the 1% level.

TABLE 2

Effect of Acephate and Monocrotophos, under Field Conditions, on the Residual Toxicity of Cypermethrin Against Bemisia tabaci Adults.

Synergists and days after application	Mortality (% ± SE)		
	Cypermethrin 0.02% a.i.	Organophosphate 0.02% a.i.	Cypermethrin 0.02% a.i. + Organophosphate 0.02% a.i.
Acephate[a]			
1	32±3	23±2	85±4*
4	25±5	8±2	74±5*
6	17±4	1±1	36±4*
Monocrotophos[a]			
1	50±4	28±4	89±3*
3	42±4	27±3	84±3*
6	16±3	11±3	35±6**
12	0	0	3±1

[a] The acephate and monocrotophos treatments were carried out in different sets of experiments and times in the summer season of 1983. The variability in the residual toxicity of cypermethrin may result from different climatic conditions.

* Significantly different at the 5% level from the sum of mortality obtained with cypermethrin and organophosphate separately.

** Significantly different at the 5% level from the mortality obtained with cypermethrin alone.

FIGURES

Fig. 1. Effect of pH, incubation time and enzyme protein level on hydrolysis of trans-permethrin by Bemisia tabaci esterase(s). Enzyme protein refers to the post-mitochondrial supernatant fraction. The glycine-sodium hydroxide buffer was used. Each arrow designates the standard assay condition.

Fig. 2. Effect of reaction temperature on hydrolysis of trans-permethrin by Bemisia tabaci esterase(s).

Fig. 3. Effect of organophosphorus insecticides in vitro on hydrolysis of trans-permethrin by Bemisia tabaci esterase(s): (●) monocrotophos; (o) methidathion; (▲) acephate; (Δ) methamidophos. The concentrations of the inhibitors are those present in the 0.2 ml enzyme-inhibitor preincubation medium (see Methods). Data are averages of four replicates with their SE values.

Fig. 4. Synergistic effect of monocrotophos and profenofos, under glasshouse conditions on cotton seedlings, on the toxicity and residual effectiveness of cypermethrin to Bemisia tabaci adults: (o—o) cypermethrin 0.004% a.i.; (●---●) profenofos 0.001% a.i.; (▲---▲) monocrotophos 0.004% a.i.; (●—●) cypermethrin 0.004% a.i. plus profenofos 0.001% a.i.; (▲—▲) cypermethrin 0.004% a.i. plus 0.004% a.i. plus monocrotophos 0.004% a.i. A logarithmic scale is used for time and a probit scale for percentage mortality.

Fig. 5. Synergistic effect of monocrotophos under glasshouse conditions on cotton seedlings, on the toxicity and residual effectiveness of cypermethrin to Bemisia tabaci adults: (o) cypermethrin without synergist; (●) cypermethrin with monocrotophos 0.004% a.i. or

acephate 0.002% a.i. Arrows indicate LC_{50} values at 3 and 10 days after treatment. The synergist alone gave no mortality. A logarithmic scale is used for concentration and a probit scale for percentage mortality.

Fig. 6. Synergistic effect of acephate, under glasshouse conditions on cotton seedlings, on the toxicity and residual effectiveness of cypermethrin to Bemisia tabaci adults: (o) cypermethrin without synergist; (●) cypermethrin with acephate 0.002% a.i. Arrows indicate LC_{50} values at 1 and 6 days after treatment. Scales for plotting as in Figure 2.

Fig. 7. Synergistic effect of methidathion, under glasshouse conditions on cotton seedlings, on the toxicity and residual effectiveness of cypermethrin to Bemisia tabaci adults: (o) cypermethrin 0.003% a.i. (Fenom); (●) cypermethrin 0.003% a.i. plus methidathion 0.025% a.i. (Fenom-S). Methidathion alone at 0.025% gave no mortality. Scales for plotting as in Figure 1.

Fig. 8. Synergistic effect of methidathion, under glasshouse conditions on cotton seedlings, on the toxicity of cypermethrin to Bemisia tabaci adults: (o) cypermethrin (Fenom); (●) cypermethrin plus methidathion - 1:8 a.i. ratio (Fenom-S). Arrows indicate LC_{50} values 7 days after treatment. Methidathion alone gave no mortality. Scales for plotting as in Figure 2.

Fig. 9. Toxicity and residual effectiveness of three commercial formulations of cypermethrin, under field conditions on cotton plants, to Bemisia tabaci adults: (o) cypermethrin 0.02% a.i. (Cymbush); (o) cypermethrin 0.02% a.i. (Fenom); (Δ)cypermethrin 0.02% a.i. and methidathion 0.16% a.i. (Fenom-S). Scales for plotting as in Figure 1.

Fig. 10. Effect of organophosphorus insecticides in vivo on hydrolysis of cis-cypermethrin by mouse liver esterase(s): (□) profenofos; (Δ) acephate; (o) methidathion; (o) monocrotophos. Enzyme assays were carried out one hour after injecting the mice with various concentrations of each test compound. Treatments involved three to five replicates of two mice each. Bars represent SE values of the mean.

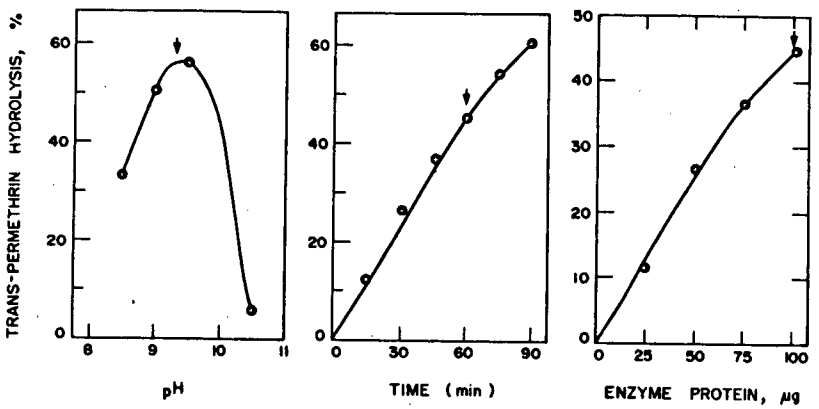
TRANS-PERMETHRIN HYDROLYSIS, %
pH
TIME (min)
ENZYME PROTEIN, µg

Fig. 1

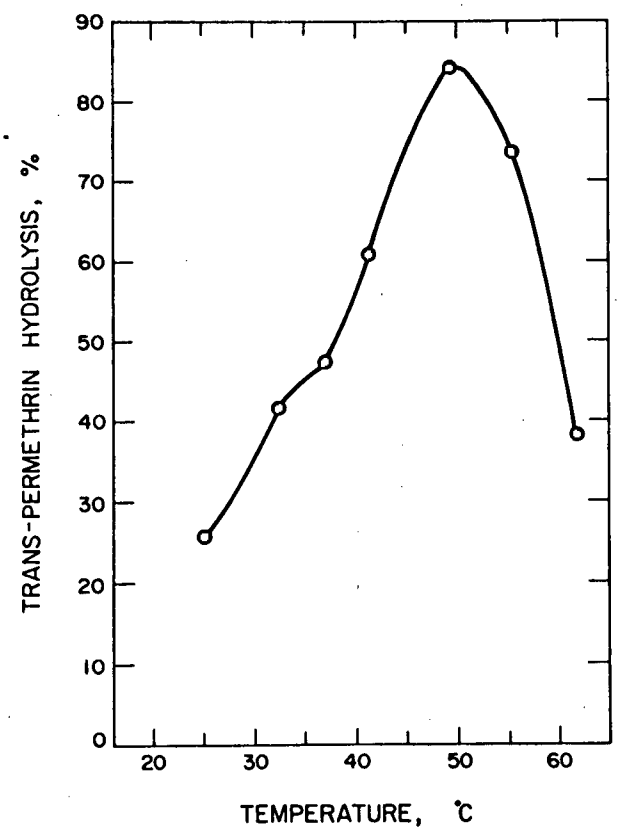
TRANS-PERMETHRIN HYDROLYSIS, %
TEMPERATURE, °C

Fig. 2

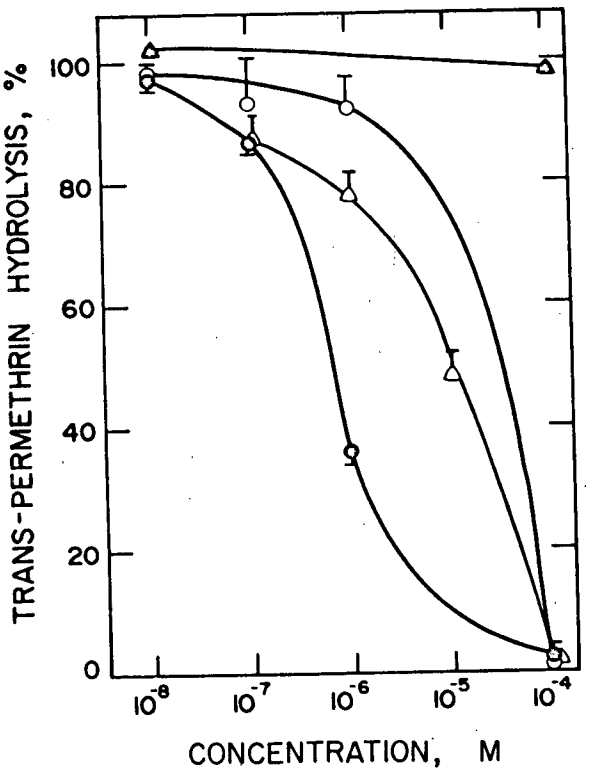
TRANS-PERMETHRIN HYDROLYSIS, %
100
80
60
40
20
0
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}
CONCENTRATION, M

Fig. 3

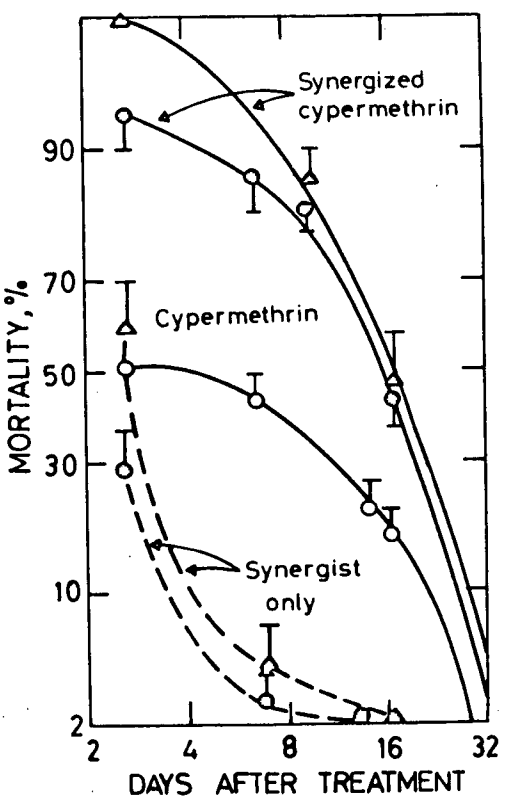
Synergized cypermethrin
Cypermethrin
Synergist only
MORTALITY, %
90
70
50
30
10
2
2
4
8
16
32
DAYS AFTER TREATMENT

Fig. 4

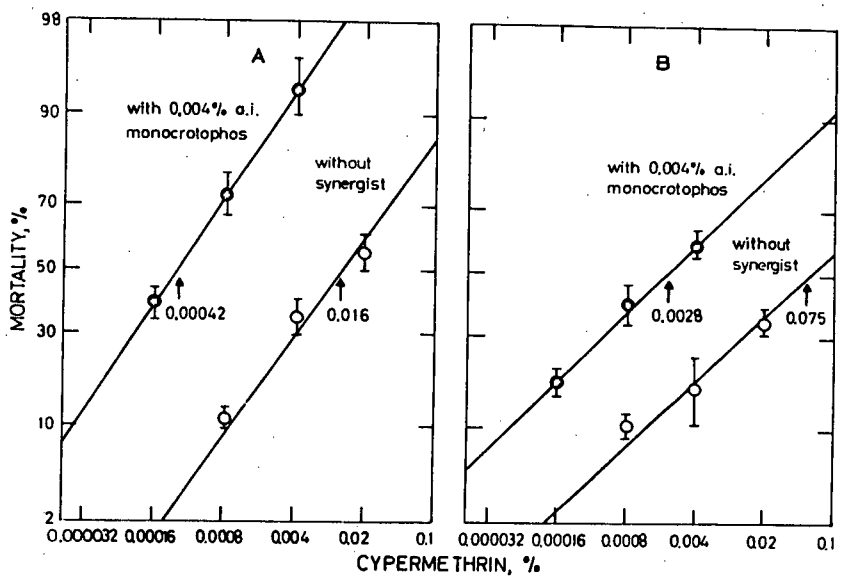
A
B
with 0,004% a.i.
monocrotophos
without
synergist
0.00042
0.016
with 0.004% a.i.
monocrotophos
without
synergist
0.0028
0.075
MORTALITY, %
98
90
70
50
30
10
2
0.000032 0.00016 0.0008 0.004 0.02 0.1
CYPERMETHRIN, %

Fig. 5

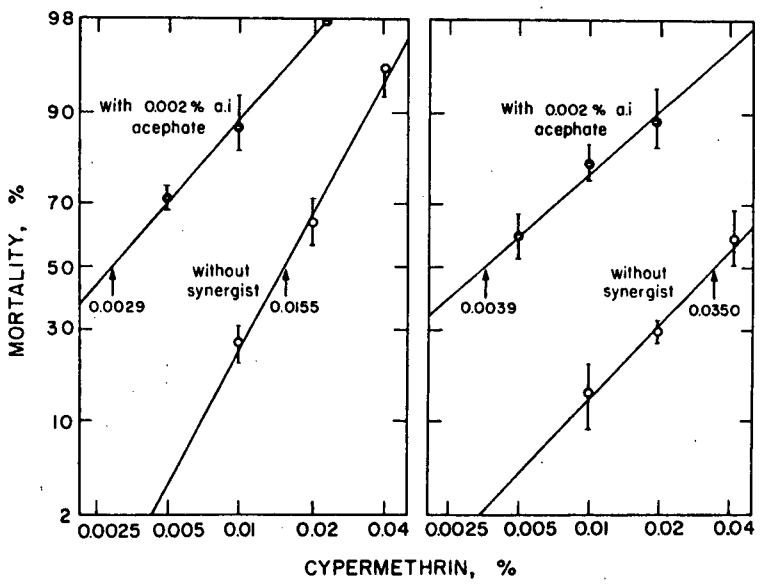
with 0.002 % a.i
acephate
without
synergist
0.0029
0.0155
with 0.002 % a.i
acephate
without
synergist
0.0039
0.0350
MORTALITY, %
98
90
70
50
30
10
2
0.0025 0.005 0.01 0.02 0.04
CYPERMETHRIN, %

Fig. 6

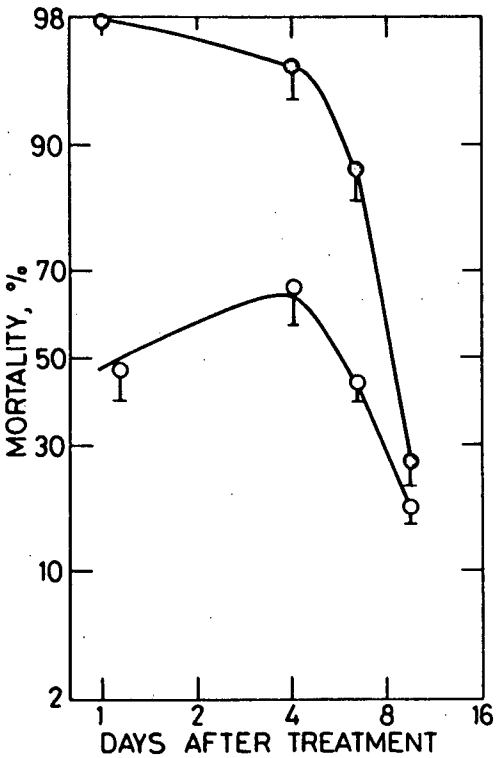

Fig. 7

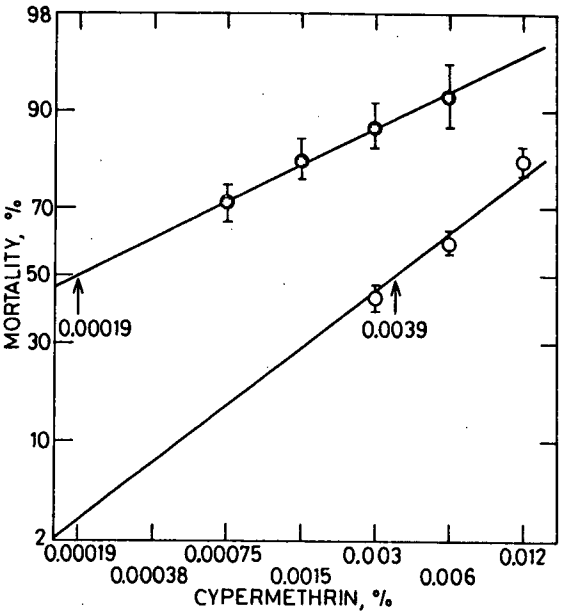

Fig. 8

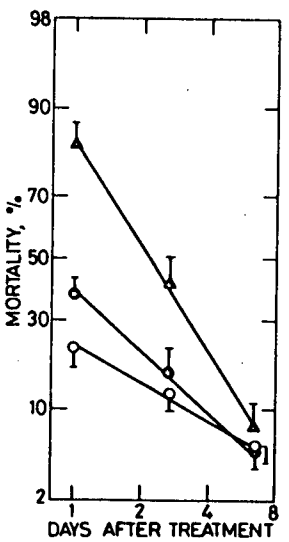

Fig. 9

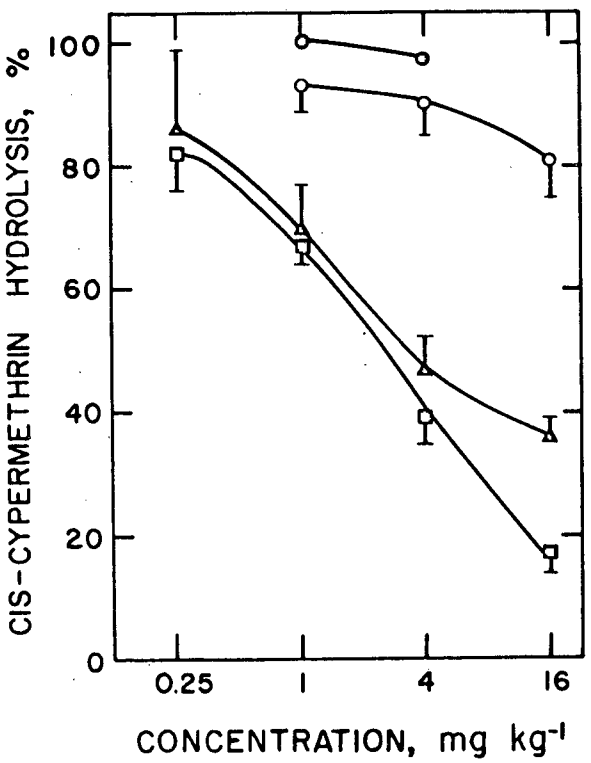
CIS-CYPERMETHRIN HYDROLYSIS, %
100
80
60
40
20
0
0.25
1
4
16
CONCENTRATION, mg kg-1

Fig. 10

CROP PROTECTION (1983) 2 (3), 335–343

Pyrethroid synergism by esterase inhibition in *Spodoptera littoralis* (Boisduval) larvae

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ABSTRACT. The susceptibility of cotton leafworm, *Spodoptera littoralis* (Boisduval) larvae to poisoning by *trans*-permethrin and *cis*-cypermethrin was increased when these pyrethroids were applied topically after the larvae had ingested profenofos, monocrotophos or azinphos-methyl for 24 h. An ingested dose of 4 nmol profenofos per larva gave a synergism factor of about threefold for both *trans*-permethrin and *cis*-cypermethrin. These pyrethroids were not synergized by oxidase inhibitors such as piperonyl butoxide, SV-1 and MPP ingested at 80 nmol/larva. Esterase preparations of larval gut hydrolysed *trans*-permethrin two to three times more rapidly than *cis*-permethrin, deltamethrin, *trans*- or *cis*-cypermethrin. Integument esterase(s) are less active but show a similar preference for *trans*-permethrin. The gut esterase(s) hydrolysing *trans*-permethrin are more sensitive *in vitro* and *in vivo* to inhibition by profenofos than by azinphos-methyl or monocrotophos. The susceptibility of *S. littoralis* larvae to pyrethroids appears to be limited by pyrethroid esterases in the gut. Organophosphorous compounds inhibiting these detoxifying enzymes serve as synergists.

Introduction

The insecticidal activity of pyrethroids is limited by detoxifying oxidases and esterases (Casida and Ruzo, 1980). Inhibitors of these enzymes may prolong the stability and enhance the potency of pyrethroids in insects, thereby acting as synergists. The synergistic activity of piperonyl butoxide, MPP and SV-1 (for chemical names *see* Table 1) results primarily from inhibiting oxidative detoxification (Casida, 1970; Ishaaya and Casida, 1980). Esterase inhibitors such as profenofos are effective synergists for the toxicity of several pyrethroids to cabbage looper larvae, *Trichoplusia ni* (Hübner) (Jao and Casida, 1974a; Gaughan, Engel and

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TABLE 1. Common and chemical names of principal compounds

Common name	Chemical name
Acephate	*O,S*-dimethyl acetylphosphoramidothioate
Azinphos-methyl	*S*-(3,4-dihydro-4-oxobenzo [*d*]-[1,2,3]-triazin-3-ylmethyl) *O,O*-dimethyl phosphorodithioate
Chlordimeform	N^2-(4-chloro-*o*-tolyl)-N^1,N^1-dimethylformamidine
cis- or *trans*-cypermethrin	(*RS*)-α-cyano-3-phenoxybenzyl (1*RS*)-*cis*-or -*trans*- 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate, or an individual isomer as specified
Deltamethrin	(*S*)-α-cyano-3-phenoxybenzyl (1*R*)-*cis*-3-(2,2,-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate
Monocrotophos	dimethyl (*E*)-1-methyl-2-methylcarbamoylvinyl phosphate
MPP (also known as Niagara 16824)	*O*-(2-methylpropyl) *O*-2-propynyl) phenylphosphonate
cis- or *trans*-permethrin	3-phenoxybenzyl (1*RS*)-*cis*- or -*trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate
Piperonyl butoxide	5-[2-(2-butoxyethoxy)ethoxymethyl]-6-propyl-1,3-benzodioxole
Profenofos	*O*-(4-bromo-2-chlorophenyl) *O*-ethyl *S*-propyl phosphorothioate
SV-1	*O,O*-diethyl *O*-phenyl phosphorothioate
Upjohn 42662	1,1,1-trichloro-*N*-(((4-chloro-2-methylphenyl)imino)methyl)-*N*-methylmethane-sulfenamide

Casida, 1980; Ishaaya and Casida, 1980). The present study evaluates the effectiveness of various oxidase and esterase inhibitors for synergizing pyrethroids in a major agricultural pest, *Spodoptera littoralis* (Boisduval) larvae.

Materials and methods

Chemicals

The labelled and unlabelled pyrethroids were obtained and purified as described previously (Shono, Ohsawa and Casida, 1979; Ishaaya and Casida, 1980, 1981). ^{14}C-acid preparations of *cis*- and *trans*-permethrin and *cis*- and *trans*-cypermethrin were 1*RS* and 1*RS*, α*RS* mixtures, respectively. Unlabelled preparations used were (1*RS trans*)-permethrin and (1*R cis*, α*S*)-cypermethrin. Emulsion concentrates of 50% profenofos (Ciba-Geigy, Basel), 40% monocrotophos and 20% azinphos-methyl (Makhteshim, Beer Sheva) were used as esterase inhibitors. Piperonyl butoxide technical grade (Pazchem, Tel Aviv), SV-1 (Ishaaya and Casida, 1980; synthesized by S. Tawata of the Berkeley Laboratory), and MPP (FMC Corporation, Middleport, New York) were used as oxidase inhibitors.

Esterase preparation and assay

Fourth-instar *S. littoralis* larvae (80 ± 5 mg body weight) were from a standard laboratory colony reared on alfalfa (Ascher and Moscowitz, 1970). The gut was

obtained by cutting off the head, excising the alimentary canal onto a wet filter paper and then removing the peritrophic membrane containing the food as described previously (Ishaaya and Casida, 1980). Most of the remaining body contents were then extruded through the anterior end of the tube to obtain the integument. Standard enzyme solutions were prepared at 0–3°C by homogenizing 10 gut walls or integuments in 8 ml distilled water. The post-mitochondrial 12 000 *g* supernatant (microsomes + soluble) fraction obtained after 15 min centrifugation was assayed for pyrethroid esterase activity. The pyrethroid esterase assays were as previously described (Ishaaya and Casida, 1980, 1981) using the following standard mixture in 2 ml of 0·1M phosphate (pH 8·0) or glycine-NaOH (pH 8·5) buffer incubated for 10 or 20 min (integument and gut preparations, respectively) at 32°C: 0·1 nmol of ^{14}C-pyrethroid (~2000 counts per minute), 2 mg of bovine serum albumin and enzyme preparations equivalent to 50 mg larval weight (approximately 250 and 600 μg protein for gut and integument 12 000 *g* supernatants, respectively).

Esterase inhibition

Inhibitor solutions for the *in vitro* assays were prepared as described previously (Ishaaya and Casida, 1980) from a stock at 100-fold the desired molarity in ethanol, by combining 0·5 ml stock solution and 49·5 ml water. The final inhibitor concentration is expressed as that present in the 0·5 ml pre-incubation medium consisting of 0·25 ml enzyme solution and 0·25 ml inhibitor solution. The enzyme reaction in 2 ml final volume at 32°C was initiated after 15 min pre-incubation by

TABLE 2. Effect of oxidase and esterase inhibitors on the toxicity of *trans*-permethrin and *cis*-cypermethrin to *Spodoptera littoralis*

Candidate synergist			Mortality (%)[a]	
Compound[b]	nmol/larva	μg/larva	*trans*-Permethrin	*cis*-Cypermethrin
None	—		9±3	7±3
Piperonyl butoxide	80	27·0	8±1	12±3
MPP	80	20·2	6±3	4±3
SV-1	80	19·7	4±3	7±4
Profenofos	2	0·75	31±9	10±2
Profenofos	4	1·50	58±6*	47±5*
Monocrotophos	2	0·45	13±1	9±7
Monocrotophos	4	0·90	57±8*	39±5*
Azinphos-methyl	4	1·27	21±9	27±5*
Azinphos-methyl	8	2·54	37±8*	34±6*

[a]Topical application of *trans*-permethrin at 0·075 μg/larva and *cis*-cypermethrin at 0·0025 μg/larva. Data are averages of at least three replicates with their SE values.

[b]The level of ingested oxidase or esterase inhibitor used in these assays gave less than 5% mortality, and in the untreated control 0% mortality. Alfalfa leaves (3–4 cm^2) were used for the *trans*-permethrin assays and cotton-leaf squares (4 cm^2) for the *cis*-cypermethrin assays (*see* Methods).

*Significantly different at 5% level from treatment with *trans*-permethrin or *cis*-cypermethrin alone.

methyl significantly increased larval susceptibility to poisoning by *trans*-permethrin and *cis*-cypermethrin (Table 2). On the other hand, oxidase inhibitors such as piperonyl butoxide, MPP and SV-1 had no effect on the toxicity of these pyrethroids. Dietary profenofos at 4 nmol/larva, the highest dose that could be used with no more than 5% mortality, synergized the toxicity of *trans*-permethrin and *cis*-cypermethrin threefold (Figure 1). It appears that esterases are more important than oxidases in detoxifying *trans*-permethrin and *cis*-cypermethrin in *S. littoralis* larvae.

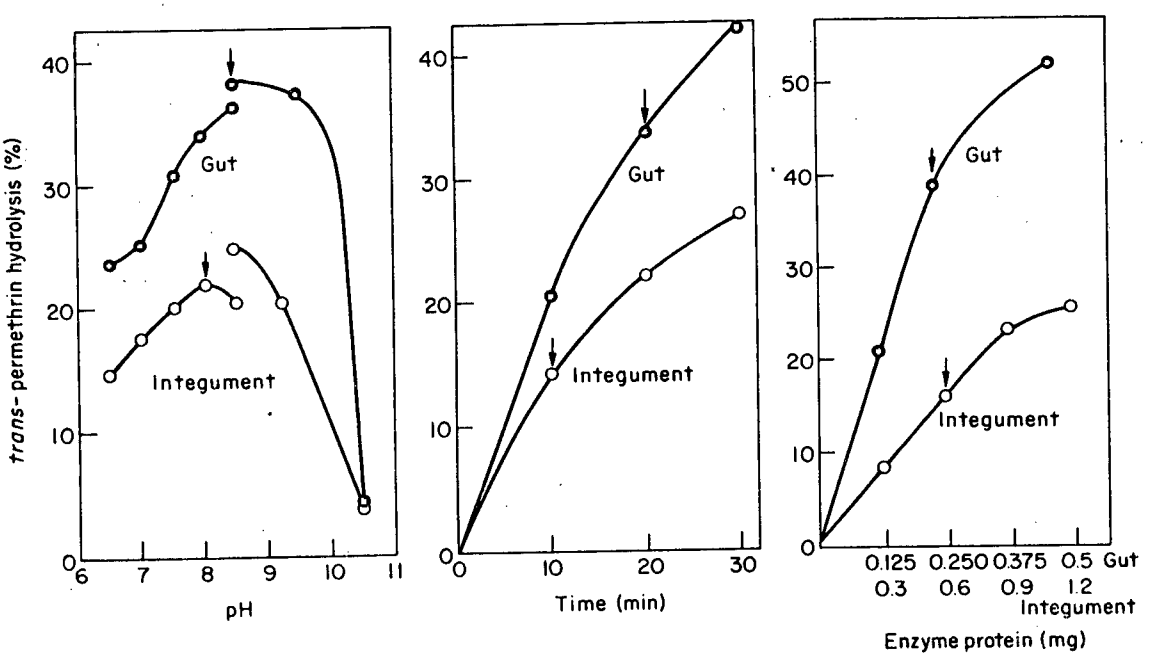

FIGURE 2. Effect of pH, incubation time and enzyme protein level on hydrolysis of *trans*-permethrin by larval gut and integumental esterase(s). Enzyme protein refers to protein level in the post-mitochondrial supernatant fraction used for assay. Each arrow designates the standard assay condition. For pH curve determination, phosphate buffer (6·5–8·5) and glycine–NaOH buffer (8·5–10·5) were used.

Properties of gut and integument pyrethroid esterases

The post-mitochondrial supernatant fractions of gut wall and integument homogenates hydrolyzed *trans*-permethrin, as shown in Figure 2 with respect to pH optima, time and enzyme concentration relationships. *Trans*-permethrin hydrolysis approximated linearity with times up to 20 min and enzyme concentrations up to 0·25 mg protein for the gut enzyme preparation and up to 10 min and 0·6 mg protein for the integument enzyme preparation. Accordingly, the enzyme assay was standardized at 0·25 mg protein and 20 min incubation for gut preparations and 0·6 mg protein and 10 min for integument preparations. The pH optima were 8 to 9 for both the gut and integument enzymes. Glycine-NaOH buffer gave slightly higher activity than phosphate buffer with both enzymes. The pH of the larval midgut is 9·5 (Ishaaya, Moore and Joseph, 1971) indicating appropriate conditions for pyrethroid esterases acting in the gut lumen. Under optimal conditions the activity was higher for the gut than for the integument esterases, approximating 1·5-fold on a per larva

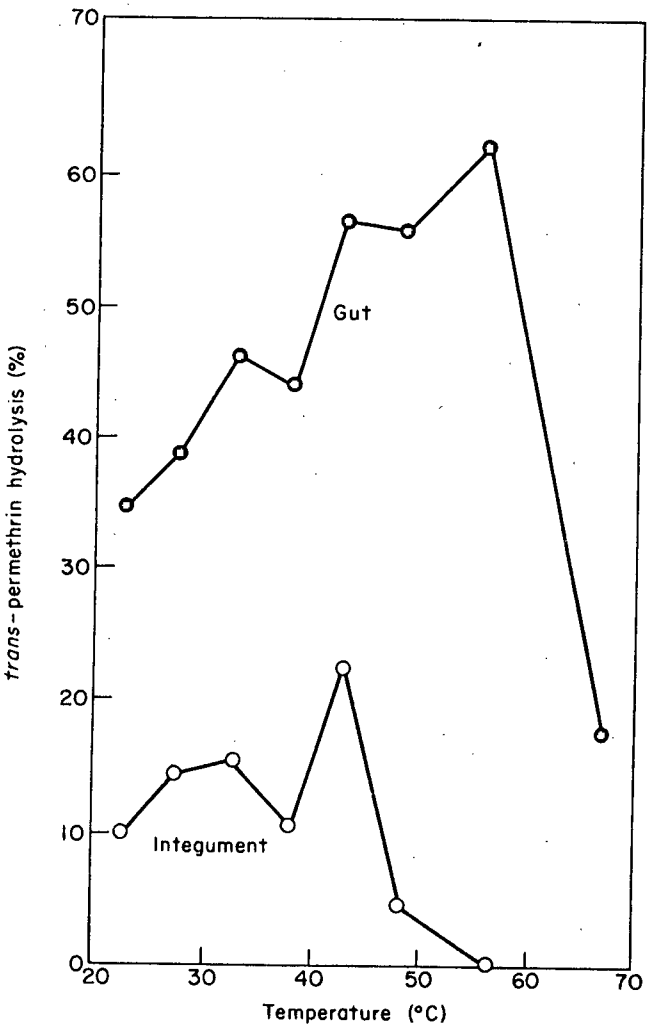

FIGURE 3. Effect of reaction temperature on hydrolysis of *trans*-permethrin by larval gut and integumental esterase(s).

basis and three- to four-fold on a per mg protein basis. The gut esterase maintained activity at higher temperatures (47–55°C) than did the integument enzyme (Figure 3). This curve relating activity to temperature suggests, but does not establish, intrinsic differences between the gut and integument enzymes and possible multiple components in both cases.

The higher esterase activity of gut than of integument preparations noted for *trans*-permethrin (Figure 2) extended also to *cis*-permethrin, *trans*- and *cis*-cypermethrin and deltamethrin (Table 3). The integument enzyme was very low in activity except on *trans*-permethrin. The gut preparations hydrolyse *trans*-permethrin much faster than *cis*-permethrin but there was relatively little difference in hydrolysis rates for the cypermethrin isomers and deltamethrin.

Inhibition of gut pyrethroid esterase(s)

Profenofos, azinphos-methyl and monocrotophos inhibited the larval gut esterase activity both *in vitro* and *in vivo* (Figure 4). The *in vitro* inhibition by the

TABLE 3. Hydrolysis of permethrin and cypermethrin isomers and deltamethrin by pyrethroid esterase(s) from *Spodoptera littoralis* gut wall and integument

Pyrethroid	Hydrolysis (%)[a] *trans*	Hydrolysis (%)[a] *cis*	Ratio *trans/cis*
Gut wall esterase(s)			
Permethrin	35·0±1·6b	16·8±3·3cd	2·1
Cypermethrin	19·0±2·1c	14·5±1·6cd	1·3
Deltamethrin	—	11·0±0·9d	
Integumental esterase(s)			
Permethrin	13·1±0·9b	1·0±0·6d	13·1
Cypermethrin	3·0±0·7c	1·1±0·7d	2·7
Deltamethrin	—	3·0±0·5c	

[a]Hydrolysis in 10 min under the standard conditions. Data are averages of 4–5 replicates with their SE values. Means followed by different letters in the same group (gut or integumental esterases) differ significantly ($P<0·01$).

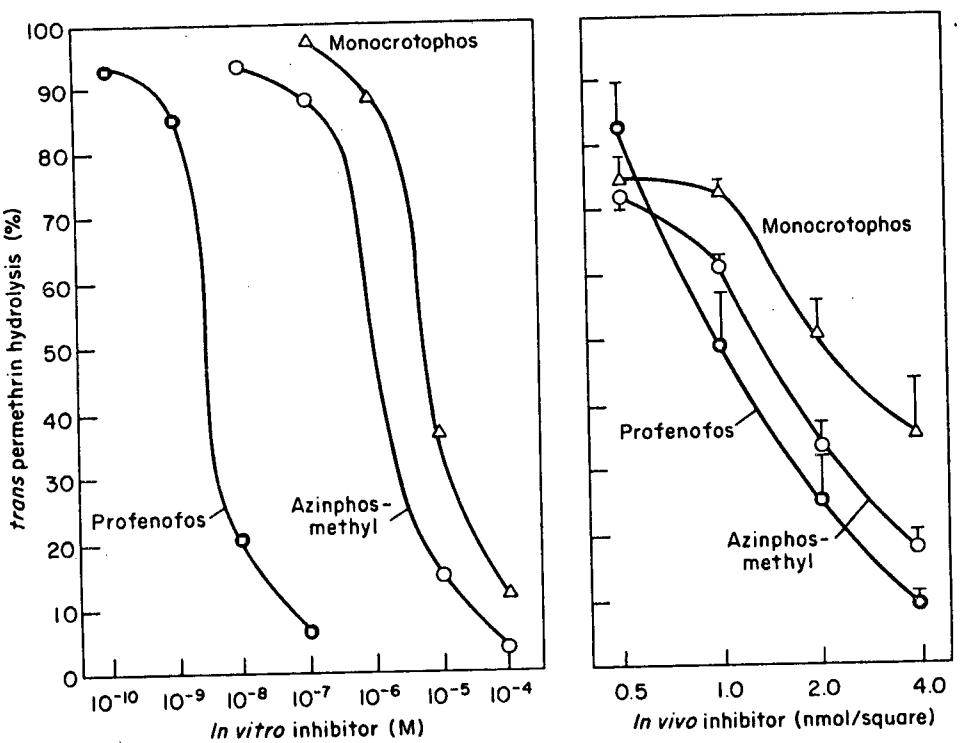

FIGURE 4. Effects of profenofos, azinphos-methyl and monocrotophos *in vitro* and *in vivo* on hydrolysis of *trans*-permethrin by larval gut esterase(s). Ranges for SE values are indicated.

phosphorothionate azinphos-methyl may result from the oxon or *O,S* isomerization impurities (Aldridge, Miles, Mount and Verschoyle, 1979) and the *in vivo* inhibition from its activation to azinphos-methyl oxon. Profenofos is the most potent compound in both cases, with 50% inhibition values of 5×10^{-9}M in the *in vitro* assay and of 1 nmol per larva for the *in vivo* assay. An *in vivo* inhibition of 90% is obtained with 4 nmol profenofos and to a lesser extent with azinphos-methyl and monocrotophos. This *in vivo* inhibitory effect on pyrethroid hydrolysis is probably responsible for the enhanced larval susceptibility to *trans*-permethrin and *cis*-cypermethrin poisoning in the presence of organophosphorus insecticides (Figure 1).

Discussion

Pyrethroid detoxification in lepidopterous larvae involves both hydrolytic and oxidative processes. Several types of evidence with lepidopterous larvae suggest a greater relative importance of esterases in metabolism of *trans*-permethrin and of oxidases in metabolism of *cis*-permethrin, i.e. *in vivo* metabolites in *Trichoplusia ni* larvae (Shono, Unai and Casida, 1978), *in vitro* metabolites in *T. ni* midgut enzyme preparations (Shono, Ohsawa and Casida, 1979), and the effect of inhibitors on loss of the parent compounds in *Spodoptera littoralis* (Holden, 1979). Esterases may be more important than oxidases in *S. littoralis* larvae, as suggested by the much better synergistic effect of esterase inhibitors than of oxidase inhibitors with *trans*-permethrin and *cis*-cypermethrin.

Pyrethroid esterases have somewhat similar properties in the three lepidopterous species that have been examined, i.e. *T. ni* (Jao and Casida, 1974b; Ishaaya and Casida, 1980), *Spodoptera eridania* Cram. (Abdel-Aal and Soderlund, 1980) and *S. littoralis* (this study). The gut wall is more active than the integument on a protein or per larva basis. Hydrolysis occurred more rapidly with *trans*-permethrin and *trans*-cypermethrin than with the corresponding *cis*-isomers; a *trans/cis* specificity was also evident but was much less prominent with *trans*-and *cis*-cypermethrin which contain the α-cyano substituent. The slower rate of ester cleavage of the *cis*- than of the *trans*- isomers paralleled their relative insecticidal activities.

The threefold magnitude of pyrethroid synergism by profenofos in *S. littoralis* (this study) can be compared with 20-fold in *T. ni* under similar conditions (Ishaaya and Casida, 1980). *Trans*-resmethrin was synergized two- to three-fold in *T. ni* with two esterase inhibitors (1-naphthyl *N*-propylcarbamate and *S,S,S*-tributyl phosphorotrithioate) and two oxidase inhibitors (MPP and piperonyl butoxide) (Jao and Casida, 1974a). Other compounds have been reported as pyrethroid synergists: these are chlordimeform and Upjohn 42662, presumably acting as oxidase inhibitors, and acephate as a possible esterase inhibitor in *Heliothis* sp. (Plapp, 1979); and various organophosphorus compounds in *T. ni* (Ishaaya and Casida, 1980), *Tetranychus urticae* Koch (Chapman and Penman, 1980), and *Chrysopa carnea* Stephens (Ishaaya and Casida, 1981), probably acting as esterase inhibitors.

Pyrethroids are often applied to insect populations which are also exposed to organophosphorus compounds, creating thereby a possibility for interactions. Suitable combinations of pyrethroids and organophosphorus compounds might lead to synergism and enhanced pyrethroid effectiveness. Care must be taken in this approach to evaluate the impact on non-target organisms as well as on the pest species.

Acknowledgement

The authors thank Mrs Sara Yablonski, Miss Anna Elsner and Miss Shoshana Hadad for excellent technical assistance. This work was supported by grant No. 249 from the United States–Israel Binational Agricultural Research and Development Fund and by grant PO1 ES00049 from the United States National Institute of Environmental Health Sciences.

Contribution No. 338-E, 1982 series, from the Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel.

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Accepted 29 January 1983

THE TOXICITY OF PYRETHROID-ORGANOPHOSPHORUS INSECTICIDE MIXTURES AGAINST SPODOPTERA LITTORALIS LARVAE

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ABSTRACT

Mixtures of one of the pyrethroids cypermethrin, fenvalerate or deltamethrin with one of the organophosphorus (OP) compounds monocrotophos, profenofos, azinphos-methyl or acephate were assayed at different ratios as 24-h dipping residues on alfalfa against Spodoptera littoralis larvae. With most of the mixtures containing various OP concentrations in excess of those of the pyrethroids, synergism was demonstrated. In the pairs fenvalerate-azinphos-methyl, deltamethrin-azinphos-methyl and deltamethrin-profenofos, however, no synergism was found. In a detailed investigation with pyrethroid concentrations causing ~20% mortality and OP concentrations giving a kill of no higher than ~10%, the above findings on synergism were amply confirmed.

A cypermethrin-monocrotophos mixture showed synergism also on cotton leaves sprayed in the field.

Publication of the Agricultural Research Organization. No. -E, 1985 series. Received ; received in final form .
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KEY WORDS: Spodoptera littoralis; synergism, pyrethroid-OP mixtures; cypermethrin; fenvalerate; deltamethrin; monocrotophos; profenofos; azinphos-methyl; acephate.

INTRODUCTION

All et al. (2) enumerated the reasons for employing mixtures of insecticides of various chemical types in agriculture, as follows: "A mixture may give best control of a complex of pests with varying susceptibilities to the different components of the mixture; insects that are resistant to one or more insecticides may be susceptible to a combination of toxicants; or synergism may be exhibited by the combinants."

The present work deals with the synergism of mixtures of pyrethroids with organophosphorus (OP) insecticides in larvae of the Egyptian cotton leafworm, Spodoptera littoralis (Boisd.). In fact, OPs, carbamates and formamidines frequently synergize pyrethroids in insects and acari. The mechanism of this synergism is as follows: pyrethroids are detoxified in insects by esterases and oxidases, as demonstrated by Casida and co-workers (6,18); Jao and Casida (18,19; see also 11) found already in 1974 that a variety of chemicals, both esterase and oxidase inhibitors, synergized some of the early photostable pyrethroids, such as resmethrin and tetramethrin, in larvae of the cabbage looper, Trichoplusia ni (Hübn.), and in the large milkweed bug, Oncopeltus fasciatus (Dallas). Plapp (24) found in 1976 that pyrethrins and tetramethrin are synergized by chlordimeform at the 1:1 ratio in larvae of the tobacco budworm, Heliothis virescens (F.). Later, in 1979, Plapp (25) extended these

findings in both H. virescens and H. zea (Boddie) to permethrin, fenvalerate and deltamethrin, which were synergized by chlordimeform, amitraz and two other formamidines. Chlordimeform was a more efficient pyrethroid synergist than the OP acephate. When tested by topical application to H. zea and H. punctigera Wellengren larvae, fenvalerate-methomyl 1:8 and 1:9.3 mixtures showed "similar joint action" - no synergism, as demonstrated by Kay (20) in 1981.

All et al. (2) found in 1977 synergism with 1:10 permethrin-parathion methyl mixtures in topical application to H. virescens, and to H. zea with 1:9 mixtures, but not with analogous fenvalerate-methyl parathion combinations. Also methomyl-permethrin and methomyl-fenvalerate showed potentiation in the latter insect. Field trials in cotton and sweet corn confirmed the enhanced toxicity of mixtures of either of the two pyrethroids with the OPs parathion-methyl and chlorpyrifos, or with methomyl. In certain cases, these mixtures were effective at rates reduced to one-tenth of the normal doses.

Reed et al. (26) demonstrated in 1977 that SAN 197 [O-ethoxy-2-ethyl-4-pyrimidinyl-O-O-dimethyl phosphorothioate] prolongs the residual effect of permethrin against females of the mosquito Aedes triseriatus (Say), but not against females of the mosquito Anopheles quadrimaculatus (Say), and the housefly, Musca domestica L. Desmarchelier (8) in 1977 pointed out the usefulness of combinations of a pyrethroid (bioresmethrin) or pyrethrins with a whole array of OPs against stored product Coleoptera.

Nolan and Bird (22) demonstrated in 1977 that pyrethroid-OP mixtures (permethrin or deltamethrin with ethion in the 1:100 ratio) proved an efficient means of controlling OP-resistant strains of the

cattle tick Boophilus microplus (Canestrini), in the field. They confirmed in laboratory tests that this effect was due to potentiation.

Hughes and Trevethan (12) in 1979 showed potentiation in a 1:5 cypermethrin-chlorfenvinphos mixture against larvae of the sheep blowfly, Lucilia cuprina (Wiedemann) both in laboratory toxicity studies and on sheep against implants of larvae.

Chapman and Penman (7) in 1980 investigated the effect of fenvalerate-azinphos-methyl combinations on mites and found ratios of 1:1, 1:2, 1:5 and 2:1 to be equally synergistic against the two-spotted spider mite, Tetranychus urticae Koch. Phosphorodithioates (such as azinphos-methyl, azinphos-ethyl and malathion) and phosphates (dichlorvos, mevinphos) proved to be efficient fenvalerate synergists against this mite.

Ishaaya and Casida (15) showed in 1980 that sublethal doses (causing no more than 5% mortality) of profenofos ingested with treated leaf discs by T. ni larvae, synergized subsequently topically applied cis-cypermethrin 20-fold and trans-permethrin fourfold. These authors (16) discovered in 1981 that in the common green lacewing, Chrysopa carnea Stephens, an insect with a natural high tolerance to pyrethroids, trans-permethrin is synergized by ~70 times by phenyl saligenin cyclic phosphonate, a potent esterase inhibitor.

Koziol and Witkowski (21) in 1982 assayed binary mixtures of methyl parathion, chlorpyrifos or malathion with permethrin in the ratios 9, 4, 2.3 and 1.5:1 against larvae of the European corn borer, Ostrinia nubilalis (Hübn.). All mixtures with methyl parathion and chloropyrifos were synergistic; with methyl parathion the 9:1 ratio showed less synergistic activity than the three other ratios, whereas

with chlorpyrifos all ratios were equally active. Malathion did not synergize permethrin.

Robertson and Smith (27) recently (1984) demonstrated synergism in larvae of the western spruce budworm, Choristoneura occidentalis Freeman, between deltamethrin and the OPs acephate, fenitrothion or chlorpyrifos; between fenvalerate and acephate, fenitrothion or phosmet; and between permethrin and chlorpyrifos. Numerous cases of synergism were located between the three pyrethroids and carbamates, such as carbaryl, aminocarb, methomyl and thiodicarb.

Ishaaya et al. (14) demonstrated in 1983 that profenofos synergizes the toxicity of cypermethrin against adults of the tobacco whitefly, Bemisia tabaci Gen., at the 1:1 ratio, by about 30 times and prolongs its activity.

On the other hand, in larvae of the red flour beetle, Tribolium castaneum (Herbst), larvae, oxidases - not esterases - seem to play an important role in metabolizing pyrethroids. Thus, several oxidase inhibitors synergized cypermethrin, fenvalerate or deltamethrin in this insect, but profenofos did not, as shown by Ishaaya et al. (17) in 1983.

Ozaki et al. (23) found in 1984 that fenvalerate, fenpropathrin and permethrin combinations with malathion or with several carbamates, as well as fenvalerate-diazinon mixtures, exhibited synergism against adult females of an OP- and carbamate-resistant strain of the green rice leafhopper, Nephotettix cincticeps Uhler.

Several studies have already been conducted with Spodoptera littoralis larvae, the subject of the present work. El-Guindy et al. (10) demonstrated in 1981 synergism for cypermethrin and fenvalerate by chlordimeform and methomyl in larvae of susceptible (S) and

resistant (R) S. littoralis strains. In the same year, Dittrich et al. (9) stated that resmethrin is synergized in S and R strains of S. littoralis by chlordimeform in feeding but not in contact-only experiments.

Saad et al. (28) found in 1981 an additive effect between cypermethrin and chlorpyrifos, and an antagonistic effect in deltamethrin mixtures with chlorpyrifos or phospholan, in this insect.

Ishaaya et al. (13) showed in 1983 that after S. littoralis larvae had ingested profenofos, monocrotophos or azinphos-methyl by feeding on treated leaves, both cis-cypermethrin and trans-permethrin subsequently applied topically were synergized. Synergism by profenofos was about three-fold.

Watson and Zidan (29) in 1983 found potentiation between cypermethrin and mephospholan or methomyl in S. littoralis.

Auda and Degheele (5) in 1985 found that with an S strain of S. littoralis fed treated leaves, the pyrethroid PP321 [Karate; α-cyano-3-phenoxybenzyl 3-(2-chloro-3,3,3-trifluoroprop-1-enyl) -2,2-dimethylcyclopropanecarboxylate] had a potentiating effect with either profenofos (in S or R) or methomyl (in S only) when the compounds were used at the LC-25 level, but only an additive effect with monocrotophos. Between cypermethrin and profenofos or methomyl there was synergism, and with monocrotophos antagonism, in the S strain, whereas in the R strain an additive effect with profenofos and methomyl, and antagonism with monocrotophos, were obtained. Auda and Degheele (5) reported deltamethrin-synergism with profenofos or methomyl and an additive effect with monocrotophos in S, and antagonism in the R strain.

MATERIALS AND METHODS

The insecticides

The pyrethroids employed were commercial formulations of cypermethrin (Cymbush 10% E.C., Makhteshim, Be'er Sheva, Israel); fenvalerate (Aqmatrine 20% E.C., Agan, Ashdod, Israel); and deltamethrin (Becis 2.5% E.C., Milchan Bros., Tel Aviv, Israel). The OP compounds were monocrotophos (Monocron 40% E.C., Makhteshim); profenofos (Curacron 50% E.C., Ciba-Geigy, Basle, Switzerland); azinphos-methyl (Cotnion 20% E.C., Makhteshim); and acephate (Orthene 75% S.P., Chevron, Richmond, CA, U.S.A.).

Bioassay

Alfalfa was dipped in aqueous dilutions of the pyrethroids alone and the OPs alone at various concentrations, and various ratios of mixtures of the two (see Table 1). The treated alfalfa was allowed to dry for 24 h and was then fed to S. littoralis larvae, weighing 200-250 mg, of an insecticide-susceptible laboratory strain grown on alfalfa at 27° C, as described by Ascher et al. (3) and Ascher and Moscowitz (4). The experiments were done in glass jars (base diam. 75 mm, height 140 mm), each containing ten larvae feeding on alfalfa over a layer of sawdust, with four replications in each treatment. The larvae were kept for 2 days on the treated alfalfa and then the number of dead and injured larvae was recorded. The results were corrected for control mortality and injury according to Abbott's (1) formula. All experiments were conducted at 27 C.

Each pyrethroid-OP concentration ratio in Table 1 was repeated two or three times on different days and synergistic and nonsynergistic mixtures were determined. Among the synergistic mixtures a pyrethroid concentration giving alone ~20% mortality and an OP-compound

concentration giving alone no more than ~10% kill were selected from each pair of compounds, for intensive further study. Each of these treatments was repeated six to nine times on different days.

Field/laboratory experiments

Cotton plants (60-80 cm high) were sprayed in the field with a "Solo" knapsack sprayer until runoff with an 0.01% a.i. aqueous dilution of the cypermethrin E.C. alone, and of the monocrotophos E.C. alone, as well as with a mixture containing 0.01% a.i. cypermethrin + 0.01% a.i. monocrotophos. Three replicates of a 3-m cotton row were used for each treatment. Twelve leaf samples were taken at various intervals after the spray from each treatment to determine residual toxicity. Each leaf sample was introduced into a rectangular (10 x 7 x 3.5 cm height) soft plastic box with a transparent cover provided with breathing holes, and offered to ten 20-40 mg S. littoralis larvae for 48-h mortality determinations as described above.

RESULTS AND DISCUSSION

Laboratory bioassays

Alfalfa feeding tests

Table 1 shows the pyrethroid-OP mixtures tested at different ratios. With cypermethrin, most mixtures containing a pyrethroid concentration showing some toxicity (3, 4 or 5 ppm) plus monocrotophos at equal concentration or in excess were synergistic, whereas when the monocrotophos concentration was lower than that of cypermethrin, no synergism could be detected. Similar synergism was obtained with cypermethrin combined with x2 excess of profenofos or x2.5 or more excess of azinphos-methyl. With acephate x3 excess or more was needed

for synergism, but much higher ratios (x10, x15) were ultimately chosen in accordance with the criteria in Materials and Methods.

[Table 1]

With the pair fenvalerate-monocrotophos only at a certain, specific ratio (7 ppm: 20 ppm) was synergism found, whereas several other ratios with monocrotophos in excess were nonsynergistic. A similar specificity held true for fenvalerate-profenofos (synergistic mixture, 5 ppm : 8 ppm). None of the fenvalerate-azinphos-methyl mixtures (the latter in excess) showed synergism. In the mixture with acephate the 60 ppm acephate concentration used with cypermethrin was employed again and synergism was obtained.

With the pair deltamethrin-monocrotophos, only 0.4 ppm deltamethrin plus 15 ppm monocrotophos was synergistic. No synergistic mixtures were located in the pairs deltamethrin-profenofos and deltamethrin-azinphos-methyl, whereas again the mixtures with high acephate concentrations were synergistic.

Table 2 shows the statistically significant results obtained with pyrethroid-OP mixtures, with pyrethroid concentrations causing ~20% mortality, and OP-concentrations giving no more than ~10% mortality.

[Table 2]

Topical application

A similar study was conducted with topical application of binary mixtures of one of the three pyrethroids cypermethrin, fenvalerate and deltamethrin with monocrotophos or azinphos-methyl. Not a single case

of synergism was encountered in all the pairs investigated. This means that pyrethroid-OP synergism is not operative through contact as it is through ingestion. A similar statement regarding pyrethroid synergism by formamidines was made by Dittrich et al.(9). Ishaaya et al. (13) demonstrated synergism when S. littoralis had ingested the OP with treated leaves for 24 h and were subsequently treated with pyrethroids by topical application (to overcome the antifeedant effect of the pyrethroids). It seems that at least the OP has to be ingested by the larvae to obtain synergism.

Field/laboratory experiments

The results of the toxicological laboratory bioassays of 1:1 cypermethrin-monocrotophos field spray residues on cotton are shown in Table 3. Synergism could be demonstrated well on days 6, 7 and 9 after the spray, when the effect of both cypermethrin and monocrotophos separately, tapered off to low values, whereas the toxicity of the mixture was still considerable.

[Table 3]

ACKNOWLEDGMENTS

The authors thank Mrs. Nadia E. Nemny for efficient help in summing up the data, Mrs. Chava Eizick for laboratory assays for the field/laboratory experiments, and Miss Shoshana Hadad for growing the Spodoptera littoralis larvae used in this work. This study was supported by Grant No. 249 from the United States - Israel Binational Agricultural Research and Development Fund (BARD).

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TABLE 1

LIST OF PYRETHROID-ORGANOPHOSPHORUS COMPOUND MIXTURES (in ppm a.i.) USED AS ALFALFA DIPPING DILUTIONS, TESTED FOR SYNERGISM IN SPODOPTERA LITTORALIS LARVAE (Cy = cypermethrin; Fen = fenvalerate; Delta = deltamethrin; Mon = monocrotophos; Prof = profenofos; Azin = azinphos-methyl; Aceph = acephate. Numbers following the abbreviations of the compounds are concentrations in ppm; *mixture chosen for further investigation.)

Cy

Synergism found in the following mixtures: Cy 3 - Mon 6; 15. Cy 4 - Mon 8*; 20. Cy 5 - Mon 5; 10; 25.
Cy 4 - Prof 8*
Cy 4 - Azin 10*; 40.
Cy 4 - Aceph 12; 16; 40*; 60*; 70; 80.

No synergism found in the following mixtures: Cy 4 - Mon 0.8; 2.
Cy 5 - Mon 2.5.
Cy 4 - Aceph 2; 4; 8.

Fen

Synergism found: Fen 7 - Mon 20*.
Fen 5 - Prof 8*.
Fen 5 - Aceph 60*.

No synergism found: Fen 4 - Mon 10; 20. Fen 5 - Mon 15.
Fen 4 - Prof 10. Fen 5 - Prof 10. Fen 7 - Prof 10.
Fen 4 - Azin 40; Fen 5 - Azin 10. Fen 7 - Azin 10.

Delta

Synergism found: Delta 0.4 - Mon 15*.
Delta 0.4 - Aceph 40*; 60*.

No synergism found: Delta 0.25 - Mon 8; Delta 0.3 - Mon 10.
Delta 0.25 - Prof 8. Delta 0.3 - Prof 10; Delta 0.4 - Prof 10; 15.
Delta 0.3 - Azin 40. Delta 0.4 - Azin 20.

TABLE 2

SYNERGISM IN SELECTED MIXTURES (IN PPM A.I. IN THE ALFALFA DIPPING DILUTIONS) OF PYRETHROID AND ORGANOPHOSPHORUS COMPOUNDS AGAINST SPODOPTERA LITTORALIS LARVAE. (All these mixtures were more toxic that the sum of mortality of the two separate components at P<0.01%, according to Duncan's Multiple Range Test.)

Insecticide, and concn in dipping liquid (ppm)		Ratio of mixture	% Mortality (±S.E.) found for each component separately		% Mortality (±S.E.) found in the mixture
Pyrethroid	OP		Pyrethroid	OP	Pyrethroid + OP
Cypermethrin, 4	Monocrotophos, 8	1:2	20.0±3.5	4.4±1.7	45.1±5.2
Cypermethrin, 4	Profenofos, 8	1:2	22.0±4.1	4.8±1.6	58.2±5.2
Cypermethrin, 4	Azinphos-methyl, 10	1:2.5	12.5±3.7	1.0±1.0	52.5±8.8
Cypermethrin, 4	Acephate, 40	1:10	22.8±5.1	4.4±1.6	48.7±5.6
Cypermethrin, 4	Acephate, 60	1:15	18.0±3.6	11.0±2.7	57.3±5.9
Fenvalerate, 7	Monocrotophos, 20	1:2.9	13.6±3.8	6.4±1.9	40.9±6.2
Fenvalerate, 5	Profenofos, 8	1:1.6	23.7±5.3	10.0±3.8	65.5±6.3
Fenvalerate, 5	Acephate, 60	1:12	20.3±3.9	13.1±4.7	71.7±4.2
Deltamethrin, 0.4	Monocrotophos, 15	1:37.5	16.0±4.0	4.8±1.5	53.7±5.7
Deltamethrin, 0.4	Acephate, 40	1:100	15.7±4.1	3.7±1.6	44.6±5.2
Deltamethrin, 0.4	Acephate, 60	1:150	21.2±3.4	11.8±2.7	57.4±5.3

TABLE 3

RESIDUAL EFFECT OF CYPERMETHRIN-MONOCROTOPHOS MIXTURES AGAINST SPODOPTERA LITTORALIS LARVAE VS THAT OF THE TWO SEPARATE COMPONENTS (Residues on sprayed cotton plants were aged in the field and bioassayed in the laboratory.)

Days after spray	Percent mortality (± S.E.)		
	0.01% a.i. cypermethrin	0.01% a.i. monocrotophos	0.01% a.i. cypermethrin + 0.01% a.i. monocrotophos
2	100	93 ± 2	100
4	79 ± 6	41 + 10	100
6	16 ± 6	13 ± 2	72 ± 7*
7	7 ± 2	5 ± 2	64 ± 9*
9	17 ± 8	5 + 2	60 ± 10*
12	6 ± 3	0	4 ± 2

*Significantly different at $P \leq 0.01$ from the sum of mortality obtained with cypermethrin and monocrotophos separately (Duncan's Multiple Range Test).

B. Pyrethroid Synergism by Oxidase Inhibitors

Pyrethroids derived from 3-phenoxybenzyl alcohol (trans- and cis-permethrin) and -cyano-3-phenoxybenzyl alcohol (trans- and cis-cypermethrin, deltamethrin and fenvalerate) are detoxicated by enzyme hydrolysis and oxidation (1-4). Esterase or oxidase inhibitors synergize the toxicity of some of these pyrethroids to several insect species. These include two dipterans (Musca domestica L. and Culex quinquefasciatus Say) (5,6), the larvae of four lepidopterans [Heliothis zea (Boddie), H. virescens (F.), Trichoplusia ni (Hubner) and Spodoptera littoralis (Boisd.)] (5-10), the pyrethroid-tolerant chrysopid Chrysopa carnea Stephens (11) and the aleurodid Bemisia tabaci (Gennadius) (12).

The first paper in this section extended these observations to Tribolium castaneum (Herbst), a major stored-product pest. Oxidase inhibitors such as piperonyl butoxide (PB), O,O-diethyl O-phenyl phosphorothioate (SV-1) and O-isobutyl O-prop-2-ynyl phenyl phosphonate (Niagara 16824) synergized the toxicity of cis-cypermethrin up to tenfold against T. castaneum larvae. A considerable synergism by PB was obtained also with cis-permethrin, trans-cypermethrin and deltamethrin. On the other hand the esterase inhibitor profenofos (9) did not synergize any of the test pyrethroids. These results indicate that in this Tribolium strain, oxidases are more important than esterases in detoxifying pyrethroids.

A novel approach was reported for pyrethroid synergism in T. castaneum larvae involving a mixture of permethrin with the juvenoid 6,7-epoxy-3-7-dimethyl-1-[3,4 (methylenedioxy)phenoxyl]-2-nonene (RO 20-3600) (13,14). In the present study (see second paper in this section), larvae of T. castaneum and of the house fly, Musca domestica vicina, were used to evaluate the toxicological properties and synergistic activity of another juvenoid with favorable persistence on plants and stored products (15-18),

ethyl[2-(phenoxyphenoxy)ethyl] carbamate (RO 13-5223). The results obtained indicate that RO 13-5223 at a dietary concentration of 100 mg kg-1 synergized the toxicity of the trans- and cis-isomers of permethrin and cypermethrin in inhibiting the growth (measured as gain in larval weight) of T. castaneum and M. domestica vicina (19). With both species the synergism factor for cis-cypermethrin was 1.5- to twofold for RO 13-5223 and about fourfold for piperonyl butoxide. Synergism was more pronounced with first than with fourth instar T. castaneum larvae. Joint application of RO 13-5223 and pyrethroids resulted in a dual effect on both T. castaneum and M. domestica vicina: increased inhibition of larval growth due to pyrethroid synergism, and progeny suppression - expressed by larval and pupal mortality - due to RO 13-5223 juvenilizing activity. These results indicate that mixtures of pyrethroids and some IGR compounds may have important practical implications in controlling insects.

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Pestic. Sci. 1983, **14**, 367–372

Synthetic Pyrethroids: Toxicity and Synergism on Dietary Exposure of *Tribolium castaneum* (Herbst) Larvae

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(*Revised manuscript received 23 September 1982*)

The potency of six dietary pyrethroids, as toxicants and inhibitors of weight gain in first- and fourth-instar *Tribolium castaneum* (Herbst) larvae, decreased in the order of *cis*-cypermethrin and deltamethrin > *trans*-cypermethrin and *cis*-permethrin > fenvalerate and *trans*-permethrin. Dosages that reduced larval weight also delayed pupation and emergence, probably due to their antifeeding activity. Three oxidase inhibitors (piperonyl butoxide, *O*,*O*-diethyl *O*-phenyl phosphorothioate, and *O*-isobutyl *O*-prop-2-ynyl phenylphosphonate), at a dietary concentration of 100 mg kg^{-1}, had little or no effect on the toxicity of *trans*-permethrin, but strongly synergised the toxicity of *cis*-cypermethrin by about 3-, 3- and 10-fold, respectively. Piperonyl butoxide also synergised the toxicity of *cis*-permethrin, *trans*-cypermethrin and deltamethrin, but not that of fenvalerate. On the other hand, an esterase inhibitor, profenofos, did not enhance the potency of any of the α-cyano-3-phenoxybenzyl pyrethroids. Oxidases appear to be more important than esterases in pyrethroid detoxification by *T. castaneum* larvae.

1. Introduction

Pyrethroids derived from 3-phenoxybenzyl alcohol (*trans*- and *cis*-permethrin) and α-cyano-3-phenoxybenzyl alcohol (*trans*- and *cis*-cypermethrin, deltamethrin and fenvalerate) are detoxicated by enzymic hydrolysis and oxidation.[1–4] Esterase or oxidase inhibitors synergise the toxicity of some of these pyrethroids to several insect species. These include two dipterans (adult *Musca domestica* L.[5] and larval *Culex quinquefasciatus* Say[6]), four lepidopteran larvae [*Heliothis zea* (Boddie),[7] *H. virescens* (F.),[7,8] *Trichoplusia ni* (Hübner,[5,9] and *Spodoptera littoralis* (Boisd.)[10]], and the pyrethroid-tolerant chrysopid *Chrysopa carnea* Stephens.[11] In contrast, the oxidase inhibitor piperonyl butoxide antagonises the toxicity of permethrin (3:1 *trans*:*cis* isomer mixture) to adults of both a susceptible and an organophosphorus-resistant strain of *Tribolium castaneum* (Herbst).[12] The present study extends these observations to *T. castaneum* larvae, a major stored-product pest, and to the effect of their dietary exposure to various synthetic pyrethroids, for which both the acute toxicity and antifeedant activity could be observed. The synergists examined were three oxidase inhibitors piperonyl butoxide (PB), *O*,*O*-diethyl *O*-phenyl phosphorothioate (I), and *O*-isobutyl *O*-prop-2-ynyl phenylphosphonate (II)],[9,13] and one esterase inhibitor, profenofos.[5,9,10]

2. Experimental methods

2.1. Chemicals

The pyrethroids of >99% purity (Table 1) were obtained from the indicated sources: (1*RS*)-*trans*- and *cis*-permethrin and (α*RS*, 1*RS*)-*trans*- and *cis*-cypermethrin from FMC Corporation (Middleport, New York); deltamethrin from Roussel-Uclaf (Paris, France); fenvalerate from

Table 1. The pyrethroids and synergists studied

Compound	Chemical name	Code used in text
Permethrin	3-Phenoxybenzyl (1*RS*)-*cis,trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate	
Cypermethrin	(*RS*)-α-Cyano-3-phenoxybenzyl (1*RS*)-*cis,trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate	
Deltamethrin	(*S*)-α-Cyano-3-phenoxybenzyl (1*R*)-*cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate	
Fenvalerate	(*RS*)-α-Cyano-3-phenoxybenzyl (*RS*)-2-(4-chlorophenyl)-3-methylbutyrate	
Piperonyl butoxide	5-[2-(2-Butoxyethoxy)ethoxymethyl]-6-propyl-1,3-benzodioxole	PB
	O,O-Diethyl *O*-phenyl phosphorothioate	I
	O-Isobutyl *O*-prop-2-ynyl phenylphosphonate	II
Profenofos	*O*-(4-Bromo-2-chlorophenyl) *O*-ethyl *S*-propyl phosphorothioate	

Shell Development Co. (Modesto, California). Synergists (Table 1) were as follows: piperonyl butoxide, technical grade (Pazchem, Tel Aviv); I (synthesised by S. Tawata of the Berkeley Laboratory); II (FMC Corporation); profenofos as a 50% emulsion concentrate (Ciba-Geigy, Basel, Switzerland).

Table 2. Effect of dietary pyrethroids on the larval weight, pupation and emergence of first-instar larvae of *Tribolium castaneum*

Pyrethroid and concentration in diet (mg kg^{-1})	Weight at day 14, relative to control[a] (%)	Pupation (and emergence), relative to control (%)	Average delay in pupation (and emergence) relative to control[a] (days)
trans-Permethrin			
0.2	115 (±3)	98 (96)	0.0 (−0.8)
1.0	108 (±8)	96 (96)	1.0 (0.8)
5.0	70 (±5)	96 (96)	4.0 (3.7)
25.0	<10**	33 (15)	19.6 (19.6)
cis-Permethrin			
0.2	86 (±2)**	98 (98)	0.6 (0.4)
1.0	82 (±2)**	100 (100)	1.2 (0.7)
5.0	48 (±2)**	100 (100)	7.6 (5.1)
25.0	All died		
trans-Cypermethrin			
0.2	71 (±8)*	100 (100)	1.6 (2.2)
1.0	37 (±6)**	88 (85)	12.3 (11.9)
5.0	All died		
cis-Cypermethrin			
0.2	39 (±8)**	50 (45)	20.4 (17.5)
1.0	All died		
Deltamethrin			
0.2	72 (±5)**	84 (84)	11.1 (12.2)
1.0	All died		
Fenvalerate			
0.2	94 (±4)	100 (100)	0.2 (0.3)
1.0	88 (±2)**	97 (97)	1.4 (1.8)
5.0	56 (±2)**	96 (96)	5.3 (6.3)
25.0	21 (±4)**	65 (28)	22.0 (23.0)

[a] The average larval weight at day 14 in the untreated control was 1.18(+0.03) mg, and the average days until pupation and emergence were 24.9 and 32.9, respectively. The experiment was continued until adult emergence was complete.

* Significantly different from control at 5% level.

** Significantly different from control at 1% level.

2.2. Toxicity and synergism assays

The *T. castaneum* larvae were from an insecticide susceptible colony[14,15] reared for the past 5 years on wheat flour containing 50 g dried yeast kg^{-1} at standardised laboratory conditions of 28°C and 70% relative humidity. For bioassays, the diet (10 g) was mixed with an acetone solution (10 ml) containing the test pyrethroid, with or without a synergist, or with acetone (10 ml) alone as the control. Following solvent evaporation and thorough mixing, the diet was distributed in 2-g portions in test vials (2 cm in diameter). Ten to 12 first-instar (0–3-h-old, white in colour) or fourth-instar [1.00 (±0.05) mg] larvae were placed in each vial and held at 28°C for determination of larval weight gain, pupation and emergence. Larval weight in the first-instar assays was determined 14 days after the start of the experiment, and in the fourth-instar assays 2 or 4 days after treatment. All data for larval weight, pupation and emergence are the means and standard errors (s.e.) for five replicates of 10 to 12 larvae for each concentration. The synergism factor was considered to be the magnitude of increased sensitivity to pyrethroid, based on IC_{50} values, after feeding on a diet containing pyrethroid and synergist. The IC_{50} value is the dietary concentration causing 50% inhibition of larval weight gain.

3. Results

3.1. Effect of pyrethroids on growth and development

Deltamethrin and *cis*-cypermethrin were the most potent compounds, resulting in total mortality of the first-instar larvae at 1 mg kg^{-1} (Table 2), and in a remarkable reduction in larval weight gain of the fourth-instar larvae at 2 mg kg^{-1} (Table 3). In both assays, *cis*-cypermethrin and *cis*-permethrin were much more effective than their *trans*-isomers. The least toxic compounds were *trans*-permethrin and fenvalerate (Tables 2 and 3). Sublethal dosages of all the pyrethroids delayed

Table 3. Effect of dietary pyrethroids on the larval weight gain, pupation and emergence of fourth-instar larvae of *Tribolium castaneum*

Pyrethroid and concentration in diet (mg kg^{-1})	Weight gain after 2 days, relative to control[a] (%)	Pupation (and emergence)[a] (%)
trans-Permethrin		
25	67 (±7)*	100 (100)
50	59 (±8)**	90 (90)
cis-Permethrin		
25	5 (±5)**	28 (28)
50	−20 (±3)**	0 (0)
trans-Cypermethrin		
4	97 (±14)	100 (100)
8	52 (±7)**	100 (100)
cis-Cypermethrin		
2	0 (±4)**	94 (94)
4	−30 (±11)**	16 (8)
Deltamethrin		
1	51 (±13)*	92 (92)
2	21 (±6)**	70 (70)
Fenvalerate		
25	39 (±10)**	98 (98)
50	20 (±3)**	98 (98)

[a] The average larval weight gain of the untreated control was 0.61 (±0.05) mg, and the pupation and emergence were 100%.

* Significantly different from control at 5% level.

** Significantly different from control at 1% level.

Table 4. Effect of piperonyl butoxide (PB) on the toxicity of dietary pyrethroids to fourth-instar larvae of *Tribolium castaneum*

Compound and concentration in diet (mg kg^{-1})		Weight gain after 4 days, relative to control[a] (%)	Pupation (and emergence) relative to control[a] (%)
Pyrethroid	PB		
—	50	98 (±3)	99 (98)
trans-Permethrin			
50	—	69 (±3)a	99 (99)
50	25	71 (±2)a	98 (98)
50	50	63 (±3)a	98 (93)
cis-Permethrin			
10	—	81 (±2)a	100 (98)
10	25	64 (±3)b	100 (100)
10	50	54 (±3)c	98 (98)
trans-Cypermethrin			
8	—	56 (±5)a	98 (85)
8	25	42 (±4)b	76 (72)
8	50	28 (±5)c	80 (73)
cis-Cypermethrin			
2	—	62 (±4)a	98 (98)
2	10	46 (±1)b	92 (55)
2	50	25 (±3)c	55 (48)
Deltamethrin			
1	—	84 (+4)a	98 (95)
1	25	77 (±3)a	96 (95)
1	50	57 (±4)b	98 (97)
Fenvalerate			
25	—	68 (±5)a	98 (98)
25	25	60 (±3)a	98 (92)
25	50	72 (±6)a	97 (97)

[a] The average larval weight gain of the untreated control was 1.45 (±0.03) mg and the pupation and emergence were 100%. Data followed by the same letter do not differ significantly from each other at the 5% level within the same group.

Table 5. Effect of two organophosphorus synergists on the toxicity of dietary *trans*-permethrin and *cis*-cypermethrin to fourth-instar larvae of *Tribolium castaneum*

Synergist and concentration in diet[a] (mg kg^{-1})		Weight gain after 4 days, relative to control[b] (%)	
		trans-Permethrin	*cis*-Cypermethrin
None	—	58 (±3)	47 (±2)
I	25	59 (±9)	15 (±2)**
	50	—	6 (±2)**
	100	62 (±3)	2 (±2)**
II	25	46 (±1)*	16 (±4)**
	50	—	2 (±2)**
	100	48 (±2)*	0**

[a] These dietary levels of I and II alone had no effect on the larval weight gain.

[b] The average larval weight gain in the untreated control was 1.43 (±0.03) mg. Dietary concentrations were 50 and 2 mg kg^{-1}, respectively, for *trans*-permethrin and *cis*-cypermethrin.

* Significantly different at 5% level from treatment with *trans*-permethrin alone.

** Significantly different at 1% level from treatment with *cis*-cypermethrin alone.

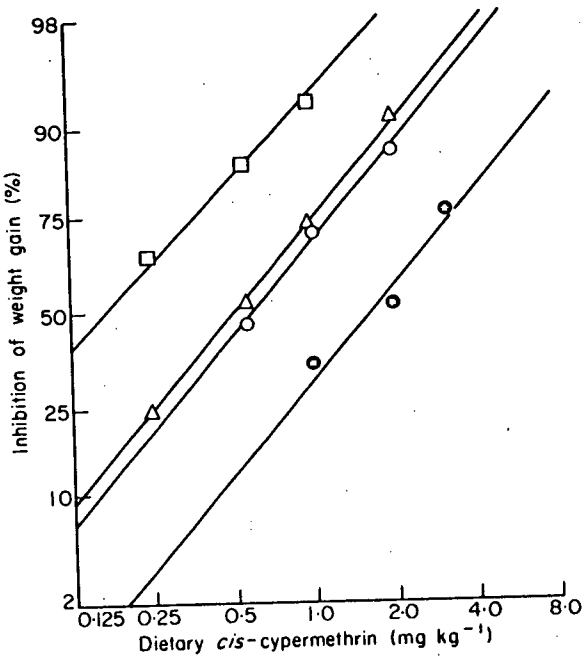

Figure 1. Synergistic effect of three oxidase inhibitors, at a dietary concentration of 100 mg kg^{-1}, on the toxicity of dietary *cis*-cypermethrin to fourth-instar larvae of *Tribolium castaneum*: (●) control (no oxidase inhibitor), IC_{50} value 1.82 mg kg^{-1}; (○) piperonyl butoxide, IC_{50} value 0.65 mg kg^{-1}; (△) *O,O*-diethyl *O*-phenyl phosphorothioate, IC_{50} value 0.55 mg kg^{-1}; (□) *O*-isobutyl *O*-prop-2-ynyl phenylphosphonate, IC_{50} value 0.19 mg kg^{-1}.

pupation and emergence (Table 2). At 25 and 50 mg kg^{-1}, *cis*-permethrin caused high larval mortality, and therefore reduced pupation, but did not affect adult emergence (Tables 2 and 3.)

3.2. Effect of oxidase and esterase inhibitors on pyrethroid toxicity

Piperonyl butoxide synergised the inhibition of larval weight gain by *cis*-permethrin, *trans*- and *cis*-cypermethrin and deltamethrin but not that by *trans*-permethrin or fenvalerate (Table 4). It also synergised the reduction in pupation and emergence resulting from exposure to *trans*- and *cis*-cypermethrin (Table 4). The two other oxidase inhibitors (I and II) strongly synergised the potency of *cis*-cypermethrin, while only II had a slight effect on the potency of *trans*-permethrin (Table 5). The synergism factor for *cis*-cypermethrin toxicity with 100 mg of synergist kg^{-1} was about 3-fold for PB and I and 10-fold for II (Figure 1). The esterase inhibitor profenofos had no synergistic effect on the cyano-pyrethroids even at the dose at which profenofos itself inhibited larval growth (Table 6).

Table 6. Effect of profenofos on the toxicity of dietary pyrethroids to fourth-instar larvae of *Tribolium casteneum*

Pyrethroid and concentration in diet (mg kg^{-1})		Weight gain after 4 days, relative to control[a] (%)	
		No profenofos	Profenofos (4 mg kg^{-1})
None	8	100	72 (±3)
trans-Cypermethrin	8	63 (±3)	58 (±3)
cis-Cypermethrin	2	63 (±2)	59 (±5)
Deltamethrin	1	82 (±3)	72 (±3)
Fenvalerate	25	67 (±5)	69 (±2)

[a] The average larval weight gain in the untreated control was 1.59 (±0.04) mg. Pupation and emergence were >90% in each case, with no effect attributable to the pyrethroid or profenofos, individually or in combination.

4. Discussion

The toxicity of the cyclopropanecarboxylates to *T. castaneum* larvae is greater for the *cis*-pyrethroids than the *trans*-pyrethroids, and greater for the cyano compounds than the non-cyano compounds. These toxicity relationships generally parallel the relative rates of hydrolysis and oxidation of these pyrethroids in many insects and in insect enzyme preparations.[3,4,9,10,16–19] Organophosphorus-sensitive esterase activity seems to be an important means for pyrethroid detoxication in *S. littoralis*,[10] *S. eridania*[18] and *T. ni*.[5,9] However, the esterase inhibitor profenofos was not synergistic with any of the cyano pyrethroids for *T. castaneum* larvae. On the other hand, the oxidase inhibitor piperonyl butoxide synergised the toxicity of *cis*-permethrin, *trans*- and *cis*-cypermethrin and deltamethrin, with little or no synergism for *trans*-permethrin and fenvalerate. I and II similarly synergised *cis*-cypermethrin but not *trans*-permethrin. These synergism results suggest that oxidases are more important than esterases in limiting the toxicity of several synthetic pyrethroids to *T. castaneum* larvae. An earlier study,[12] with adults rather than larvae of *T. castaneum*, revealed that PB antagonises instead of synergises the toxicity of permethrin as a *cis–trans* mixture.

The delay in pupation and emergence at sublethal pyrethroid doses is associated with a drastic reduction in larval growth, possibly due to antifeeding effect.[20]

Acknowledgements

The authors thank Mrs Sara Yablonski for excellent technical assistance. This paper constitutes contribution No. 408-E, 1982 series, from the Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel. The research was supported by grant No. I-249-81 from the United States-Israel Binational Agricultural Research and Development Fund (BARD), and by grant PO1 ES00049 from the United States National Institute of Environmental Health Sciences.

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PYRETHROID SYNERGISM AND PREVENTION OF EMERGENCE IN *TRIBOLIUM CASTANEUM* AND *MUSCA DOMESTICA VICINA* BY THE INSECT GROWTH REGULATOR RO 13-5223

I. ISHAAYA*, SARA YABLONSKI*, K.R.S. ASCHER** and J.E. CASIDA***

Ethyl [2-(4-phenoxyphenoxy)ethyl] carbamate (RO 13-5223) at a dietary concentration of 100 mg kg^{-1} synergized the toxicity of the *trans*- and *cis*-isomers of permethrin and cypermethrin in inhibiting the growth (measured as gain in larval weight) of *Tribolium castaneum* and *Musca domestica vicina*. With both species the synergism factor for *cis*-cypermethrin with 100 mg kg^{-1} synergist was 1.5- to twofold for RO 13-5223 and about fourfold for piperonyl butoxide. Synergism was more pronounced with first instar than with fourth instar *T. castaneum* larvae. Methoprene was not a pyrethroid synergist with *T. castaneum* larvae, so the synergistic effect of RO 13-5223 appears to depend on its structural features and not its insect-growth-regulator activity. Joint application of RO 13-5223 and pyrethroids resulted in a dual effect on both *T. castaneum* and *M. domestica:* increased inhibition of larval growth due to pyrethroid synergism, and progeny suppression – expressed by larval and pupal mortality – due to RO 13-5223 juvenilizing activity.

KEY WORDS: Insect growth regulator, RO 13-5223; methoprene, pyrethroid synergism; *Musca domestica vicina*, pyrethroid synergism; piperonyl butoxide, pyrethroid synergism; RO 13-5223, pyrethroid synergism; *Tribolium castaneum*, pyrethroid synergism.

INTRODUCTION

The insecticidal activity of pyrethroids is limited by metabolic hydrolysis and oxidation (3,5,8,9,16,17,24,27). Larvae of three lepidopterous species, *Trichoplusia ni, Spodoptera eridania* and *Spodoptera littoralis,* hydrolyze the *trans*-pyrethroids faster than the corresponding *cis*-isomers, correlating with their relative insecticidal

Publication of the Agricultural Research Organization. No. 795-E, 1983 series. Received March 21, 1984; received in final form July 9, 1984.

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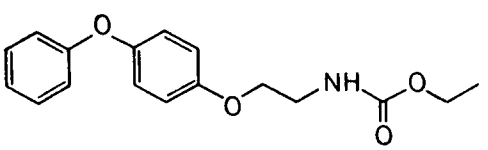
activities (1,11,12,14). Under laboratory conditions esterase inhibitors such as profenofos synergized the toxicity of *cis*-cypermethrin about 20-fold against *T. ni* (12) and about threefold against *S. littoralis* (11). Another potent esterase inhibitor and malathion synergist, phenyl saligenin cyclic phosphonate (7), synergized the toxicity of *trans*-permethrin more than 60-fold against *Chrysopa carnea* larvae (13). On the other hand, oxidases seem to be more important than esterases for pyrethroid detoxification in *Tribolium castaneum* larvae, a major pest of stored products, since inhibitors of oxidases (but not of esterases) synergized the toxicity of several pyrethroids (15).

A novel approach for pyrethroid synergism was reported in *T. castaneum* larvae involving a mixture of permethrin with the juvenoid 6,7-epoxy-3,7-dimethyl-1-[3,4-(methylenedioxy)phenoxy]-2-nonene (RO 20-3600) (23,28). In the present study larvae of *T. castaneum* and of the house fly, *Musca domestica vicina,* were used to evaluate the toxicological properties and synergistic activity of another potent juvenoid with favorable persistence on plants and stored products (6,18,19,21), ethyl [2-(4-phenoxyphenoxy)ethyl] carbamate (RO 13-5223).



Formula: RO 13-5223, ethyl [2-(4-phenoxyphenoxy)ethyl] carbamate.

MATERIALS AND METHODS

Chemicals

Pyrethroids of >99% purity from sources described previously (15) were used; they had the following isomeric composition: 1RS for *trans*- and *cis*-permethrin; 1RS,αRS for *trans*-cypermethrin; 1R,αS for *cis*-cypermethrin and deltamethrin; and 2RS,αRS for fenvalerate. Compounds assayed as potential synergists were: RO 13-5223 (50% W.P. from Dr. Maag Ltd., Dielsdorf, Switzerland), piperonyl butoxide (Pazchem Ltd., Tel Aviv, Israel) and Altosid (60% methoprene E.C., Palimport Ltd., Tel Aviv).

Toxicity and synergism assays

The red flour beetle, *Tribolium castaneum* (10,15), and the oriental house fly, *Musca domestica vicina* (2), were from insecticide-susceptible colonies reared at the Bet Dagan laboratories for at least 10 years. *T. castaneum* larvae were reared on wheat flour containing 5% dried yeast at standardized laboratory conditions of 28°C and

70% relative humidity. For bioassay, 10 g of the diet was mixed with 10 ml of acetone solution of the test pyrethroid and/or the synergist; 10 ml of acetone alone was used for the untreated control. Following solvent evaporation and thorough mixing, 2-g portions of the diet were introduced into test vials 2 cm in diameter. Thereafter, 10-12 larvae of the 4th instar (1.00 ± 0.05 mg) or 1st instar (0-3-h-old, white in color) were placed in each vial and maintained at 28°C. Larval weight gain, pupation and emergence were determined as described previously (15).

The house fly larvae were reared on wheat bran containing 3% milk powder (basic diet) and mixed with an equal portion (w/w) of water. For bioassay, the desired amount of pyrethroid was dissolved in 0.5 ml of acetone and 50 µl of Tween 80, diluted with 24.5 ml of water containing the synergist, and mixed thoroughly with 25 g of basic diet. A similar amount of acetone and Tween 80 was used for the untreated control. The diet was divided into four 12-g portions and kept in jars 5 cm in diameter. Twelve 2-day-old 2nd-instar larvae (3-4 mg) were placed in each jar and maintained at 28°C. Larval weight gain was determined 2 days later, and pupation and emergence were followed.

The synergism factor for both *T. castaneum* and *M. domestica vicina* was considered to be the magnitude of increased sensitivity to pyrethroid, based on IC_{50} values (concentration needed for 50% inhibition of weight gain) after feeding on a diet containing both pyrethroid and synergist. The synergistic effect of RO 13-5223 was compared with that of piperonyl butoxide, a classical pyrethroid synergist, which acts through inhibition of oxidase activity in *T. castaneum* larvae (15).

RESULTS

Pyrethroid synergism and prevention of pupation and emergence in Tribolium castaneum *by RO 13-5223*

RO 13-5223 at a dietary concentration of 100 mg kg^{-1} synergized to various extents the potency of *trans*- and *cis*-permethrin, *trans*- and *cis*-cypermethrin, deltamethrin and fenvalerate in inhibiting the weight gain of 4th-instar *T. castaneum* larvae within 4 days after treatment (Tables 1 and 2). The synergistic effect of RO 13-5223 with all the test pyrethroids (except *trans*-permethrin) seems to be lower than that obtained with piperonyl butoxide (Tables 1 and 2). A more pronounced effect was obtained when RO 13-5223 or piperonyl butoxide was tested together with *cis*-cypermethrin on freshly hatched *T. castaneum* larvae (Table 2). In these assays dietary concentrations of 40 and 80 mg kg^{-1} RO 13-5223 synergized the larval growth inhibition obtained by 0.05 mg kg^{-1} *cis*-cypermethrin, the magnitude of their effects resembling those with 10 and 20 mg kg^{-1} of piperonyl butoxide, respectively. Log concentration-probit inhibition curves based on larval weight gain (Fig. 1) revealed that RO 13-5223 and piperonyl butoxide at a dietary concentration of 100 mg kg^{-1} synergized *cis*-cypermethrin at its IC_{50} value by 1.8- and 3.6-fold, respectively; all values were significantly different (P=0.01) from those of cypermethrin alone.

TABLE 1

INFLUENCE OF RO 13-5223 AND PIPERONYL BUTOXIDE (PB) ON THE EFFECT OF DIETARY PYRETHROIDS ON LARVAL WEIGHT GAIN, PUPATION AND EMERGENCE OF FOURTH-INSTAR *TRIBOLIUM CASTANEUM* LARVAE

Dietary level (mg kg^{-1})			*Weight gain after 4 days relative to control (%)*[a]	*Pupation and emergence relative to control (%)*[a]
Pyrethroid	RO 13-5223	PB		
trans-Permethrin				
50	–	–	71±2[b]	98 (98)
50	100	–	54±3[c]	0 (0)
50	–	100	68±4[b]	100 (87)
cis-Permethrin				
10	–	–	80±2[b]	100 (100)
10	100	–	68±4[c]	0 (0)
10	–	100	63±4[c]	98 (88)
trans-Cypermethrin				
10	–	–	51±3[b]	97 (95)
10	100	–	37±4[c]	0 (0)
10	–	100	22±3[d]	75 (62)
cis-Cypermethrin				
1	–	–	73±2[b]	98 (92)
1	100	–	65±5[b]	0 (0)
1	–	100	40±5[c]	77 (72)
Deltamethrin				
1	–	–	80±3[b]	100 (100)
1	100	–	72±4[b]	0 (0)
1	–	100	52±4[c]	92 (85)
Fenvalerate				
50	–	–	60±3[b]	90 (85)
50	100	–	37±3[c]	0 (0)
50	–	100	26±5[c]	80 (67)
None				
–	100	–	99±3	0 (0)
–	–	100	103±2	101 (93)

[a] Data are the mean and SE values of five replicates of 10-12 larvae each. Fourth-instar larvae (1.00±0.05 mg) were introduced into the medium and maintained until adult emergence was complete. The average larval weight gain of the untreated control was 1.60±0.05 mg and the pupation and emergence were 99%.

b, c and d Represent data which differ significantly from each other at $P=0.05$ within the same group.

TABLE 2

INFLUENCE OF RO 13-5223 AND PIPERONYL BUTOXIDE (PB) ON THE EFFECT OF DIETARY *CIS*-CYPERMETHRIN ON LARVAL WEIGHT, PUPATION AND EMERGENCE OF FRESHLY HATCHED *TRIBOLIUM CASTANEUM* LARVAE

Dietary level (mg kg^{-1})			*Larval weight at 14 days relative to control (%)*[a]	*Pupation (and emergence) relative to control (%)*[a]
cis-Cypermethrin	RO 13-5223	PB		
0.05	–	–	85±1	100 (100)
0.05	20	–	74±5	0
0.05	40	–	60±3*	0
0.05	80	–	54±3*	0
0.05	–	5	84±5	95 (95)
0.05	–	10	66±3*	100 (100)
0.05	–	20	58±3*	98 (95)
–	80	–	93±3	0
–	–	20	92±3	100 (98)

[a]Data are the mean and SE values of five replicates of ten larvae each. Freshly hatched larvae (0-3-h-old) were introduced into the medium and maintained until adult emergence was complete. Weight of 14-day-old larvae, pupation and emergence are expressed as percentage of control. The average larval weight of the untreated control was 2.15±0.08 mg with 100% pupation and emergence.

*Differ significantly from *cis*-cypermethrin at P=0.01.

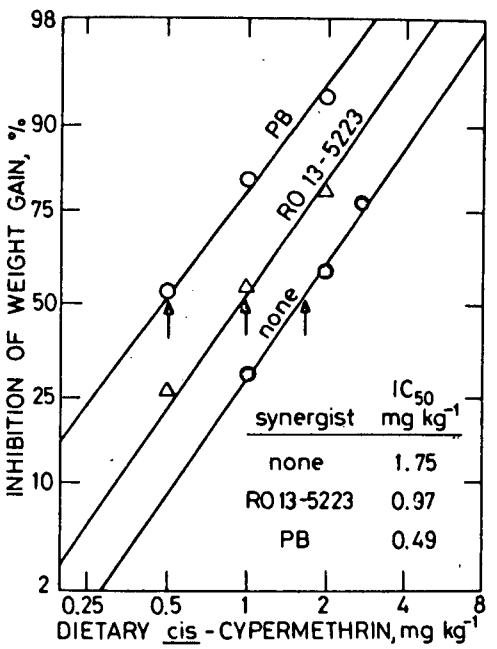


Fig. 1. Influence of RO 13-5223 and piperonyl butoxide (PB) at a dietary concentration of 100 mg kg^{-1} on larval weight inhibition of dietary *cis*-cypermethrin to fourth-instar *Tribolium castaneum* larvae. The data are averages of 10-15 replicates of 12 larvae each. All values obtained by addition of RO 13-5223 and piperonyl butoxide to cypermethrin differ significantly (P = 0.01) from those of cypermethrin alone.

RO 13-5223, in addition to its pyrethroid synergistic effect, inhibited pupation and emergence (Tables 1 and 2) and may suppress further propagation of the pest. Methoprene, on the other hand, had no effect on the toxicity of either *trans*-permethrin or *cis*-cypermethrin when assayed similarly on *T. castaneum* larvae (Table 3).

TABLE 3

INFLUENCE OF METHOPRENE ON THE EFFECT OF DIETARY *TRANS*-PERMETHRIN AND *CIS*-CYPERMETHRIN ON WEIGHT GAIN OF FOURTH-INSTAR *TRIBOLIUM CASTANEUM* LARVAE

Methoprene, dietary level (mg kg^{-1})[a]	*Weight gain after 4 days relative to control (%)*[b]	
	trans–Permethrin	*cis*-Cypermethrin
–	70±3	44±2
25	72±3	38±2
50	77±3	43±2
100	-	41±5

[a]These dietary levels of methoprene had no effect on the larval weight gain.

[b]Data are mean and SE values of five replicates of ten larvae each. The average larval weight gain in the untreated control was 1.52±0.05 mg. Dietary concentrations were 50 and 2 mg kg^{-1} for *trans*-permethrin and *cis*-cypermethrin, respectively.

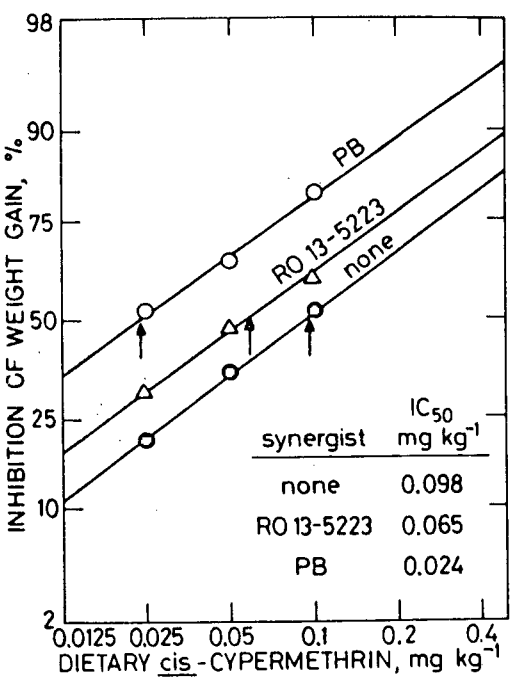


Fig. 2. Influence of RO 13-5223 and piperonyl butoxide (PB) at a dietary concentration of 100 mg kg^{-1} on larval weight inhibition of dietary *cis*-cypermethrin to 2-day-old *Musca domestica vicina* larvae. The data are averages of 16 replicates of ten larvae each. All values obtained by addition of RO 13-5223 and piperonyl butoxide to cypermethrin differ significantly ($P = 0.05$ and $P = 0.01$, respectively) from those of cypermethrin alone.

Pyrethroid synergism and prevention of emergence in Musca domestica vicina *by RO 13-5223*

RO 13-5223 at dietary concentrations of 25 and 100 mg kg^{-1} synergized the toxicity of *trans*-permethrin more strongly than that of *cis*-cypermethrin toward house fly larvae (Table 4), similarly to the trend obtained with *T. castaneum* larvae (Table 1). In addition to its pyrethroid synergizing property, RO 13-5223 inhibited about 95% of house fly adult emergence at a dietary concentration of 100 mg kg^{-1} and about 50% at 25 mg kg^{-1} (Table 4). According to log concentration-probit

TABLE 4

INFLUENCE OF RO 13-5223 AND PIPERONYL BUTOXIDE (PB) ON THE EFFECT OF DIETARY *TRANS*-PERMETHRIN AND *CIS*-CYPRERMETHRIN ON LARVAL WEIGHT GAIN AND EMERGENCE OF *MUSCA DOMESTICA VICINA*

Dietary level (mg kg^{-1})			*Weight gain after 2 days relative to control (%)*[a]	*Emergence relative to control (%)*[a]
Pyrethroid	RO 13-5223	PB		
trans-Permethrin				
2	–	–	66 ± 2^{b}	97 ± 2^{b}
2	25	–	45 ± 4^{c}	50 ± 7^{c}
2	100	–	46 ± 4^{c}	3 ± 2^{d}
2	–	25	35 ± 3^{d}	91 ± 4^{b}
2	–	100	36 ± 4^{cd}	90 ± 3^{b}
cis-Cypermethrin				
0.05	–	–	60 ± 2^{b}	86 ± 2^{b}
0.05	25	–	58 ± 5^{b}	46 ± 5^{d}
0.05	100	–	48 ± 2^{c}	7 ± 1^{e}
0.05	–	25	47 ± 3^{c}	91 ± 3^{b}
0.05	–	100	36 ± 2^{d}	68 ± 3^{c}
None				
–	25	–	96 ± 3^{b}	42 ± 9^{c}
–	100	–	93 ± 2^{b}	5 ± 1^{d}
–	–	25	111 ± 3^{b}	101 ± 3^{b}
–	–	100	96 ± 2^{b}	99 ± 1^{b}

[a]Data are the mean and SE values of 12-24 replicates of 12 larvae each. Two-day-old larvae (3-4 mg) were introduced into the medium and maintained until adult emergence was complete. Weight gain and emergence are expressed as percentage of control. The average larval weight gain of the untreated control was 15.9±0.8 mg with 100% emergence.

b, c, d and e Represent data which differ significantly from each other at $P=0.05$ within the same group.

inhibition curves (Fig. 2), RO 13-5223 and piperonyl butoxide at a dietary concentration of 100 mg kg^{-1} synergized the toxicity of *cis*-cypermethrin by about 1.5- and fourfold, respectively; all values differ significantly from those of cypermethrin alone at P=0.05 and P=0.01 (see Fig. 2). These results resembled in their magnitude those obtained with *T. castaneum* larvae (Figs. 1, 2).

DISCUSSION

Oxidases play an important role in pyrethroid detoxification in both *T. castaneum* and *M. domestica vicina* larvae and piperonyl butoxide is a useful pyrethroid synergist for both species. Oxidase inhibitors such as SV-1 (*O,O*-diethyl *O*-phenyl phosphorothioate) and Niagara 16824 [*O*-(2-methylpropyl)*O*-(2-propynyl) phenylphosphonate], in addition to piperonyl butoxide, synergize the toxicity of various pyrethroids to *T. castaneum* larvae while esterase inhibitors such as profenofos do not (15). Mixed function oxidases in the house fly detoxify insecticides in general (4,20,22) and pyrethroids in particular (25,26,27).

RO 13-5223 exhibits strong IGR activity (2,6,10,18,19,21) and in this respect is a more favorable adjuvant than piperonyl butoxide. The potent juvenoid methoprene is not a synergist in *T. castaneum;* hence, the synergistic effect of RO 13-5223 is probably due to structural features other than those conferring IGR activity. Both pyrethroids and RO 13-5223 combine high potency toward insects with relatively low mammalian toxicity (5,6,18,19). The present study indicates that the joint application of these compounds results in dual effects on both *T. castaneum* and *M. domestica vicina: (a)* increased inhibition of larval growth due to the synergized pyrethroid activity, and *(b)* suppression of new progeny, as expressed by inhibition of pupation and emergence, due to the IGR activity of RO 13-5223.

ACKNOWLEDGMENTS

The authors thank Mrs. Zamira Mendelson and Miss Shoshana Hadad for excellent technical assistance. The research was supported by grant No. I-249-81 from the United States – Israel Binational Agricultural Research and Development Fund (BARD) and by grant P01 ES00049 from the U.S. National Institute of Environmental Health Sciences.

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Pyrethroid Detoxification and Synergism in Insects

This is a review paper presented at the IUPAC Congress in Kyoto, 1982 and published in: Miyamoto, J. and Kearney, P.C. (Eds.) "Pesticide Chemistry: Human Welfare and the Environment." Vol. 3, pp. 307-310, 1983, Pergamon Press, New York, NY. The paper summarizes biochemical and toxicological studies involved in pyrethroid synergism by esterase and oxidase inhibitors in various insects, carried out by the investigators of this project and by other workers. Detoxifying enzymes and their importance for selecting suitable synergists for pyrethroids, are presented and discussed.

PYRETHROID DETOXIFICATION AND SYNERGISM IN INSECTS

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Abstract - Pyrethroid esterases of Spodoptera littoralis and Trichoplusia ni gut walls are more active on a per larva basis than those of the corresponding integuments in hydrolyzing trans-permethrin and cis-cypermethrin. In general the trans isomers are hydrolyzed more extensively than the cis isomers with both the gut and integument preparations. Profenofos fed to T. ni larvae at a level inhibiting the gut pyrethroid esterases by 65% with trans-permethrin and over 90% with cis-cypermethrin increases the toxicity of topically-applied trans-permethrin by 4-fold and of cis-cypermethrin by 20-fold. Similar assays with S. littoralis result in an increase of about 3-fold in the toxicity of both compounds. Phenyl saligenin cyclic phosphonate applied topically to Chrysopa carnea larvae synergizes the toxicity of trans-permethrin by 68-fold. Oxidase inhibitors including piperonyl butoxide and two others have no appreciable effect on the toxicity of several pyrethroids to S. littoralis and T. ni larvae. On the other hand only oxidase but not esterase inhibitors are effective synergists for the toxicity of pyrethroids to Tribolium castaneum larvae. Apparently the predominant pathway of pyrethroid detoxification, whether hydrolytic or oxidative, depends largely on the insect species and to a lesser extent on the pyrethroid involved.

INTRODUCTION

The insecticidal activity of pyrethroids is limited by metabolic hydrolysis and oxidation (1). Inhibitors of the detoxifying esterases and oxidases may prolong the stability and enhance the potency of pyrethroids in insects, thereby serving as synergists.

Although early studies with houseflies emphasize the importance of insect oxidases in detoxifying pyrethroids (2-4), there is increasing evidence with several insects including lepidopterous larvae that esterases also play a major role (5-14). Pyrethroid esterases have somewhat similar properties in larvae of three lepidopterous species, i.e., Trichoplusia ni Hübner (7, 12), Spodoptera eridania Cramer (11) and Spodoptera littoralis (Boisd.) (14). Except for S. eridania the gut wall esterases are more active than those of the integument on a protein or on a per larva basis. Hydrolysis occurs more rapidly with trans-permethrin and trans-cypermethrin than with the corresponding cis-isomers. The slower rate of ester cleavage of cis- than of the trans-isomers parallels their relative insecticidal activities. In contrast, larvae of Chrysopa carnea Stephens, a lacewing with remarkable natural tolerance to pyrethroids, hydrolyze trans-permethrin more slowly than cis-permethrin, which again is in agreement with the relative toxicity of the isomers (13). On the other hand, in Tribolium castaneum (Herbst) larvae, oxidases seem to be more important than esterases for pyrethroid detoxification since oxidase inhibitors (but not esterase inhibitors) synergize the toxicity of several pyrethroids (15). This report summarizes some of our recent studies on pyrethroid detoxification by insect esterases and pyrethroid synergism by esterase and oxidase inhibitors.

MATERIALS AND METHODS

Chemicals

(1RS)-cis- and -trans-Permethrin and (1RS,αRS)-cis- and -trans-cypermethrin were used as unlabeled and ^{14}C-acid preparations. Deltamethrin is a single isomer (1R,cis,αS) and fenvalerate was used as the racemate (2RS,αRS). Profenofos, monocrotophos, azinphosmethyl, sulprofos, DEF, and phenyl saligenin cyclic phosphonate (PSCP) (a potent inhibitor of malathion carboxyesterase and synergist for malathion) (16) were used as esterase inhibitors. PB (piperonyl butoxide), SV-1 (O,O-diethyl O-phenyl phosphorothioate) and MPP [also known as Niagara 16824, O-(2-methylpropyl) O-(2-propynyl) phenylphosphonate] were used as oxidase inhibitors, although the two phosphorus compounds (or closely related materials) also have some activity as esterase inhibitors (5, 12).

Esterase Preparation and Assay

Gut, integument or whole larval pyrethroid esterases from T. ni, S. littoralis and C. carnea were assayed on trans- and cis-isomers of ^{14}C-acid labeled pyrethroids under optimal conditions as described previously (12-14). For in vitro inhibition assays, the inhibitor and enzyme preparation were incubated for 15 min at 32°C prior to adding more buffer and substrate for assay. For in vivo esterase inhibition, each larva was fed or treated topically with the inhibitor, determining the pyrethroid esterase activity from gut, integument or whole larvae after 24 hr (12-14). The conditions for separate studies with T. ni larvae and Musca domestica L.adults are given elsewhere (6).

Toxicity and Synergism Assays

Larvae of T. ni, S. littoralis and C. carnea were fed for 24 hr on segments of leaves treated with esterase or oxidase inhibitors or they were treated topically (12-14). The pyrethroid was then applied topically on the dorsum just behind the head capsule and mortality was determined after 48 hr. The ingested esterase and oxidase inhibitors gave no more than 5% mortality. Synergism assays with T. castaneum larvae were carried out by adding the synergist and the pyrethroid simultaneously to the diet of fourth-instar larvae and determining inhibition of larval weight gain four days later (15). Test conditions for housefly adults are given in ref. 10. The magnitude of synergism was considered to be the increased sensitivity to pyrethroid, based on LD_{50} or LC_{50} values, and was related in some cases to the magnitude of pyrethroid esterase inhibition in these larvae.

RESULTS AND DISCUSSION

Substrate Specificity of Esterases (Table 1)

Pyrethroid esterases from T. ni and S. littoralis larval gut and integument post mitochondrial fractions consistently hydrolyze trans-permethrin and -cypermethrin more extensively than the corresponding cis isomers. The gut enzymes are more active than the integument enzymes in hydrolyzing the permethrin and cypermethrin isomers. trans-Permethrin is hydrolyzed to a greater extent than trans-cypermethrin. The isomer specificity of pyrethroid esterases for more rapid hydrolysis of the trans-isomers is also evident with S. eridania (11), Periplaneta americana L. (9), Heliothis virescens (F.) and Heliothis zea (Boddie) (8) and M. domestica (6). On the other hand, C. carnea hydrolyzes the cis-isomers of both permethrin and cypermethrin to a greater extent than the trans-isomers (13).

Esterase Inhibitors Synergizing Pyrethroid Toxicity (Table 2)

Profenofos (a potent in vitro pyrethroid esterase inhibitor) at a dietary level of 4 nmol/lettuce disc/larva, the highest dose that could be used with no more than 5% mortality, synergizes the toxicity of cis-cypermethrin by ~ 30-fold and of trans-permethrin by 4-fold. This specificity agrees with the greater sensitivity to profenofos inhibition of the gut esterase(s) hydrolyzing cis-cypermethrin (> 90%) compared with those hydrolyzing trans-permethrin (~ 65%) (12). Similar assays with S. littoralis larvae result in 3-fold synergism with both trans-permethrin and cis-cypermethrin. Two additional organophosphorus compounds, azinphosmethyl and monocrotophos, inhibit trans-permethrin esterases in S. littoralis to a lesser extent than profenofos (14) both in vitro and in vivo. PSCP applied topically to C. carnea larvae prior to pyrethroid treatment synergizes trans-permethrin by 68-fold reducing an LD_{50} value of 17,000 µg/g to 250 µg/g, i.e., the remarkable difference of 16,750 µg/g in the LD_{50} value is due to esterase inhibition (13). M. domestica adults show only a small degree of synergism with esterase inhibitors (10) particularly relative to the high levels of synergism with PB.

Oxidase Inhibitors Synergizing Pyrethroid Toxicity

Oxidase inhibitors such as PB and SV-1 are not effective synergists for trans-permethrin and cis-cypermethrin when applied to S. littoralis (14) or to T. ni (12). In T. castaneum larvae, PB, SV-1 and MPP strongly synergize the potency of cis-cypermethrin, while only MPP but not the other compounds has a slight effect on the potency of trans-permethrin (Table 3). The synergism factor for dietary cis-cypermethrin toxicity with 100 mg kg^{-1} dietary synergist is about 3-fold for PB and SV-1 and 10-fold for MPP. PB also synergizes the toxicity to T. castaneum larvae of cis-permethrin, trans-cypermethrin and deltamethrin but not that of fenvalerate (15). Other compounds reported to be pyrethroid synergists, probably acting as oxidase inhibitors, are chlordimeform and Upjohn 42662 [1,1,1-trichloro-N-(((4-chloro-2-methylphenyl)-imino)methyl)-N-methylmethanesulfenamide] in Heliothis sp. (17).

Balance of Esterase and Oxidase Detoxification

The synergism results suggest that esterases are more important than oxidases in limiting the toxicity of several synthetic pyrethroids in S. littoralis, T. ni and C. carnea larvae and oxidases are more important than esterases in T. castaneum larvae and both esterases and oxidases are important in M. domestica adults. Apparently the predominant pathway of pyrethroid detoxification in insects, whether hydrolytic or oxidative, depends largely on the species and to some extent on the pyrethroid involved.

Cautionary Note

Pyrethroids are often applied to insect populations also exposed to organophosphorus compounds, creating thereby a possibility for interactions. Suitable combinations of pyrethroids and

TABLE 1. Hydrolysis of Permethrin and Cypermethrin Isomers by Pyrethroid Esterases from Larval Trichoplusia ni, Spodoptera littoralis and Chrysopa carnea and Adult Musca domestica

Enzyme source	Pyrethroid	Hydrolysis,% trans	Hydrolysis,% cis	Ratio, trans/cis	Ref.
Trichoplusia ni					
Gut wall	permethrin	37	13	2.8	6
	permethrin	28	9	3.1	12
	cypermethrin	15	9	1.7	12
Integument	permethrin	19	3	6.3	12
	cypermethrin	16	6	2.7	12
Spodoptera littoralis					
Gut wall	permethrin	35	17	2.1	14
	cypermethrin	19	15	1.3	14
Integument	permethrin	13	1	13.0	14
	cypermethrin	3	1	3.0	14
Chrysopa carnea	permethrin	25	56	0.4	13
	cypermethrin	11	35	0.3	13
Musca domestica	permethrin	38	9	4.2	6

TABLE 2. Toxicity and Synergism by Esterase Inhibitors of Topically-Applied trans-Permethrin and cis-Cypermethrin to Larval Trichoplusia ni, Spodoptera littoralis and Chrysopa carnea and Adult Musca domestica

Insect	LD_{50}, µg/g trans-permethrin	LD_{50}, µg/g cis-cypermethrin	Synergism factor[a] trans-permethrin	Synergism factor[a] cis-cypermethrin
Trichoplusia ni	0.3	0.05	4(1)	~ 30(3)
Spodoptera littoralis	1.8	0.09	3	3
Chrysopa carnea	17,000		68	
Musca domestica	1.0	0.11	0.9	2.3

[a]The synergist was ingested profenofos for T. ni (12) and S. littoralis (14), topical PSCP for C. carnea (13), and an average of topical profenofos, sulprofos and DEF for M. domestica adults and T. ni larvae (results in parentheses) (10).

TABLE 3. Effects of Three Oxidase Inhibitors on the Toxicity of Dietary trans-Permethrin and cis-Cypermethrin to Fourth-Instar Tribolium castaneum larvae (15).

Compound	Dietary level (mg kg^{-1})[b]	Weight gain after 4 days, rel. to control (%)[a] trans-permethrin	Weight gain after 4 days, rel. to control (%)[a] cis-cypermethrin
None	-	58	47
PB	25	60	-
	50	53	18
SV-1	25	59	15
	50		6
	100	62	2
MPP	25	46	16
	50		2
	100	48	0

[a]Dietary levels of 50 mg kg^{-1} for trans-permethrin and 2 mg kg^{-1} for cis-cypermethrin.

[b]Synergist levels used had no effect on larval weight gain.

organophosphorus compounds might lead to synergism and enhanced pyrethroid effectiveness. Care must be taken in this approach to evaluate the impact on nontarget organisms as well as on the pest species.

Acknowledgment - The authors thank Mr. K.R.S. Ascher for fruitful discussions and Mrs. Sara Yablonski and Miss Anna Elsner for excellent technical assistance. This paper constitutes contribution No. 489-E, 1982 series, from the Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel. The research was supported by Grant No. I-249-81 from the United States-Israel Binational Agricultural Research and Development Fund (BARD) and by Grant PO1-ES00049 from the United States National Institute of Environmental Health Sciences.

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C. Effect of Mineral Oil on Pyrethroid Action

Several types of mineral oils interfere efficiently with aphid transmission of non-persistent viruses and are used as such or in combination with other pesticides for controlling non persistent aphid-borne viruses (1-6). In some cases mineral oils increase the toxicity and persistence of contact insecticides and serve as useful additions to various insect control agents (7-10). The mechanism of this phenomenon has not yet been established. Mineral oil may increase the pick-up of the toxicant by the insect or reduce its evaporation and/or dissipation. In some insect species mineral oil inhibits respiration (8) and this, in turn, may synergize the toxicity of insecticides acting on the nervous system.

The present study considered the effect of a light-medium range mineral oil, "Virol", on the chemical residue levels and on the toxicity of fenpropathrin applied against adults of the whitefly, Bemisia tabaci, under high- and low-volume spray conditions (11). Addition of 1% a.i. Virol to 0.03% a.i. fenpropathrin applied under high volume spray conditions resulted in a higher mortality of B. tabaci adults than that obtained by the two materials applied separately. The LD_{50} value of fenpropathrin when applied with 1% Virol (0.013 ug cm^{-2}) was approximately fivefold lower than when applied alone (0.062 ug cm^{-2}. On the other hand, addition of Virol to fenpropathrin under low volume spray conditions had no effect either on the toxicity or on the residual level of fenpropathrin. Thus, addition of mineral oil to fenpropathrin may have important practical implications in controlling the whitefly, only when applied with high-volume sprays. Assumptions on the possible mechanisms involved in pyrethroid synergism by mineral oils are discussed. The paper presented in this section was submitted recently for publication in the "Journal of Economic Entomology."

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Manuscript prepared for
publication in:
J. Econ. Entomol.
preferable section B.

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Effect of the Mineral Oil "Virol" on Toxicity and Chemical Residue of Fenpropathrin when Applied against Adults of the Whitefly, Bemisia tabaci (Homoptera: Aleyrodidae), as High and Low Volume Sprays.

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ABSTRACT Addition of 1% a.i. Virol (a light-medium range oil) to 0.03% a.i. fenpropathrin applied under high volume spray conditions, resulted in a higher mortality of Bemisia tabaci (Gennadius) adults than that obtained by the two materials applied separately. The LD_{50} value of fenpropathrin when applied with 1% Virol (0.013 µg cm^{-2}) was approximately five-fold lower than when applied alone (0.062 µg cm^{-2}). On the other hand, addition of Virol to fenpropathrin under low volume spray conditions had no effect either on the toxicity or on the residual level of fenpropathrin. Thus, addition of mineral oil to fenpro- pathrin may have important practical implications in controlling the whitefly B. tabaci only when applied with high volume sprays.

Mineral oils have long been used as effective insecticides for controlling citrus pests, especially mites and scales (de Ong et al. 1927, Grunberg 1967, Jeppson and Carman 1974, Schroeder et al. 1977), and several other agricultural pests (Chapman et al. 1962, Smith and Salkeld 1966). Several types of mineral oils interfere efficiently with aphid transmission of non-persistent viruses and are used as such or in combination with other pesticides for controlling non persistent aphid-borne viruses (Bradley et al. 1962, Vanderveken 1977, Loebenstein and Raccah 1980, Simons and Zitter 1980, Raccah et al. 1983, Gibson and Cayley 1984). In some cases mineral oils increase toxicity and persistence of contact insecticides and serve as a useful addition to various insect control agents (Whollen and Sawyer 1954, Grunberg 1967, Ahmed and Gardiner 1967, 1968). The mechanism of this phenomenon has not yet been established. Mineral oil may increase the pick-up of the toxicant by the insect or reduce its evaporation and/or dissipation. In some insect species mineral oil inhibits respiration (Grünberg 1967) and this, in turn, may synergize the toxicity of insecticides acting on the nervous system. Vegetable oils such as those from cotton seed or soybean are a useful addition to ultra low volume formulations for regulating the number of drops and their volume median diameters (VMD), which are in many cases important factors for increasing the efficacy of insecticides in aerial application (McDaniel 1982).

The present study considers the effect of a light-medium range mineral oil, "Virol", on the chemical residue levels and

on the toxicity of fenpropathrin applied against adults of the whitefly, Bemisia tabaci, under high and low volume spray conditions. Fenpropathrin is today one of the important synthetic pyrethroids for controlling B. tabaci (Gennadius), a very severe pest of cotton and vegetable crops in Israel is used as a test compound.

Materials and Methods

Chemicals. Emulsifiable concentrates of fenpropathrin 10% (Smash 10%, Agan Chemical Manufacturers, Ltd, Ashdod, Israel) and of Virol 80% (summer mineral oils of light-medium range, Pazchem Chemicals, Tel Aviv, Israel) were applied in high and low volume sprays on cotton seedlings against adults of the whitefly, Bemisia tabaci.

Rearing and Assays. The whitefly was reared on cotton seedlings in a glasshouse at 26±2°C. For high volume application assays, cotton seedlings 10-15 cm tall were sprayed until run off with an electric sprayer with fenpropathrin and/or Virol. Fifteen whitefly adults in five replicates, confined in leaf cages were exposed to treated plants at various intervals after application and kept under glasshouse conditions, at 26±2°C for 24 h mortality determination and chemical residue analysis. For low volume application assays, cotton seedlings were placed individually in a spray chamber and treated with aqueous emulsion sprays of fenpropathrin and fenpropathrin+oil mixture with a "Mini Ulva"

rotary atomizer (Micron Sprayers Ltd., Herefordshire, England) at a flow rate of 5 ml min^{-1} and 5500 rpm. The quantity of insecticide on the leaf surface was determined and the low volume application rates were adjusted to $\sim$ 0.08 µg cm^{-2}. These conditions simulate low volume conditions in the field of 50 l ha^{-1}.

Chemical analysis. A sample of ten leaf disks was collected by excising two 5 cm diameter disks from each pot using a leaf punch sampler. The leaf disks were rinsed in an ultrasonic bath with 5 ml distilled analytical grade acetone and the acetonic solution was stored at -20°C until analysis.

An analytical standard of fenpropathrin [(RS)- α- -cyano-3-phenoxybenzyl 2,2,3,3-tetramethylcyclopropane-carboxylate], produced by Sumitomo Chemical Co. Ltd. was used for the gas-liquid chromatography analysis.

The instrument for analysis was a Packard Becker Model 419 gas chromatograph equipped with a ^{63}Ni electron capture detector, having a 1m x 2mm ID glass column packed with 3% XE-60 on 80/100 mesh chromosorb W-HP. Operating parameters were: injection port 220°C, column 190°C, detector 240°C, with a nitrogen carrier flow of 60 ml min^{-1}.

Results and Discussion

The light-medium range mineral oil Virol increased considerably the toxicity of fenpropathrin and prolonged its activity when applied on cotton seedlings under high volume

spray conditions (Table 1). The LD_{50} value of the mixture was about five-fold lower than that of fenpropathrin (Fig. 3). The increase in toxicity obtained with the fenpropathrin+oil mixture (Table 1) could not be explained solely by the increased level of fenpropathrin residue (Fig. 1). At day 10 (Table 1), 96% mortality of whitefly adults was obtained at a residue level of 0.035 µg cm^{-2} with the fenpropathrin+oil mixture application, while similar mortality with fenpropathrin alone was obtained one day after application at a residue level of 0.2 µg cm^{-2} (Fig. 1). These results are consistent with those in previous reports indicating that, in some cases, addition of mineral oil to an insecticide increases the latter's toxicity and persistence (Whoolen and Sawyer 1954, Grünberg 1967, Ahmed and Gardiner 1967, 1968). The increased level of fenpropathrin residues observed in all the analyses of the fenpropathrin+mineral oil treatments could have occurred due to a reduced level of evaporation and/or dissipation. The greater toxicity of the fenpropathrin+mineral oil mixture may result, in addition to a greater fenpropathrin residue, from a higher pick-up of the toxicant and/or from a synergistic effect of the combination of mineral oil and fenpropathrin.

On the other hand, when similar assays were repeated under low volume spray conditions, producing droplets of 100 um VMD and a residue of 0.08 ug cm^{-2} fenpropathrin on the leaf surface, no increased effect of Virol was observed on either whitefly mortality or fenpropathrin residue on the leaves (Table 2, Fig. 2). In this case the LD_{50} values of

fenpropathrin applied alone and as a mixture with Virol were similar (Fig. 3). The residual effect of fenpropathrin applied under low volume spray conditions was much more efficient than under high volume, as expressed by the LD_{50} values (Fig. 3). These results concur with those published previously, indicating higher residue levels and greater efficiency of low volume applications (Wheeler et al. 1967, Saini and Dorough 1970, Austerweil et al. 1981). On the other hand, the residual effect of fenpropathrin+mineral oil mixtures of both high and low volume applications was similar in their potency to whitefly adults (Fig. 3).

Our results indicate that addition of mineral oil to fenpropathrin may have important practical implications for controlling the whitefly, B. tabaci, only when applied under high volume spray conditions.

Acknowledgments

The authors thank K.R.S. Ascher for valuable suggestions, Mrs. Zmira Mendelson and Mrs Bracha Steiner for excellent technical assistance; Agan Chemicals for supplying fenpropathrin; and Pazchem Chemicals for supplying Virol. The project was supported in part by Agan Chemicals and the Cotton Council of Israel and by grant No. I-249-81 from the United States-Israel Binational Agricultural Research and Development Fund (BARD). This article is a contribution from the Agricultural Research Organization, Bet Dagan, Israel. No. 1547-E, 1985 series.

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Table 1. Effect of Virol on the toxicity of fenpropathrin against whitefly adults under glasshouse conditions using high volume spray

Days after application	Mortality, %[a]		
	Fenpropathrin 0.005% a.i.	Virol 1% a.i.	Fenpropathrin 0.005% a.i. + Virol 1% a.i.
1	97± 3	97± 3	100
3	75±10	54±10	100*
7	43±11	40±10	91± 7*
10	28± 7	6± 4	96± 2**
14	14± 6	8± 5	66±13**
17	2± 2	0	36±10**

[a]Data are means ± SE values of five replicates of 15 whitefly adults each. Cotton seedlings were sprayed until runoff with fenpropathrin, with Virol, or with both. The residual effect was determined at various times after applications. Mortality in the untreated controls ranged between 0-2%.

*Significantly different at 5% level from mortality obtained with fenpropathrin treatment.

**Significantly different at 5% level from cumulative mortality obtained with fenpropathrin and virol treatments given separately.

Table 2. Effect of Virol on the toxicity of fenpropathrin against whitefly adults under glasshouse conditions using low volume spray

Days after application	Mortality, %[a]		
	Fenpropathrin 0.03% a.i.	Virol 5% a.i.	Fenpropathrin 0.03% a.i. + Virol 5% a.i.
1	100	60±6	100
4	98±6	22±3	84±5
7	26±5	8±5	47±9
13	4±2	0	10±5

[a]Data are means and SE values of five replicates of 15 whitefly adults each. Cotton seedlings were sprayed at low volume with 0.03% fenpropathrin, 5% virol, or the two together. The sprays were carried out with a rotary atomizer as described in Methods. The residual effect was determined at various times after application.

Figure Captions

Fig. 1. Effect of Virol on surface residues of fenpropathrin applied as a high volume spray on cotton seedlings. Cotton seedlings were sprayed until run-off with 0.005% a.i. fenpropathrin (o), or with 0.005% a.i. fenpropathrin plus 1% a.i. Virol (o). Data are averages of 5 replicates with their SE values. Conditions and assays as in Table 1.

Fig. 2. Effect of Virol on surface residues of fenpropathrin applied as a low volume spray on cotton seedlings. Cotton seedlings were sprayed with 0.03% a.i. fenpropathrin (o), or with 0.03% a.i. fenpropathrin plus 5% a.i. Virol (o). Data are averages of 5 replicates with their SE values. Conditions and assays are as in Table 2.

Fig. 3. LD_{50} values expressed in ug cm^{-2} leaf surface of fenpropathrin applied alone or together with Virol as high (H.V.) and low volume (L.V.) sprays. The LD_{50} values were calculated from the data presented in Tables 1 and 2 and Figs. 1 and 2.

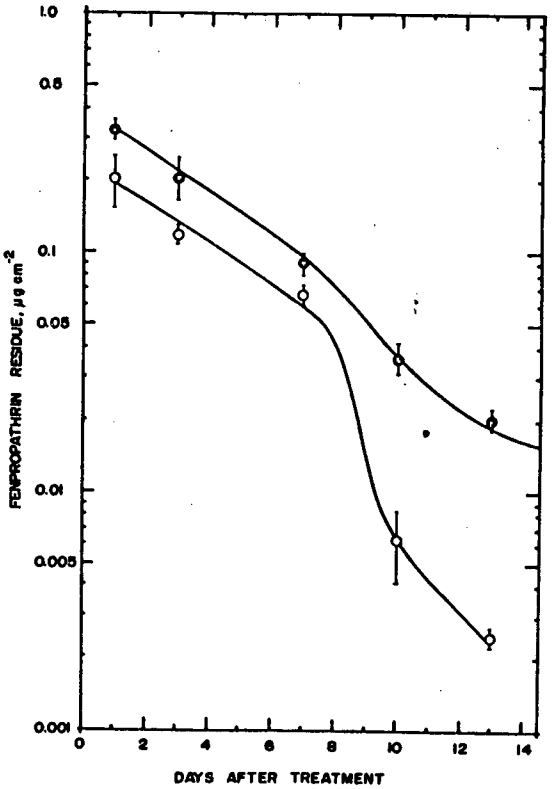
1.0
0.5
0.1
0.05
0.01
0.005
0.001
FENPROPATHRIN RESIDUE, µg cm-2
0
2
4
6
8
10
12
14
DAYS AFTER TREATMENT

Fig. 1

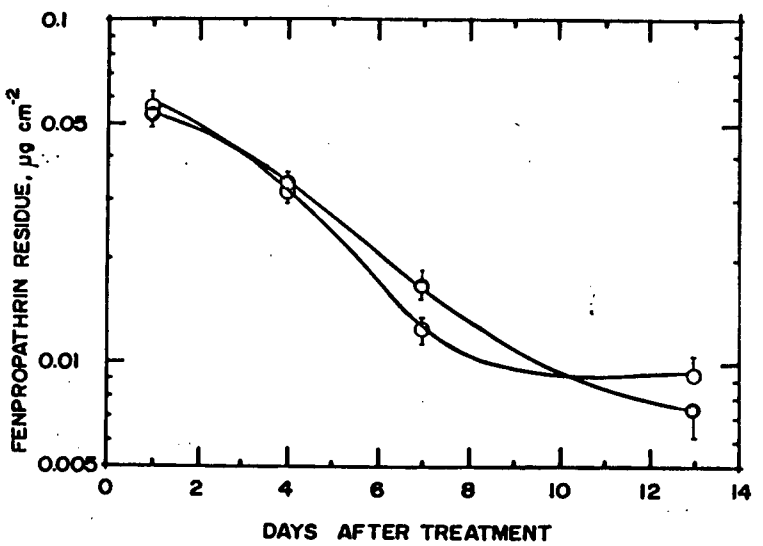
0.1
0.05
0.01
0.005
FENPROPATHRIN RESIDUE, µg cm-2
0
2
4
6
8
10
12
14
DAYS AFTER TREATMENT

Fig. 2

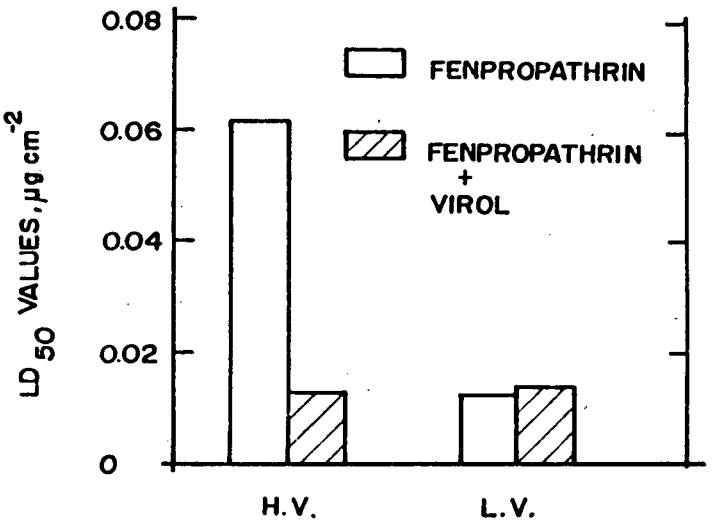
0.08
0.06
0.04
0.02
0
LD 50 VALUES, µg cm-2
FENPROPATHRIN
FENPROPATHRIN + VIROL
H.V.
L.V.

Fig. 3

DESCRIPTION OF COOPERATION

This work should be regarded as an integrated research project in all respects, with each associate group contributing complementary effort as required. During the four years of the actual work of this project, the laboratory at Berkeley concentrated its efforts on studying the chemistry of pyrethroids and their synergists in order to provide the basis for biochemical, toxicological and field studies at Bet Dagan. The Berkeley laboratory provided the staff at The Volcani Center with labeled and unlabeled pyrethroids and with synergists and other related compounds which were either synthetized or purified at Berkeley. Throughout the collaborative research program, there has been continuous correspondence and exchange of views. The joint effort made by both parties is evident in the research publications reported herein.

During the research project, Prof. Casida visited at Bet Dagan in September 1982 and Dr. Ishaaya visited at Berkeley in October 1981 and May 1984. The purpose of these cooperative work sessions was to discuss thoroughly the lines of research pursued, to evaluate and analyze the results and to consult on new approaches to be taken. These meetings catalyzed the research work and strengthened collaboration between the two groups of scientists working on this prgram. In the light of the successful collaboration evolved from professional as well as from personal relations, the parties of the project are looking forward to collaborating on a new BARD project.

EVALUATION OF THE RESEARCH ACHIEVEMENTS INCLUDING BENEFITS TO AGRICULTURE, WITH RESPECT TO THE ORIGINAL RESEARCH PROPOSAL

This project was designed to examine the biochemical, toxicological and practical aspects of pyrethroid synergism in some important agricultural pests. During the past four years, studies

were conducted to evaluate biochemical and toxicological effects of synergists acting by inhibiting pyrethroid hydrolytic or oxidative reactions in insects. In three important cotton pests, Trichoplusia ni, Spodoptera littorralis and Bemisia tabaci, esterase inhibitors were efficient synergists to pyrethroids. On the other hand, in Tribolium castaneum and Musca domestica vicina, oxidase (and not esterase) inhibitors acted as synergists to pyrethroids. Apparently, the predominant pathway of pyrethroid detoxification in insects, whether hydrolytic or oxidative, depends largely on the species and to some extent on the pyrethroid involved.

Biochemical assays for evaluating pyrethroid detoxification and synergism have been developed, and the inhibition level of detoxifying enzymes was used for selecting efficient synergists to pyrethroids. Monocrotophos, methidathion and acephate synergized considerably the toxicity of various pyrethroids against B. tabaci and S. littoralis under both laboratory and field conditions.

Optimization of a synergist for pyrethroids must consider its possible effect on mammalian toxicity. The compounds examined are more effective synergists in whiteflies than in mice. The selectivity of monocrotophos and methidathion may result in part from their poor activity as inhibitors of the mouse liver pyrethroid esterase(s). Mice also appear to depend on oxidases as well as esterases for pyrethroid detoxification. This limited comparison of whiteflies and mice indicates the possibility of selective synergism in insects compared with mammals.

A novel approach of using IGR compounds as synergists for pyrethroids has been tested in Tribolium and house fly larvae. A joint application of the IGR compound RO 13-5223 and pyrethroids results in a dual effect on both insect species, as expressed by increased inhibition of larval growth due to pyrethroid synergism and a strong reduction in adult emergence due to RO 13-5223 juvenilizing activity.

In some cases mineral oils increase toxicity and persistence of contact insecticides and serve as a useful addition to various insect control agents. Our results indicate that addition of light-medium range oil increases the residues and toxicity of fenpropathrin when applied in high-volume sprays on cotton seedlings against the whitefly, B. tabaci. On the other hand, no such effect was observed when similar assays were carried out with low volume sprays. Possible mechanisms of pyrethroid synergism by mineral oil and practical implications of these assays were discussed in the present report.

LIST OF PUBLICATIONS UNDER BARD SPONSORSHIP

a. Research Papers (including those in press and in preparation)

Ascher, K.R.S., Eliyhu, Miriam, Ishaaya, I., Zur, M. and Ben-Moshe, E. Toxicity of mixtures of pyrethroids with organophosphorus insecticides to Spodoptera litoralis (Boisduval) larvae under laboratory and field conditions, in preparation.

Ishaaya, I., Ascher, K.R.S. and Casida, J.E. (1983) Pyrethroid synergism by esterase inhibition in Spodoptera littoralis. Crop Protection 2, 335-344.

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Ishaaya, I. and Casida, J.E. (1983) Pyrethroid detoxification and synergism in insects. In: Miyamoto, J. and Kearney, P.C. (Eds.) "Pesticide Chemistry: Human Welfare and the Environment." Vol. 3, pp. 307-310. Pergamon Press, New York, NY.

Ishaaya, I., Elsner, A., Ascher, K.R.S. and Casida, J.E. (1983) Synthetic pyrethroids: Toxicity and synergism on dietary exposure of Tribolium castaneum (Herbst) larvae. Pestic. Sci. 14, 367-372.

Ishaaya, I., Mendelson, Z., Ascher, K.R.S. and Casida, J.E. (1985) Cypermethrin synergism by pyrethroid esterase inhibitors in adults of the whitefly Bemisia tabaci. Pestic. Biochem. Physiol., submitted.

Ishaaya, I., Yablonski, S., Ascher, K.R.S. and Casida, J.E. (1984) Pyrethroid synergism and prevention of emergence in Tribolium castaneum and Musca domestica vicina by the insect growth regulator RO 13-5223. Phytoparasitica 12, 99-108.

b. Papers Presented at Scientific Meetings

Ishaaya, I. and Ascher, K.R.S. (1983) Synergized pyrethroids against Bemisia tabaci. Abstract of a paper presented at the 2nd Meeting on Whiteflies in Field Crops and Vegetables, (Rehovot, Israel). Phytoparasitica 11, 67.

Ishaaya, I. and Ascher, K.R.S. (1984) Synergized cypermethrin for controlling the whitefly, Bemisia tabaci, in cotton. Abstract of a paper presented at the 3rd Meeting on Whiteflies in Field Crops and Vegetables (Tel Aviv, Israel). Phytoparasitica 12, 139-140.

Ishaaya, I., Ascher, K.R.S. and Casida, J.E. (1983) Pyrethroid synergism by esterase inhibitors in cotton pests. Proc. Xth Int. Congr. Pl. Protection, Brighton, England, p. 230.

Ishaaya, I. and Casida, J.E. (1982) Pyrethroid detoxification and synergism in insects. Fifth Int. Congr. Pestic. Chem. (IUPAC)

Abstract volume, Kyoto, Japan, p. Va-9.

Ishaaya, I., Mendelson, Z., Ascher, K.R.S. and Casida, J.E. (1985) Mixtures of synthetic pyrethroids and organophosphorus compounds for controlling the whitefly, Bemisia tabaci. Abstract of a paper presented at the 4th Meeting on Whiteflies in Field Crops, Vegetables and Orchards, (Bet Dagan, Israel). Phytoparasitica 13, 76-77.