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FINAL REPORT

PROJECT No. I-249-80

Pyrethroid Synergists: Biochemical and Toxicological Aspects

I. Ishaaya, E. Casida, K.R. Ascher

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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 R0 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 R0 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

* 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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(1984) Phytoparasitica 12, 99-108.

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 synthesized 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 program. 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 littoralis 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 R0 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 R0 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 littoralis (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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Synthetic pyrethroids: Toxicity and synergism on dietary
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adults of the whitefly Bemisia tabaci. Pestic. Biochem.
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b. Papers Presented at Scientific Meetings

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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.