SYNERGISM OF ORGANOPHOSPHATE AND PYRETHROID TOXICITY BY DIBROM IN INSECTICIDE-SUSCEPTIBLE AND –RESISTANT TOBACCO BUDWORMS, HELIOTHIS VIRESCENS

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Abstract

The activity of dibrom as a synergist of organophosphate and pyrethroid toxicity was evaluated in laboratory strains of the tobacco budworm, Heliothis virescens (F.). In the first series of experiments, a maximum non-lethal dose of dibrom was applied topically to the abdominal dorsum of fifth instars, then 30 minutes later, a dose corresponding to the LD_{50} of either cypermethrin or profenofos was applied to the midthoracic dorsum. In tests with an insecticide-susceptible strain, dibrom synergized the toxicity of cypermethrin (synergism ratio= 1.76), but not profenofos. In contrast, in bioassays with dibrom and insecticide-resistant larvae, no synergism of cypermethrin toxicity was detected, but dibrom significantly synergized the toxicity of profenofos in larvae from strains resistant to organophosphates and pyrethroids (synergism ratios= 1.46 and 2.89, respectively). Similar results were measured in bioassays where an LD_{50} of dibrom was co-applied with low doses of either cypermethrin or profenofos. These findings confirm previous results suggesting that resistance is esterase-based in these strains, and illustrate the potential for use of organophosphates as synergists of pyrethroid or organophosphate toxicity in insecticide-resistant strains of the tobacco budworm.

Introduction

Development of insecticide resistance is a major limitation to the use of chemical management strategies for insect pests, and is one of the predominant reasons that the tobacco budworm, Heliothis virescens, is difficult to control (Sparks 1981, 1993). There are three major mechanisms of insecticide resistance and all are expressed in H. virescens that resist organophosphate (OP) and pyrethroid insecticides. Reduced cuticular penetration has been shown as a minor mechanism conferring low levels of resistance to organophosphates (Szeicz et al., 1973, Kanga and Plapp, 1994), as well as pyrethroids (Ottea et al., 1995). Decreased sensitivity of acetylcholinesterase (AChE) the target site for OPs, has also been associated with OP resistance in this pest (Brown, 1991, Brown and Bryson, 1992; Harold and Ottea, 1997). Similarly, electrophysiological and genetic studies have shown that reduced neuronal sensitivity is expressed in pyrethroid-resistant H. virescens. (McCaffery, 1991, Ottea et al., 1995, Park et al., 1999).

Enhanced metabolic detoxication of insecticides plays a major role in insecticide-resistant H. virescens. In particular, elevated activities of esterases (ESTs) have been shown to be associated with resistance to OP and pyrethroid insecticides in H. virescens (Konno et al., 1989, 1990, Goh et al., 1995, Zhao et al, 1996, Harold and Ottea, 1997), and related pests such as Helicoverpa armigera (Gunning et al., 1996, 1997). The overall objective of this research was to test the hypothesis that the toxicities of OP and pyrethroid insecticides can be synergized by compounds that inhibit the activity of these resistance-associated esterases. Here we report results from initial studies using the organophosphate, dibrom as a candidate synergist.

Materials and Methods

Chemicals

Profenofos(O-(4-bromo-2-chlorophenyl)-O-ethyl-S-propyl-phosphorothioate; 89% purity) was donated by Novartis (Greensboro, NC) and dibrom (O, O-dimethyl-1,2-dibromo-2,2-dichloroethylphosphate; 98% purity) was provided by AmVac (Newport Beach, CA). Cypermethrin (technical grade) was obtained from FMC Corporation (Princeton, NJ).

Insects

Insecticide-susceptible and -resistant laboratory strains of *H. virescens* were studied. The reference-susceptible strain (LSU) was established in 1977 (Leonard et al., 1988) and has been reared in the laboratory since that time without exposure to insecticides. The pyrethroid-resistant (Pyr-R) strain was derived from a field collection made in August 1995 from the Red River Research Station (Bossier City, LA). Insects from this collection were reared for one generation, then selected in subsequent generations as fifth stadium larvae with cypermethrin (1.75 μg/larva). Survivors were crossed with LSU insects and progeny were selected as third stadium larvae with 1 μg cypermethrin/larva, a dose corresponding to 21 times the LD_{50} for LSU larvae (Shan et al. 1997. Similarly, an OP-resistant (OP-R) strain was established by repeatedly selecting larvae from this field-collection with profenofos (2.5 μg/larva; Harold and Ottea, 1997).

All larvae were reared in 1-oz cups containing a pinto bean-based semi-synthetic diet (Leonard et al., 1988). Adults were reared in 3.8-l cardboard cartons covered with cotton gauze as a substrate for oviposition and were provided with sucrose (10% in water) as a carbohydrate source. Both larvae and adults were held at 27°C, 70% relative humidity, and a photoperiod of 14:10 hr (light: dark). Larvae were separated following head capsule slippage at the end of the fourth stadium, and fifth stadium (day 1) insects (180±20 mg) were selected for biological assays.

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 2:967-969 (2000)
National Cotton Council, Memphis TN

Biological Assays

In initial tests, insecticide susceptibility of fifth stadium (day 1) larvae from the three strains was measured following topical application of 1 µl profenofos, cypermethrin or dibrom (in acetone) onto the thoracic dorsum. Control larvae were treated with 1 µl of acetone. The dose-mortality response of larvae was measured with at least 5 doses of insecticides (≥10 larvae/dose) and replicated thrice.

Two protocols were used to assess the activity of dibrom as a synergist of profenofos or cypermethrin toxicity. In the first series of experiments, a maximum non-lethal dose of dibrom was applied topically to the abdominal dorsum of fifth instars, then 30 minutes later, a dose corresponding to the LD_{50} of either cypermethrin or profenofos was applied to the midthoracic dorsum. In the second series of tests, a dose corresponding to the LD_{50} of dibrom and the LD_{20} of either cypermethrin or profenofos were co-applied to the thoracic dorsum of larvae. At least 30 insects/treatment were used for these tests and control larvae received 2 µl of acetone.

Treated larvae were held in 1-oz cups with diet and maintained at 27°C, 70±5% relative humidity, and a photoperiod of 14:10 (light: dark) hr. Mortality was recorded 24, 48, and 72 hr posttreatment using absence of coordinated movement within 30 sec after being prodded with a pencil as the criterion. Mortality was maximal at the 48 hr reading; therefore, data from this timepoint are reported. For all tests, control mortality did not exceed 2%. Dose-mortality data were analyzed by probit analysis (Finney, 1971) using a microcomputer-based program (SAS, 1985). Results from synergists bioassays were analyzed using either Students t-test (protocol 1) or chi-square (protocol 2) with $\alpha = 0.05$.

Results and Discussion

Dibrom was only moderately toxic to susceptible (LSU-S) H. virescens (Table 1). The LD_{50} estimated for dibrom was about 10 times greater than for profenofos and over 100 times greater than that for cypermethrin. In addition, strains that resisted both profenofos and cypermethrin also resisted dibrom (Table 1). In fact, there was greater resistance to dibrom than to either cypermethrin or profenofos in both organophosphate- and pyrethroid-resistant strains.

Application of a non-toxic dose of dibrom followed 30 minutes later by an LD_{50} of cypermethrin or profenofos resulted in increased toxicity compared with insecticide alone treatments (Table 2). Further, levels of increase varied depending on the strain and the insecticide studied. In tests with the susceptible, LSU insects, increases in toxicity were greater with cypermethrin (SR=1.76) than with profenofos (SR= 1.33). In contrast, in tests with larvae of the organophosphate- and pyrethroid-resistant strains, the toxicity of profenofos was synergized to a greater extent than that of cypermethrin. The greatest increase in toxicity (almost 3-fold) was measured with Pyr-R larvae in assays with dibrom and profenofos.

Similar results were measured in assays where dibrom and profenofos or cypermethrin were applied simultaneously (Table 3). Coapplication of dibrom significantly synergized the toxicity of both cypermethrin and profenofos in larvae from the susceptible, LSU strain. In addition, mortality was significantly higher for OP-R and Pyr-R larvae treated with both profenofos and dibrom than the sum of mortalities from tests with these inseciticides applied individually. However, no synergism of cypermethrin toxicity by dibrom was detected in larvae from either of the resistant strains.

These results confirm previous findings that resistance is, at least in part, esterase-mediated in the resistant strains tested. In addition, they suggest that the toxicity of available, OP insecticides can be enhanced by either pre-application or co-application of OP synergists. However, because OPs also attack and are metabolized by esterases in mammals, the human health hazards associated with mixing OPs must be considered but are largely unknown.

Acknowledgments

We thank AmVac, Cotton Incorporated, and the Louisiana Agricultural Experiment Station for funding this study.

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Table 1. Toxicity of insecticides in susceptible and –resistant strains of H. virescens

	Profenofos		Cypermethrin		Dibrom	
Strain	**LD_{50}[1]**	**RR[2]**	**LD_{50}**	**RR**	**LD_{50}**	**RR**
LSU-S	1.2	1	0.1	1	11.4	1
OP-R	25	21	1.3	13	680	60
Pyr-R	10	8.3	1.0	10	580	51

[1] expressed in μg insecticide/larva
[2] RR= Resistance Ratio= LD_{50}(test strain/LSU-S)

Table 2. Effect of dibrom on the toxicity of profenofos and cypermethrin1

	Percentage Mortality					
	Profenofos			Cypermethrin		
Strain	**alone**	**+dibrom**	**SR[2]**	**alone**	**+dibrom**	**SR**
LSU	50.0	66.7	1.33	26.7	46.9	1.76*
OP-R	33.3	48.7	1.46*	54.3	62.5	1.15
Pyr-R	31.0	89.7	2.89*	51.7	68.2	1.32

[1] Profenofos and cypermethrin (at doses corresponding to LD_{50}s) were administered to the thoracic dorsum of larvae 30 min. following application of the maximal, nonlethal dose of dibrom on the abdominal dorsum. No mortality was measured in tests with dibrom alone.
[2]SR= synergism ratio= % mortality (insecticide + dibrom/insecticide alone). Asterisks denote SRs that are significantly greater than 1 (Students t-test; α =0.05)

Table 3. Effect of co-application of dibrom on the toxicity of profenofos and cypermethrin[1]

	Percentage Mortality		
Treatment	**LSU**	**Pyr-R**	**OP-R**
Dibrom	53	57	47
Profenofos	20	17	17
Profenofos+ dibrom	93*	100*	77*
Cypermethrin	13	27	20
Cypermethrin + dibrom	77*	93	57

[1] Profenofos and cypermethrin (at doses corresponding to LD_{20}s) and dibrom (at doses corresponding to LD_{50}s) were co-applied to the thoracic dorsum of larvae. Asterisks denote mortality that is significantly greater than that expected as the sum of individual mortalities for the components of the mixture (chi square; α=0.05)