Synergism of Pyrethroids by Several Compounds in Larvae of the Diamondback Moth (Lepidoptera: Plutellidae)

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ABSTRACT Synergism of several pyrethroids in both susceptible (FS) and resistant (BC) *Plutella xylostella* (L.) larvae by several inhibitors of detoxifying enzymes and a compound reported to affect the insect nerves was determined. Only permethrin was synergized consistently and obviously in the resistant BC strain by the esterase inhibitors triphenyl phosphate and *S,S,S*-tributyl phosphorotrithioate. Piperonyl butoxide (pb), a microsomal oxidase inhibitor, synergized all four pyrethroids, although to different degrees, in both FS and BC strains. Pretreatment of the BC strain with pb increased the effectiveness of fenvalerate by 15-fold, deltamethrin 13-fold, permethrin 6-fold, and cypermethrin 3-fold. A tank-mix formulation of pb, Butacide, when mixed and applied simultaneously with these pyrethroids at varying ratios, also showed synergistic action. Among the four pyrethroids tested, fenvalerate was most drastically synergized by Butacide. A 1:1 to 5:1 ratio for Butacide and pyrethroids seemed optimal for the control of resistant *P. xylostella*. The response of the BC strain to deltamethrin and fenvalerate was affected somewhat by the pretreatment of 1,1-di-(4-chlorophenyl) ethanol. Finally, the possible consequences of large-scale applications of synergists, such as the appearance and intensification of certain resistance mechanisms and effects on mammals, are discussed.

SYNERGISTS that interfere with detoxification of insecticides are of practical importance in achieving more efficient control of insects, increasing the spectrum of activity of an insecticide, and restoring the activity of an insecticide against resistant strains of insects (Metcalf 1967). Recently, the successful development of new tank-mix formulations of piperonyl butoxide (pb), one of the most important commercialized methylenedioxyphenyl synergists, has enhanced the prospects of greatly expanding the uses of many insecticides, especially pyrethroids and carbamates, for agricultural insect control.

We determined the synergism of some pyrethroids in both susceptible and resistant diamondback moth, *Plutella xylostella* (L.) by several compounds known as inhibitors of detoxifying enzymes, and one of the carbinol analogues of DDT reported by Sawicki (1978) to affect the nerves of insects. Prospects and possible consequences of large-scale application of synergists in pest control are discussed.

Materials and Methods

The susceptible (FS) and resistant (BC) strains of diamondback moth were of the same origin as given by Liu et al. (1982a). Pupae or mature larvae of the BC strain were collected from the field and reared in the laboratory on rape seedlings (Liu and Sun 1982). Larvae of the first generation were used for bioassay.

Technical grade pb (80%) (Food Machinery Corp.) and Butacide 8 EC (91%) (Fairfield American Corp.) were used as microsomal oxidase inhibitors. The esterase inhibitors S,S,S-tributyl phosphorotrithioate (TBPT) (70.5% technical grade) and triphenyl phosphate (TPP) (analytical grade) were manufactured by Chemagro Co. and E. Merck, respectively; 1,1-di-(4-chlorophenyl) ethanol (DMC) (82% technical grade) was manufactured by Sherwin–Williams Corporation.

For the experiment involving Butacide, commercial pyrethroid formulations were used. These were fenvalerate 20% EC (Sumitomo), deltamethrin 2.8% EC (Roussel Uclaf), cypermethrin 5% EC (*cis*:*trans*, 40:60) (Dow Chemical), and permethrin 10% EC (*cis*:*trans*, 40:60) (Shell Chemical). For the other experiments, 95% technical grade fenvalerate (Sumitomo) and 95% technical grade cypermethrin (*cis*:*trans*, 50:50) (ICI) were used instead of the commercial formulations.

Fourth instars were sprayed by a Shandon spraygun with acetone solutions of the insecticides; mortality was recorded 24 h later. Thirty to 40 larvae were treated for each concentration; two replicates were done. At least four concentrations and a control were included in each bioassay, except when DMC was tested. In the latter experiment, three or four concentrations were used. Temperature ranged between 22 and 28°C throughout the study. Bioassays whose results were to be compared directly were carried out simultaneously to minimize the influence of variable

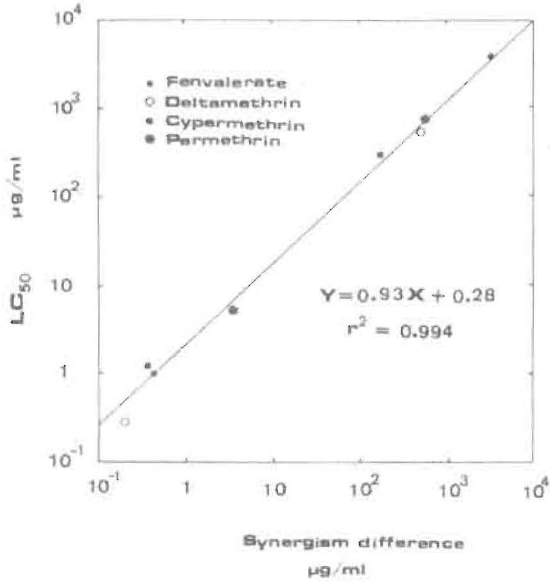

Fig. 1. Relationship between synergism difference produced by pb on fenvalerate, deltamethrin, cypermethrin, and permethrin and LC_{50}'s of these pyrethroids to both susceptible and resistant strains of diamondback moth.

temperature. Results were corrected by Abbott's formula (Abbott 1925) and subject to probit analysis (Finney 1971).

Results

Synergism produced by esterase inhibitor STBPT and TPP in FS strain was limited. Nearly a 2-fold increase of toxicity of fenvalerate and deltamethrin at LC_{50} was noted (Table 1). Permethrin w the only compound synergized consistently by th synergists in the resistant BC strain, a result simi to that reported by Liu et al. (1981). This co have been due to a more rapid hydrolysis of pe methrin compared to the other α-cyano-3-ph noxybenzyl esters (Casida and Ruzo 1980).

Piperonyl butoxide (pb) was unmistakably e fective in synergizing the toxicity of all four pyr throids, though to varying extents, against bo susceptible FS and resistant BC strains of di mondback moth (Table 2). A significant linear r lationship was established between the LC_{50}'s the four pyrethroids and the synergism produc by pb against both FS and BC strains (Fig. When pb was used, the resistant BC strain tole ated large additional quantities of pyrethroids, e pecially fenvalerate. In contrast, the FS strain pr treated with pb tolerated little addition pyrethroids.

A further experiment was performed to te whether a new tank-mix formulation of pb, B tacide, when used simultaneously with, instead before, the insecticides, would produce simil synergistic phenomena. In the FS strain, Butaci mixed with pyrethroids at ratios of 1:1, 5:1, an 10:1 produced limited synergism (Fig. 2). Amon the four pyrethroids tested, deltamethrin was aga synergized more than the rest. For the resistan BC strain, fenvalerate toxicity was most drasticall increased by Butacide. At a concentration of 25 μg/ml, fenvalerate killed only 3% of the resistan larvae. Inclusion of Butacide at ratios of 1:1 an 5:1 increased mortality to 53 and 87%, respectiv ly. At a 1:1 ratio, Butacide increased the mortali of resistant diamondback moth treated with 1,00 μg/ml fenvalerate from 23 to 98%. The effectiv

Table 1. Synergism of fenvalerate, deltamethrin, cypermethrin, and permethrin by TBPT and TPP in FS and B strains of diamondback moth

Treatment	FS		BC		RR[b]
	LC_{50} (95% FL) μg/ml	Slope ± SE	LC_{50} (95% FL) mg/ml	Slope ± SE	
Fenvalerate					
Alone	1.64 (0.91–3.20)	2.45 ± 1.38	3.39 (2.64–4.34)	0.92 ± 0.30	2,067
+ TBPT	1.22 (0.09–1.71)	1.69 ± 0.34	3.79 (2.86–5.01)	1.71 ± 0.24	3,107
+ TPP	0.88 (0.54–1.42)	1.31 ± 0.30	3.90 (2.79–5.45)	1.48 ± 0.30	4,432
Deltamethrin					
Alone	0.23 (0.18–0.30)	2.52 ± 0.53	0.50 (0.38–0.65)	1.81 ± 0.26	2,174
+ TBPT	0.17 (0.11–0.25)	1.78 ± 0.50	0.97 (0.71–1.32)	1.56 ± 0.25	5,706
+ TPP	0.12 (0.04–0.28)	1.07 ± 0.34	0.80 (0.58–1.10)	1.49 ± 0.24	6,667
Cypermethrin					
Alone	2.10 (1.70–2.59)	3.33 ± 0.60	0.27 (0.22–0.33)	3.07 ± 0.57	129
+ TBPT	2.21 (1.14–4.29)	1.91 ± 0.56	0.25 (0.21–0.30)	1.29 ± 0.58	113
+ TPP	2.59 (1.74–3.84)	2.38 ± 0.66	0.21 (0.17–0.25)	3.44 ± 0.60	81
Permethrin					
Alone	2.11 (1.69–2.71)	3.04 ± 0.60	0.63 (0.49–0.80)	2.27 ± 0.37	299
+ TBPT	2.49 (2.05–3.03)	3.71 ± 0.65	0.24 (0.18–0.32)	1.96 ± 0.36	96
+ TPP	2.15 (1.54–2.97)	1.92 ± 0.50	0.21 (0.16–0.28)	1.87 ± 0.35	98

Two replicates with 30 to 40 larvae per replicate were used for each concentration.

[a] Resistance ratio = LC_{50} of BC strain/LC_{50} of FS strain for corresponding treatment.

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SE	RR[b]
.30	2,067
.24	3,107
.30	4,432
.26	2,174
.25	5,706
.24	6,667
.57	129
.58	113
.60	81
.37	299
.36	96
.35	98

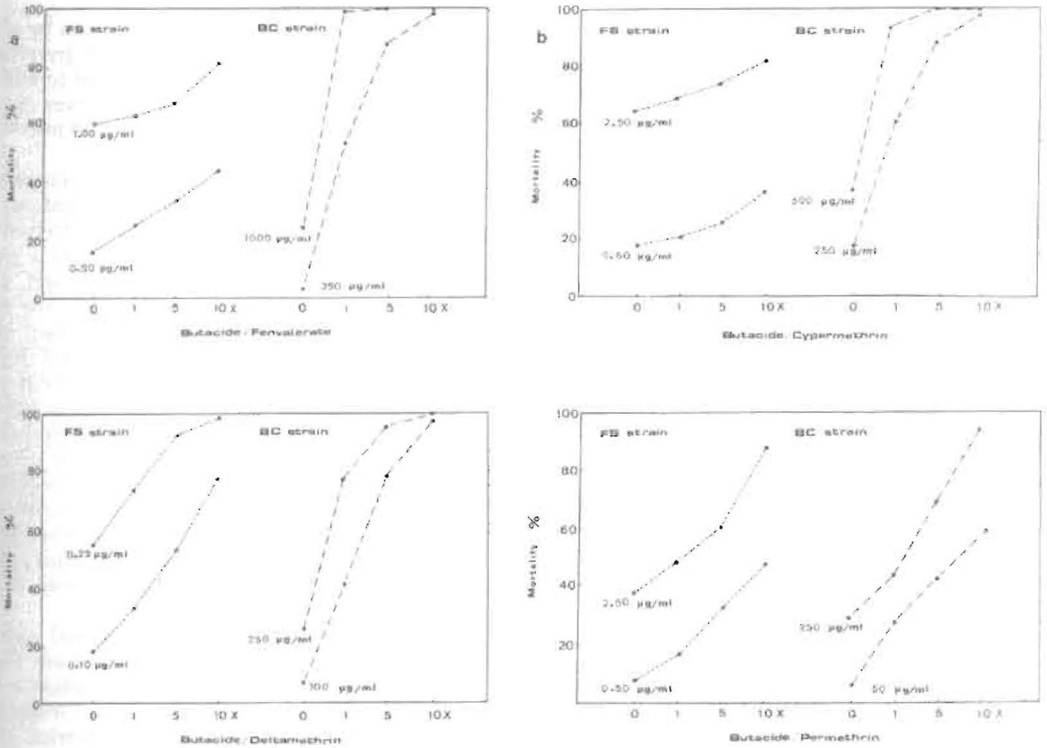

Fig. 2. Synergism of fenvalerate, deltamethrin, cypermethrin, and permethrin by Butacide in FS and BC strains of diamondback moth.

ness of deltamethrin and cypermethrin was also increased significantly by mixing with Butacide at all ratios tested. This synergism, however, was much less pronounced for permethrin.

When diamondback moth larvae were pretreated with DMC, a potent DDT-dehydrochlorinase inhibitor, their response to the pyrethroids appeared to change (Fig. 3). This change was very limited for the FS strain. However, it was seemingly significant, in terms of LC_{50} difference be-

Table 2. Synergism of fenvalerate, deltamethrin, cypermethrin, and permethrin by pb in FS and BC strains of diamondback moth

Treatment	FS				BC			
	LC_{50} (95% FL) μg/ml	Slope ± SE	SD[a] μg/ml	SR[b]	LC_{50} (95% FL) mg/ml	Slope ± SE	SD mg/ml	SR
Fenvalerate								
Alone	0.94 (0.82–1.07)	3.96 ± 0.48			3.39 (2.64–4.43)	0.92 ± 0.20		
+ pb	0.53 (0.41–0.69)	2.35 ± 0.41	0.41	1.8	0.22 (0.15–0.33)	1.10 ± 0.22	3.17	15.4
Deltamethrin								
Alone	0.26 (0.18–0.40)	2.28 ± 0.48			0.50 (0.38–0.65)	1.81 ± 0.26		
+ pb	0.06 (0.04–0.08)	1.85 ± 0.36	0.20	4.3	0.04 (0.028–0.056)	1.47 ± 0.26	0.46	12.5
Cypermethrin								
Alone	1.14 (0.87–1.49)	2.39 ± 0.41			0.27 (0.22–0.34)	3.07 ± 0.57		
+ pb	0.79 (0.55–1.14)	1.53 ± 0.36	0.35	1.4	0.10 (0.077–0.13)	2.22 ± 0.50	0.17	2.7
Permethrin								
Alone	5.09 (4.33–5.98)	2.73 ± 0.31			0.63 (0.49–0.80)	2.27 ± 0.37		
+ pb	1.86 (1.32–2.63)	1.91 ± 0.43	3.23	2.7	0.11 (0.088–0.14)	2.41 ± 0.40	0.52	5.7

Two replicates with 30 to 40 larvae per replicate were used for each concentration.

[a] Synergism difference = LC_{50} of unsynergized treatment − LC_{50} of synergized.

[b] Synergism ratio = LC_{50} of unsynergized treatment/LC_{50} synergized treatment.

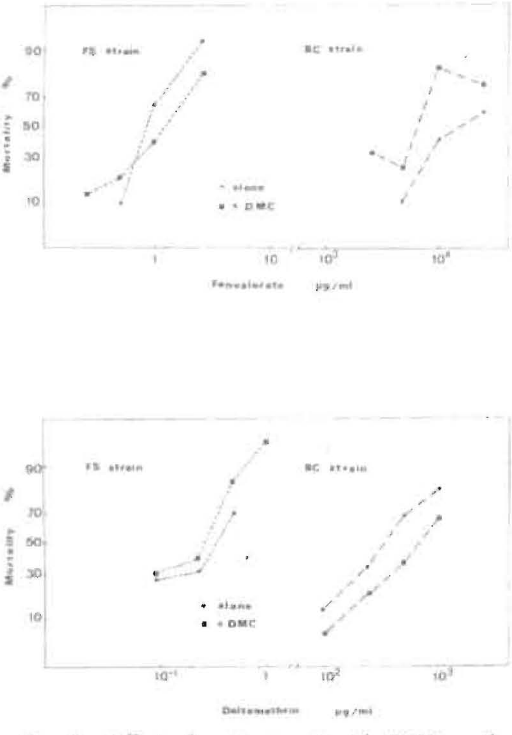

Fig. 3. Effect of pretreatment with DMC on the response to fenvalerate and deltamethrin in FS and BC strains of diamondback moth.

tween the pyrethroid alone and pyrethroid + DMC treatments, for the resistant BC strain.

Discussion

Brindley (1977) proposed using synergist difference as an estimate of microsomal oxidase activity in vivo in insects. Thus, the role of microsomal oxidation in the overall resistance to pyrethroids in this insect pest can be easily and better appreciated (Fig. 1). Nevertheless, the synergism ratio is still useful for practical purposes. Table 2, for example, shows that pretreatment of resistant diamondback moth with pb could increase the effectiveness of fenvalerate by 15-fold, deltamethrin 13-fold, permethrin 6-fold, and cypermethrin 3-fold.

The optimal ratios of mixtures of Butacide with pyrethroids for the control of resistant diamondback moth appear to be from 1:1 to 5:1. Under grower conditions, a ratio of 5:1 of pb:pyrethroid was suggested to be most effective for the control of resistant Colorado potato beetles, *Leptinotarsa decemlineata* (Say) (G. M. Ghidiu, personal communication). However, slight phytotoxicity to eggplant and tomato was observed when pb was mixed with some pyrethroids at a ratio of 4:1 (Anonymous 1982).

Though no plausible explanation can be offere for the observed effect of DMC on pyrethroi compounds similar to those suggested to affect unknown site of action on insect nerves (Sawic 1978) might be sought to synergize the insecticid at target level.

Potentially, any modification of the processes absorption, distribution, biotransformation, an excretion of an insecticide and its interaction wi the site of action in insects that permits a greate quantity of the active compound into and, subs quently, a more efficient attack upon the targ site would result in apparent enhancement of to icity, or synergism. However, Sun and Johns (1972) used the term "quasisynergism" to descri the enhancement of insecticidal action resultin from an increase of insect cuticle permeabili The term synergism has been reserved generall for the improvement of toxicity by a synergist th interferes with the detoxifying metabolism of a insecticide. Many types of compounds are know to have synergistic action; methylenedioxyphen compounds are the most important ones (Casid 1970).

DDT-dehydrochlorinase, microsomal oxidase esterases, and glutathione transferases are the en zymes often cited as involved in the enhanced me tabolism of insects resistant to many insecticide (Oppenoorth and Welling 1976). Dittrich (198 suggested that screening and development of syn ergists which would effectively block resistanc mechanisms were among industry's efforts to cop with insecticide resistance. However, pb is cu rently the only synergist available for agricultur use. Toxicologically acceptable esterase and gl tathione transferase inhibitors have yet to be de veloped.

Use of synergists to cope with a resistance prob lem is expected to be more satisfactory where on dominant mechanism exists for the resistance to number of insecticides, such as esterase hydrolys for organophosphorus resistance in *Culex pipien quinquefasciatus* Say (Georghiou et al. 1975), *My zus persicae* (Sulzer) (Devonshire and Moor 1982), and *Nephotettix cincticeps* Uhler (Oza 1969). Optimal synergists may vary with the inse species and the insecticides (Jao and Casida 197 Chang and Jordan 1983).

Chang and Jordan (1983) also reported signifi cant in vivo inhibition by carbaryl and pirimipho methyl of permethrin-hydrolyzing enzymes in th larvae of porina moth, *Wiseana cervinata*. Thi work suggests the possible use of mixtures of or ganophosphorus or carbamate insecticides wit some pyrethroids to obtain more than an additiv effect.

Compounds that produce an effect at the targe site level may be of potential value as synergist Recently, chlordimeform, reported to strongl synergize pyrethroids applied to *Heliothis* sp. o cotton (Plapp 1979), has been suggested to be target-site synergist by Chang and Plapp (1983).

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A possible consequence of large-scale application of synergists in the field for insect control may be the emergence and subsequent intensification of certain known or even unknown resistance mechanisms. Ranasinghe and Georghiou (1979) concluded that selection of *C. p. fatigans* Wiedemann with insecticides and insecticide/synergist combinations yielded strains with distinctly different resistance characteristics. They stated that the phenomenon of synergism may permit the extended use of an insecticide where alternative resistance mechanisms are either absent or of low efficiency in the target population. Yet, use of an insecticide plus a synergist may allow the selection of alternative resistance pathways if appropriate genes are present in the population. In the case of diamondback moth, use of pb might eventually accelerate and intensify the suspected insensitive nerve resistance mechanism for pyrethroids (Liu et al. 1982b), and the insensitive acetylcholinesterase resistance mechanism for organophosphorus and carbamate insecticides (Sun et al. 1983).

In practice, farmers may choose to use these kinds of synergists indiscriminately with all their insecticides. The long-term consequence of applying synergists at rates up to 10-fold of the dose of the insecticides would be very difficult to assess. Routinely monitoring populations of important insect pests in the field for known primary resistance mechanisms to each major group of insecticides used since the introduction of synergists may be required in order to gather information on any long-term changes in response.

Most insecticide synergists have low acute and subacute toxicity to mammals (Casida 1970). However, fundamental similarities between microsomal oxidases facilitate the ability of most synergists to inhibit the enzymes from both insect and mammalian sources (Wilkinson 1976). Synergists are suspected of increasing the persistence of insecticide residues. Thus, pesticides of low toxicity might become substantially more toxic and hazardous. In addition, pb was reported to have been cocarcinogenic in mice (Epstein et al. 1967).

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