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PYRETHROID RESISTANCE IN THE TOBACCO BUDWORM, HELIOTHIS VIRESCENS (F.)

THE UNIVERSITY OF ARIZONA M.S. 1983

PYRETHROID RESISTANCE IN THE TOBACCO BUDWORM, *Heliothis virescens* (F.)

by

Michael Paul Jensen

A Thesis Submitted to the Faculty of the

DEPARTMENT OF ENTOMOLOGY

In Partial Fulfillment of the Requirements
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STATEMENT BY AUTHOR

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ABSTRACT

Selection of larvae of the tobacco budworm, Heliothis virescens (F.), using permethrin at the LD_{80} level produced strong tolerance towards this compound. After eleven generations of continuous selection pressure, the LD_{50} in the F_{12} generation had increased 36.8-fold compared to the LD_{50} of the F_1. In a companion study, larvae were selected with a permethrin:chlordimeform mixture (1:1) at the LD_{80} level during ten of eleven generations. By the F_{12} generation, the degree of tolerance to either permethrin or the mixture was scarcely different from levels established in the F_1 generation.

In a preliminary study, Bacillus thuringiensis Berliner (Dipel®) was examined for its potential as a synergist for the pyrethroid insecticides, permethrin and cypermethrin. Mortality from either chemical treatment was increased by 2-fold when larvae fed on Dipel®-treated-diet for twenty-four hours prior to dosing.

INTRODUCTION

Tobacco Budworm

From the standpoint of potential crop losses, the tobacco budworm (TBW), Heliothis virescens (Fabr.), belongs to one of the most important families of insects in the world, the Noctuidae. The immature stage is responsible for damage to leaves and fruits of host plants such as soybean, cotton, tobacco, field and sweet corn, tomato, sorghum, peanut, and many species of minor crops. In addition to the direct damage done by their feeding, the larvae are reported to be responsible for the mechanical transmission of virus, fungal and bacterial rots, causing additional damage and crop losses especially under moist conditions (Kogan et al. 1978).

Description

Freshly laid eggs are whitish or cream colored, approximately 1 mm in size and slightly oblong in shape. As the embryo develops, the egg assumes a purplish hue and eventually turns dark gray prior to hatching (Werner, Moore, and Watson 1979).

Very young caterpillars are yellowish or reddish, with large black bumps on the body. Later instars reach a length of 40 mm and vary in color from pale green to dark

brown, often with a pattern of paler markings on the back and sometimes with a pronounced dark band on the sides. Third instar or older larvae have tiny spines, like those on the integument that extend onto the slightly enlarged dorsal bumps on the first, second, and eighth segments behind the true legs (Werner et al. 1979).

Adults are approximately 22 mm long with three oblique dark bands on the fore wings. Their color ranges from light olive-green to dark brown in newly emerged moths and eventually turn to a straw color as scales become dull and fall off (Jensen, personal observation).

Life History

Adult females that emerge in the spring may lay up to 3,000 eggs on host plants. The eggs are laid singly on the upper surfaces of the leaves and hatch in 2-16 days depending on the temperature (Butler and Hamilton 1976). It was reported that the rate of egg, larval, and pupal development increased up to 34°C. The amount of time required for larval development ranged from 56 to 11 days progressing from 15 to 34°C. At 36°C the developmental rate was retarded and at temperatures above this mortality resulted in all the immature stages. In a similar study, Fye and McAda (1972) subjected laboratory-reared larvae, pupae, and moths to four constant and four varied temperature treatments. No general correlation could be made in comparing developmental

times for the larvae between the constant and varied treatments. However, as the temperature was increased, the duration of the immature stages declined. Five instars were usually required to complete larval development but a sixth instar was occasionally necessary at lower temperatures. With adults, a reduction in fecundity correlated with an increase in temperature although longevity of the moths also decreased at the higher temperatures.

Tollefson and Watson (1981) studied TBW feeding behavior and developmental rates on field cotton in Phoenix, Arizona during June, July, and August. In June, larvae fed extensively on squares since the square to boll ratio was still high. In July and August, larvae fed on significantly more bolls and the developmental time was shortened. The average duration of the prepupal and pupal stages in the soil was similar for all infestation periods (Tollefson and Watson 1981). After larval development is completed, the prepupa leaves the plant and burrows into the soil to a depth of approximately 5 cm. deep (Potter 1979). Eight to twelve days are necessary for adult emergence during the summer and there is a preovipositional period of 2-5 days. The generation time averages from 3-4 weeks (Kogan et al. 1978, Werner et al. 1979).

Insecticide Tolerance

The bollworm, Heliothis zea (Boddie), and TBW are two of the most damaging mid-to-late-season pests of cotton throughout the United States. In recent years, the predominant species has been TBW, particularly in fields where insecticides were applied. This may be due, in part, to its lower susceptibility to most insecticides relative to H. zea. McPherson, Newsom, and Roussel (1956) were among the first researchers to compare insecticide tolerances between these two species. They demonstrated that the bollworm was more susceptible to DDT (1,1,1-trichloro-2,2-bis[p-chlorophenyl]ethane) and a BHC (1,2,3,4,5,6-hexachlorocyclohexane)-DDT-sulfur mixture than larvae of the TBW. It was also reported that bollworm and TBW larvae of the first three instars were readily controlled by DDT when applied at the recommended rate but that the later instars survived treatment. Gast (1959) also reported that early instar budworms and bollworms were more susceptible to DDT poisoning than later instars. He attributed the difference to the rate of penetration of the toxicant, with greater permeability occurring in younger larvae.

At the same time, reports of inadequate control of the bollworm and TBW with chlorinated insecticides were becoming numerous. In Louisiana, Graves, Roussel, and Phillips (1963) found that high levels of resistance had

developed to DDT and endrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-endo-5,8-dimenthanonaphthalene) in bollworm larvae. Concurrently, dosage-mortality data obtained by Brazzel (1963) showed that a Texas strain of TBW was also highly resistant to DDT compared with a strain from Florida. He also found a reduced effectiveness of endrin, carbaryl (1-naphthyl methylcarbamate), toxaphene (chlorinated camphene containing 67-97% chlorine) plus DDT (2:1) and Strobane® (terpene polychlorinates [65% chlorine]) plus DDT (2:1) in TBW as compared to the bollworm.

Adkisson (1967) followed the development of resistance of TBW to mixtures of toxaphene or Strobane® plus DDT. Using the LD_{50} (the dose that kills 50 percent of test animals) as an index, the tolerance of TBW during the period 1963-65 increased approximately 6-fold to toxaphene plus DDT and 22-fold to Strobane® plus DDT. The same author in a later paper found that the tolerance of TBW during the period 1961-65 increased more than 200-fold to endrin and approximately 180-fold to carbaryl. It was suggested that cross-resistance to DDT or toxaphene plus DDT may have been responsible for the resistance because the use of endrin, and especially of carbaryl, was limited in Texas (Adkisson 1968).

Penetration and metabolism were studied by Vinson and Brazzel (1966) as the mechanisms for resistance of TBW to DDT. They reported that resistance in one strain was due to increased metabolism, but was due to reduced penetration of the insecticide in another strain. Also, Vinson (1967) showed that a TBW strain that exhibited penetration resistance to DDT was susceptible to DDT when reared on a diet low in ascorbic acid. Furthermore, comparative analysis of the cuticles of larvae from susceptible and resistant strains indicated that protein and lipid content were higher in resistant insects and that there was a greater degree of sclerotization (Vinson and Law 1971).

As resistance developed to the chlorinated hydrocarbon insecticides, organophosphorus compounds, primarily methyl parathion (0,0-dimethyl 0-[p-nitrophenyl] phosphorothioate) came into use (Sparks 1981). This prompted a study by Carter and Phillips (1968) in which ten cycles of selection with methyl parathion were conducted against a laboratory colony of bollworms. Their results indicated that the response of the bollworm had progressed through vigor tolerance, periods of increases in LD_{50} and decreases in slope of the dosage-mortality regression (d-mr) line, to a period showing a sharp increase in homogeneity and a marked shift of the d-mr line to the right. The final population showed an approximate 8 to 10-fold tolerance to

methyl parathion. Shortly thereafter, methyl parathion resistance in TBW appeared in Texas. Wolfenbarger and McGarr (1970) reported the buildup of resistance to methyl parathion and monocrotophos (dimethyl phosphate ester with [E]-3-hydroxy-N-methylcrotonamide) by TBW in the Rio Grande valley. Plapp (1972) briefly summarized the effectiveness of methyl parathion in 1969 and 1970 populations of TBW. Levels of tolerance jumped from 45-fold at the LD_{50} level to 100-fold at the LD_{90} level. He concluded that methyl parathion was no longer an effective insecticide for Heliothis spp. control under Texas conditions. A year earlier, Plapp (1971) had reported indications of cross-resistance to certain other insecticides in a methyl parathion-resistant strain of TBW from central Texas. In comparing LD_{50} values of several insecticides against field-collected, resistant strains of TBW, Wolfenbarger (1973) also suggested that resistance to methyl parathion confers some level of cross-resistance to other compounds available for budworm control.

Bull (1981) reviewed many comparative studies on the metabolism of compounds in organophosphate-susceptible and-resistant larvae of TBW. These studies have shown that in organophosphate-resistant larvae, there appears to be a general enhancement of several important detoxifying enzyme systems, when compared to susceptible strains. It was

concluded that increased detoxication contributed at least in part to resistance.

At the time when methyl parathion resistance in field populations was becoming apparent, new synthetic pyrethroids began to appear in the literature. Cantu and Wolfenbarger (1970) recommended resmethrin (5-benzyl-3-furyl methyl 2,2-dimethyl-3-[2-methylpropenyl]cyclopropane carboxylate) for use against TBW in cotton. When compared to methyl parathion and monocrotophos, the new pyrethroids were over 100-fold more active when topically-applied. However, these compounds were sensitive to sunlight and therefore ineffective in the field. Several years later a new photostable pyrethroid, Niagara NIA-33297 (m-phenoxybenzyl cis, trans-[±]-3-[2,2-dichloro-vinyl]-2,2-dimethyl cyclopropane carboxylate) later to be named permethrin, was produced by Elliott et al. (1973) and was evaluated for effectiveness in the laboratory and field (Davis, Harding, and Wolfenbarger 1975). Laboratory tests indicated that the new compound was more toxic than methyl parathion against TBW and bollworm. In field tests, permethrin gave control of Heliothis spp. equal to methomyl(S-methyl N-[(methylcarbamoyl)oxy]thioacetimidate) and monocrotophos. These authors also reported that field-collected strains of TBW had an LD_{50} value at least 50-fold higher than the laboratory strain. Other laboratory studies have also observed

this differential susceptibility, even before pyrethroids were registered for field use (Harding et al. 1977; Davis, Wolfenbarger, and Harding 1977). In Arizona, Crowder, Tollefson, and Watson (1979) reported that there was a decrease in susceptibility to pyrethroids in TBW that already possessed high levels of tolerance to methyl parathion. This phenomenon has been recently confirmed in California (Twine and Reynolds 1980) and South Carolina (Brown, Bryson, and Payne 1982).

Ishida, Miller, and Kennedy (1983) reported a wide variance in TBW susceptibility to permethrin and bioethanores methrin (5-benzyl-3 furylmethyl[±]-trans-3-cyclopentadienylmethyl 1-2,2-dimethyl-cyclopropane carboxylate) from several counties in California. A greater degree of tolerance was found in larvae collected from cotton as compared to larvae from commercial flowers. It was suggested that the differential susceptibility probably reflects the greater use of these compounds in field agriculture. Plapp (1981) showed that the tolerance present in field-collected larvae disappeared within ten generations in the laboratory. He concluded that the apparent "tolerance" to pyrethroids may only reflect differences associated with laboratory rearing of the budworm and not acquired resistance. To date there have been no reports of pyrethroid failure in the field which have been attributed to resistance.

Chemical and Microbial Insecticides

Synthetic Pyrethroids

Pyrethrum is made from the dried flowers of *Chrysanthemum cinerariaefolium* and *C. coccineum* which belong to the family Compositae. The powder has been used as an insecticide from ancient times and the original home of the pyrethrum flower is said to have been the Middle and Near East (Matsui and Yamamoto 1971). The insecticidal principals are called pyrethrins and occur in the highest concentration in the disc flowers at the time of full bloom. They are esters and thus consist of an alcohol and acid moeity. The natural pyrethrins are made up of six components derived from combinations of two different acids and three different alcohols (Head 1973).

Synthetic pyrethroids are analogs of pyrethrum and are more stable than the natural pyrethrins due to the lower reactivity of the side chains. Otieno and Pattenden (1980) reported that changes in the structural constitution and stereochemical detail of pyrethrins occurred rapidly when they are exposed to light and heat, acid or base, and microbial activity. Although natural pyrethrins have been used sparingly against agricultural pests because of their poor residual ability and high cost, synthetic pyrethroids have presently undergone great expansion. In the last decade a large number of synthetic pyrethroids have been

discovered by various workers (see for review: Elliott, Janes, and Potter 1978). These new synthetic compounds are more stable than the natural pyrethrins and their characteristics include outstanding potency to insects, low toxicity to mammals and high biodegradability (Elliott 1976).

Mode of Action in Insects

Although their precise mode of action in insects is not established, the pyrethroids are recognized as nerve poisons that do not interact with acetylcholinesterase as do organophosphates and carbamates (Elliott et al. 1978). The mode of action of pyrethroids was studied by means of electrophysiological techniques upon cockroach giant fiber preparations (Narahashi 1971, 1976). From these experiments Narahashi showed that allethrin (cis, trans-[±]-2,2-dimethyl-3-[2-methyl proppenyl] cyclopropanecarboxylic acid ester with [±]-2-allyl-4-hydroxy-3-methyl-2-cyclopenten-1-one) modified axonal conduction within the central nervous system by altering the permeability of the nerve membrane to sodium and potassium ions. At least four changes were noted in the resting membrane potential and action potential after exposure to allethrin: (1) the membrane potential is gradually and slightly decreased, (2) the negative after-potential that follows the spike action-potential is increased in amplitude and prolonged in duration,

(3) repetitive after discharges are often produced by a single stimulus, and (4) the amplitude of the action potential is surpressed and eventually blocked (Narahashi 1976).

Similar effects upon giant fiber axons were found by Burt and Goodchild (1971). However, these authors suggested that the fatal lesions caused by pyrethrin I may be within the ganglia rather than being associated with axonal conduction. It was observed that changes in the amount of spontaneous activity in the sixth abdominal ganglion of the cockroach closely paralleled the course of poisoning. Also, after treatment of the insects with an LD_{95} (the dose that kills 95 percent of test animals) of pyrethrin I they became prostrate within two hours after treatment while the giant fibers showed few abnormalities until after four hours. Narahashi (1976) stated that the synapses may be initial target sites for pyrethroids because they are affected at much lower concentrations than would be necessary to elicit a response in nerve fibers. Burt and Goodchild (1977) studied the molecular structure of pyrethroids in association with toxicity to whole cockroaches and to their giant fiber axons. They suggested that the nerve axon may not be a critical site of action for pyrethroids, and that additional processes occurring outside the nervous system, but part of the total toxic mechanism, may be dominant in determining the relationship of structure to toxicity. They

concluded that there must be more susceptible sites of action within the nervous system associated with insecticidal action.

Clements and May (1977) found that the pyrethroids containing the three phenoxybenzyl and ∝ cyano-3-phenoxybenzyl esters may cause four distinct actions upon the peripheral nervous system and associated organs in the locust, Schistocerca gregaria (Forskal). These actions are: (1) prolonged firing of the crural nerve without associated muscle contractions, (2) repetitive after discharges with associated muscle contractions, (3) sustained muscle contractions, and (4) blockage of neurally evoked contractions. They suggested that the impulse generator region of the cell, rather than the axon, was the primary site of action. It was thought that this type of effect was determined by the molecular structure of the pyrethroid. Knockdown was linked to one particular response, and it was suggested that this type of action caused by a pyrethroid, and not the rate of penetration into the insect, determines whether or not it will have knockdown activity.

Gammon (1978) used chronically implanted electrodes in a free walking cockroach to study the action of allethrin at 15 and 32°C. He observed that at both temperatures there were hyperexcitant actions on the peripheral nervous system, but the primary effects of allethrin on the central nervous system were only found at the higher temperature. Topical

application of a LD_{95} dose at 32°C was 10-fold the LD_{95} at 15°C, showing a negative temperature coefficient. He suggested that the greater sensitivity of the peripheral nervous system to hyperexcitation by allethrin at low temperature could account for its greater toxicity at reduced temperature. To further demonstrate the effect of allethrin on peripheral nerves, Gammon (1979) injected doses of the sodium channel blocker, tetrodotoxin, which blocks the peripheral but not the central nervous system, and observed complete protection against an LD_{95} dose of allethrin. He concluded that tetrodotoxin protects cockroaches by reversibly blocking those peripheral nerves normally hyperexcited by allethrin. This protection, however, depends critically on the insect being prostrate at the moment of allethrin administration.

To determine whether an insecticide is a central or peripheral nerve poison, Miller (1979) observed that many types of damage to the house fly central nervous system resulted in the uncoupling of flight motor activity, but that no amount of damage to peripheral nerves will cause uncoupling. Adams and Miller (1980) used a recent neurophysiological technique that records muscle potentials from the flight muscles of house flies. With this technique, they showed that in addition to peripheral effects, the pyrethroids also had significant actions on the central

nervous system. They found that most pyrethroids uncoupled the house fly flight motor but that DDT and its analogs did not.

Chlordimeform

The formamidine pesticides have been used commercially to control various phytophagous mites, some insects in the orders Lepidoptera and Hemiptera and some parasitic ticks at the egg and early larval stages (Hollingworth 1976; Lund, Hollingworth, and Yim 1979). Formamidines are harmless against many other arthropods including beneficial non-target species (Lund et al. 1979). The representative of the formamidines receiving the most attention is chlordimeform (CDM) (N´-[4-chloro-o-tolyl]-N,N-dimethyl formamidine), commercially known as Fundal® and Galecron®.

The first successful deployment of CDM by Dittrich (1966) opened up new possibilities in mite control, especially in greenhouses. It killed adult spider mites when applied as a vapor and as a spray, being equally effective against organophosphate-tolerant carmine spider mites, Tetranychus telarius (L.), and non-tolerant two-spotted spider mites, T. urticae (Koch). Besides having acaricidal properties, CDM showed strong ovicidal effects against insects and mites (Dittrich 1967). Since these initial studies, the application of CDM has expanded into many areas of acarine and particularly of insect control.

The insecticidal and miticidal actions of CDM are perplexing. CDM produces toxicity and death at low doses in only a few cases such as eggs and very young larvae (Harris and Gore 1971, Zied et al. 1968, Gemrich et al. 1976), and adults (Watanabe and Fukami 1977). This has led a number of investigators to suggest that sublethal behavioral effects may be important in affording plant and animal protection. For example, Gladney, Ernst, and Drummond (1974) and Stone, Atkisson, and Knowles (1974) reported that CDM causes detachment of cattle ticks from the host. Doane and Dunbar (1973) found that CDM caused larvae of the elm spanworm, Ennomos subsignarius (Hübner), to drop off treated foliage, and that good protection was present after 15 days from treatment even though larvae were active. In feeding tests with the gypsy moth, Porthetria dispar (L.) and E. subsignarius, the same authors observed that larvae of both species showed strong preference for untreated foliage. Gemrich et al. (1976) found that in adult mites distinctive behavioral patterns of avoidance such as "walk off" or "spindown" were associated in response to treatment by the formamidines. Thus, the behavioral affects attributed to CDM may be partly responsible for plant protection aside from direct mortality.

Mode of Action in Insects

A wide variety of biochemical and pharmacological actions of formamidines have been reported including mitochondrial uncoupling, local anesthesia, neuromuscular effects, monoamine oxidase inhibition, and other aminergic effects (see for reviews: Lund et al. 1979, Beeman 1982). One of the first studies reporting a possible mode of action of CDM in insects was described by Abo-Khatwa and Hollingworth (1972). It was demonstrated that CDM uncoupled electron transport and oxidative phosphorylation in German cockroach mitochondria with a potency similar to that of 2, 4-dinitrophenol. These authors, however, stated that CDM-induced symptoms indicated "extensive involvement of the nervous system in poisoning" which cannot be explained by uncoupling. Beeman and Matsumura (1974) proposed that CDM's monoamine oxidase inhibitory potency was related to its toxicity in American cockroaches. In addition to the inhibition of monoamine oxidase in vitro, it was found that the biogenic amines norepinephrin and serotonin accumulated in poisoned insects. It was then suggested that CDM may be a false transmitter due to the fact that this compound was shown to be a strong cardiostimulant in the same animal (Beeman and Matsumura 1974). This stimulatory action on the heart is known to be mimicked by a number of biogenic amines

including epinephrine, serotonin, and octopamine (Krijgsman and Krijgsman 1951, Collins and Miller 1977).

The recent interest in CDM's mode of action partially results from the idea that if CDM inhibits monoamine oxidase, it may therefore act upon synapses utilizing indolamines or catecholamines as transmitters. Beeman and Matsumura (1978) assayed CDM and several neuroactive amines for anorectic activity in starved cockroaches. Of the nine compounds tested, CDM was the most potent, causing a 78 percent reduction in food consumption. It was also found that octopamine acted as a strong anorexigenic agent. Lund et al. (1979) reported that sub-lethal concentrations of CDM can protect tomato plants from feeding damage caused by tobacco hornworm, Manduca sexta (L.). Larvae were not deterred from feeding but rapidly showed slight tremors and dropped from the plants when exposed to sub-lethal levels of CDM. Neurophysiological studies indicated that this behavior arose through stimulatory actions of CDM in the central nervous system. It was concluded that the increase in tremors and excited symptoms were caused by a synaptically-active material which was non-cholinergic.

The lighting response of photocytes in the firefly, Photinus pyralis (L.), is believed to be controlled by octopaminergic neurons (Nathanson 1979). Assays of CDM on the lantern of adult firefly showed that topical

application of CDM caused the light organ to glow (Lund et al. 1979). The same authors, after comparing the actions of CDM with those of amphetamine and norepinephine, suggested that the action of CDM was postsynaptic rather than presynaptic. Hollingworth and Murdock (1980) found that in addition to CDM, two closely related analogs (or metabolites) also caused the light organ of the firefly to glow. They, too, suggested that this action was postsynaptic and probably involved membrane-bound receptors since cyproheptadone blocked the glows by both demethyl CDM and octopamine.

In addition to its excitatory role in the firefly lantern, Evans and O'Shea (1977) reported that octopamine was the neurotransmitter of a single identified neuron in S. gregaria. They showed that this neuron contained octopamine and innervates a muscle where it functions as a neuro-modulator; this function was mediated by the release of octopamine. This led to further studies with the locust since there appeared to be some relationship between the actions of octopamine and CDM. Evans and Gee (1980) demonstrated that both CDM and demethyl CDM can mimic the effects of octopamine in potentiating twitch tension and increasing the rate of relaxation of tension induced in the extensor tibia muscle. This perhaps is the clearest evidence of a site of action of a formamidine and indicates a possible mode of action for these pesticides.

Bacillus thuringiensis

Taxonomically, *Bacillus thuringiensis* (*Bt*) is classified under the *B. cereus* group, a common, widely distributed soil saprophyte with an extensive host range including insect species from the orders Coleoptera, Hymenoptera, and Lepidoptera (Heimpel and Angus 1963). Distinguishing itself from other species of spore-forming bacilli, *Bt* also produces an insecticidal "endotoxin" or crystalline parasporal body. Hannay (1953) described this inclusion as a diamond-shaped crystal which is formed in the sporangium during sporulation. This proteinaceous crystal is the principal insecticidal component in commercial preparations of *Bt*, and being a stomach poison must be ingested to be effective.

Biological Activity

Heimpel (1967) outlined the different toxins produced by strains of *Bt* and there are three toxins having demonstrated insecticidal activity. They are: (1) alpha-exotoxin, produced by the *B. cereus* group; (2) beta-exotoxin; and (3) delta-endotoxin produced specifically by *Bt*. These toxins, in addition to being insecticidal, exert many different and detrimental side effects in susceptible hosts at non-lethal concentrations (Heimpel 1967).

Kim et al. (1972) studied the effects of the beta-exotoxin on cells from adult cotton bollworm ovaries. They found that the growth of cells was significantly retarded

after treatment. Retardation in growth was attributed to inhibition of RNA synthesis and that 50 percent inhibition was attained only 4-6 hours after inoculation. Bugerjon, Grison, and Cals (1969) demonstrated the teratological effect of the beta-endotoxin on the Colorado potato beetle, Leptinotarsa decemlineata (Say). These malformations usually involved atrophy of the buccal parts, eyes, and antennae, but some morphogenic abnormalities such as claws on distal segments of the antennae and protuberences on the eyes and palps could be found.

Effects of the beta-exotoxin on different stages of Lepidoptera were studied by Ignoffo and Gregory (1972). It was found that mouth-part development in H. zea, H. virescens, Trichoplusia ni (Hübner), Spodoptera exigua (Hübner), Estigmene acrea (Drury), and Pectinophora gossypiella (Saunders) was prevented after mature larvae fed on beta-exotoxin-treated diets. They also observed that development was affected in neonatal H. zea and mature T. ni larvae from exposure to diet surfaces treated with beta-exotoxin. They claimed that most mortalities resulted from failure to molt and/or to discard exuvia. A reduction in fecundity and longevity was also observed in moths which developed from mature larvae that fed on treated diet.

Dulmage and Martinez (1973) reared TBW on diets containing various concentrations of the spore/delta-endotoxin

complex of Bt var. kurstaki. They found that by increasing the complex concentration, development times would increase, pupal weights of surviving larvae decreased, and the number of larvae available to complete the cycle and reach adulthood was reduced. In all cases, the changes were directly proportional to the concentration of the complex in the diet. In a related study, Dulmage, Graham, and Martinez (1978) tested the relationship between length of exposure to the toxin and survival. Larvae of TBW were fed various levels of the delta-endotoxin-treated diet, from 1-3 days, and then transferred to fresh, untreated diet. They observed a strong capacity for larvae to recover from the effects of the toxin, although as the length of exposure increased the capacity decreased. Observations on larvae held to emergence indicated that recovery from the toxin was complete.

The histopathological effects of Bt have been studied in a few insect species. Griego, Fancher, and Spence (1980) examined the histopathological effects of ingested Bt crystal endotoxin on the tobacco hornworm, Manduca sexta (L.), midgut. One hour after ingestion, microvilli showed pathological effects and after four hours exposure to the toxin, nearly complete destruction of the goblet and columnar cells had taken place. In the silkworm, Bombyx mori (L.), breakdown of cells of the midgut by the delta-endotoxin causes leakage of the gut contents into the

hemolymph. As a consequence of this leakage, the pH of the hemolymph rises sharply and causes general paralysis, as contrasted with gut paralysis observed in the majority of susceptible insects (Heimpel and Angus 1959). It was suggested that the site of action is in the midgut and that all of the midgut epithelia are sensitive. With the European corn borer, Ostrinia nubialis (Hübner), ingestion of the delta-endotoxin caused midgut epithelial cells to slough off into the lumen resulting in gut paralysis (Sutter and Raun 1967). It was also found that bacterial spores germinated readily after they were ingested by the larvae. The resulting vegetative rods penetrated the peritrophic membrane and attacked the disorganized midgut epithelium and eventually penetrated the basement membrane. When the gut contents mixed with the hemolymph, the larvae died.

General

Arthropod Resistance to Pyrethroids

A survey of resistance from 1967 to 1976 found that the number of species of insects and acarines in which resistant strains have evolved had increased by 62.5 percent to a total of 364 (Georghiou and Taylor 1976). Of these, 225 are of agricultural importance and 139 of medical/veterinary importance. Of these, only six resist pyrethroids (Georghiou and Taylor 1976), probably reflecting the limited use of these compounds compared with the major

established insecticide groups (Georghiou and Taylor 1976, Elliott et al. 1978). Within the last few years, however, pyrethroids have become the predominant insecticides in cotton, rice, and other important crops. As a result, several papers have indicated that resistance has already become a problem in some areas.

Studies on house flies have provided a valuable source of knowledge in understanding pyrethroid resistance mechanisms. Farnham (1973) investigated the genetics of a resistant strain of house fly by isolating the individual resistant chromosomes. Pyrethroid resistance was attributed to four factors: (1) pen, which reduced the rate of penetration but gave no resistance to DDT; (2) Kdr-NPR, showed a great delay in knockdown by natural pyrethins and DDT; (3) py-ses, gave resistance to natural pyrethrins but was susceptible to synergized pyrethrins; and (4) py-ex, gave slight resistance to natural pyrethrins but was found to give strong resistance when synergized. Farnham and Sawicki (1976) found that selecting a dimethoate-resistant strain of house fly with pyrethrin:piperonyl butoxide increased resistance to pyrethroids while selection with bioresmethrin:piperonyl butoxide had little effect. Keiding (1976) showed that moderate to high levels of pyrethroid resistance had developed in house fly populations on Danish farms when intensive use of pyrethroid aerosols or space

sprays (pyrethrins:piperonyl butoxide, bioresmethrin, bioresmethrin:piperonyl butoxide, tetramethrin:piperonyl butoxide, tetramethrin:resmethrin) were used. He attributed resistance as being due to the intensity of selection pressure applied by the regular treatment. Elliott et al. (1978) stated that pyrethrum resistance in Danish house flies was a result of widespread use of DDT. The recessive gene, Kdr (Knock-down resistance), persisted at low frequencies while flies were controlled by cyclodiene and organophosphate insecticides. As pyrethroid usage increased after 1971, Kdr was selected in a homozygous condition, which resulted in failure of fly control. It was suggested that a similar situation may be found elsewhere if pyrethroids are used extensively against other pests that had been resistant to DDT.

The Kdr-NPR strain of house fly described earlier by Farnham was recently investigated to determine the mechanism for Kdr. Osborne and Hart (1979) reported that significantly higher concentrations of permethrin were required to affect sensory nerve activity in Kdr larvae. Miller, Kennedy, and Collins (1979) also found that much greater concentrations of tetramethrin (N-[hydroxymethyl-1-cyclohexene-1,2-dimethyl vinyl]-2,2-dimethylcyclopropane carboxylate) or cismethrin (5-benzyl-3-furylmethyl [+]-cis-3-[2,2-dimethylvinyl]-2,2-dimethylcyclopropane carboxylate)

were needed to uncouple flight motor potentials in Kdr adults. It was concluded that resistance appears to be widespread in the house fly nervous system, involving sensory, motor, and central neural elements. DeVries (1979) also reported a non-metabolic mechanism of resistance in a trans-permethrin-selected strain (147-R) of house fly. This strain was previously selected for resistance to fenthion (0,0-dimethyl 0-[4-methylthio-m-tolyl]phosphorothioate) and trans-resmethrin (5-benzyl-3-furylmethyl[+]-trans-3-[2,2-dimethylvinyl]-2,2-dimethyl cyclopropane carboxylate). It exhibited cross-resistance to all pyrethroids tested as well as to DDT and a DDT analog.

Priester and Georghiou (1980a) examined knockdown and penetration rate between larvae of pyrethroid resistant and susceptible strains of Culex quinquefasciatus (Say). Susceptible larvae were knocked down within 10-20 minutes by a concentration of 0.01 parts per million (ppm) whereas resistant larvae were only partly affected by concentrations as high as 10 ppm. Variations in penetration rate were attributed to activity differences between the resistant and susceptible strains. It was concluded that penetration was not a factor in resistance. In a following paper, the same authors (1980b) reported that laboratory selection of larvae of C. quinquefasciatus with either (1R)-cis-permethrin or (1R)-trans-permethrin resulted in 4100 and 450-fold

resistance factors, respectively. These strains were found to be cross-resistant to DDT and to all 30 pyrethroids tested. However, they were not significantly cross-resistant to dieldrin (1,2,3,4,10,10-hexachloro-6,7-epoxy,1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-exo-5,8-dimethanophthalene), temephos (0,0´-[thiodi-p-phenylene]0,0,0´,0´, tetramethyl-phosphorothioate), propoxur (o-isopropoxyphenyl methyl carbamate) and two organotin compounds. The resistance could not be suppressed with piperonyl butoxide or DEF® (S,S,S-tributyl phosphorotrithioate) which indicated that some non-metabolic mechanism, such as Kdr, may be an important component of resistance to pyrethroids as well as to DDT in this mosquito. Omer, Georghiou, and Irving (1980) observed that high levels of DDT resistance in larvae of Anopheles stephensi Liston accompanied moderate levels of cross-resistance to permethrin. A non-metabolic mechanism was suggested when DDT and pyrethroids failed to be synergized. Measurements of miniature excitatory post-synaptic potentials (mepps) from motor terminals in larvae represent the spontaneous and continuous release of neuro-transmitter from nerve terminals. Concentrations of (1R)-cis-permethrin needed to cause an increase in mepps in susceptible and resistant larvae were 5×10^{-11}M and 1×10^{-9}M, respectively. It was suggested that the observed resistance was due, in part, to changes in the nervous system of the resistant

strain which resulted in lower sensitivity to the toxic actions of permethrin.

Two South African resistant strains of cattle tick, Boophilus decoloratus (Koch), were examined for pyrethrum cross-resistance. One strain of tick resistant to sodium arsenite and -BHC showed little cross resistance to pyrethrins, but a strain resistant to sodium arsenite, γ-BHC and DDT was highly cross-resistant to pyrethrins (Whitehead 1959). In Australia, Nolan, Roulston, and Wharton (1977) studied cross-resistance to several pyrethroids from a DDT-resistant strain of cattle tick, B. microplus (Can). They detected cross-resistance to permethrin, cypermethrin (-cyano-3-phenoxybenzyl[±]-cis, trans-3-[2,2-dichlorovinyl]-2,2-dimethyl cyclopropane carboxylate) decamethrin (αcyano-3-phenoxybenzyl [1R]-cis-3-[2,2-dibromovinyl]-2,2-dimethyl-cyclopropane carboxylate) and fenvalerate (αcyano-3-phenoxybenzyl [±]-2-[4-chorophenyl]-3-methylbutyrate) in levels ranging from 2-10 fold. The mechanism of pyrethroid resistance in this tick was examined by Nicholson et al. (1980). Biochemical studies showed that no large metabolic differences were found between pyrethroid-resistant and susceptible strains, although the resistance ratio between the strains was not stated. Site-sensitivity studies showed that the nervous system of the pyrethroid-resistant strain was less responsive to permethrin than the susceptible strain.

An Egyptian field strain of the cotton leafworm, S. littoralis (Boisd), showed about a 4-fold resistance factor to permethrin, and a Dutch glasshouse strain of the beet armyworm, S. exigua, was about 400-fold resistant (Fullbrook and Holden 1980). The metabolism of permethrin and its rate of penetration into the larva were compared in resistant and susceptible strains of S. exigua. Fullbrook and Holden (1980) found that decreased penetration of the insecticide was not a factor contributing to resistance. As the temperature was decreased from 30° to 10°C, the tolerance to permethrin also decreased showing a strong negative temperature coefficient. Permethrin was synergized by a factor of eight, which was thought to account for partial resistance. A second component of resistance was thought to be temperature-related and possibly associated with the site of action of the insecticide. In S. littoralis, no significant difference was established from penetration and metabolic studies with permethrin and cypermethrin between resistant and susceptible strains (Holden 1979). It was suggested that permethrin resistance in this insect was non-metabolic. This was subsequently investigated by Gammon (1980). He found a significant difference in abdominal nerve cord poisoning by permethrin between resistant and susceptible larvae, the time of onset of repetitive firing being greater in resistant larvae. However, cypermethrin

did not cause such repetitive discharges. It should be noted that both susceptible and resistant strains were equally susceptible to cypermethrin, and that the 4-fold resistance to permethrin at 30°C disappeared at 10°C. Qualitative differences had been suggested in explaining susceptibility to cypermethrin but not permethrin. Also, since no difference was recorded in the time taken to show nerve blockage by permethrin between larvae, it was unlikely that nerve blockage played a major role in resistance (Gammon 1980).

Liu, Tzeng, and Sun (1982) reported high levels of resistance to four major insecticide groups in a field-collected strain of the diamondback moth, Plutella xylostella (L.). Compared with a susceptible strain, resistance ratios (rr) ranged greatly from 110 in permethrin to > 50,000 in methyl parathion. The resistance level to permethrin was considerably lower than those of the other three ∝ cyanopyrethroids which were 984, 2235, and 2880 for cypermethrin, decamethrin, and fenvalerate, respectively. It was stated that a short life cycle, high fecundity, and intensive insecticide pressure would all facilitate the development of multiple resistance at high levels.

Two field strains of the brown plant hopper, Nilaparrata lugens, Stal, were selected for malathion (diethyl mercaptosuccinate S-ester with 0,0-dimethyl

phosphorodithioate) and MIPC resistance in the laboratory (Chung, Sun, and Hung (1982). Resistance to their respective compounds induced rr from 120 to 170-fold to permethrin when compared to a susceptible strain; however, only a 5-fold rr was observed with fenvalerate. This same correlation was found when these insecticides were tested against four field strains. Rr from 70 to 120 and from 1.6 to 3.0 were found with permethrin and fenvalerate, respectively. The authors noted that since synthetic pyrethroids were recommended for rice insect control in late 1980, field populations should not have been directly exposed to this group of compounds. They suggested that any permethrin resistance detected in the field must represent cross-resistance from insecticides used previously, which included organochlorines, organophosphates, and carbamate compounds.

It has been reported that the common green lacewing, Chrysopa carnea, Stephens, is highly tolerant to pyrethroid insecticides (Shour 1979). Ishaaya and Casida (1981) demonstrated that hydrolysis was a major factor in the seemingly natural resistance towards pyrethroids. More recently, Bashir (1982) showed that both hydrolytic and oxidative mechanisms played major roles in resistance. It was suggested that the explained tolerance to permethrin was due to hydrolysis at the 2' position, whereas with most insects studied, hydroxylation occurs at the 4' position.

Synergism

Synergism is referred to those cases where the toxicity of two compounds together is greater than that expected from the sum of their effects when applied separately (O'Brien 1967). In entomology, this could mean the combination of two or more insecticides or an insecticide and a non-toxic substance. The same description can also apply to the combined actions of micro-organisms and insecticides (Benz 1971).

The best known and most important class of compounds specifically used as insecticide synergists are derivatives of methylene dioxyphenol (Wilkinson 1976), sesamex and piperonyl butoxide being typical examples. These compounds have been used since the 1940s when it was found that sesame oil would synergize the actions of pyrethrins. Sun and Johnson (1960a) compared various pyrethrin synergists with organoclorine and organophosphate insecticides against the house fly. Sesamex proved the most potent for pyrethrin and several other important insecticides. However, clear-cut examples of antagonism were also apparent.

Carbamates are also known to be synergized by methylene dioxphenyl derivatives (Moorefield 1960; Fukuto et al. 1962; Wilkinson, Metcalf, and Fukuto 1966) and by organothiocynates (El-Sebae, Metcalf, and Fukuto (1964). Piperonyl butoxide was found to be effective in increasing

susceptibility of resistant house flies to carbamates (Georghiou 1962, Georghiou and Metcalf 1961).

Like their natural predecessors, pyrethroids are synergized by methylene dioxyphenyl compounds. It was suggested by Sun and Johnson (1960b) that these compounds inhibited oxidative metabolism of insecticides. It was later thought that methylene dioxyphenyl synergists prevented both oxidases and esterases from metabolizing pyrethroids in insects (Casida 1970, Yamamoto 1973). Jao and Casida (1974) supported this hypothesis by demonstrating that a variety of chemicals, both esterase and oxidase inhibitors, can synergize pyrethroids.

The formamidines have recently been suggested as potential synergists for pyrethroids against Heliothis pests (Plapp 1976; 1979) and S. littoralis (Dittrich, Gisin, and Studer 1981). All et al. (1977) reported improved toxicity of pyrethroid mixtures with methyl parathion and methomyl against larvae of H. zea and TBW. Koziol and Witkowski (1982) tested binary mixtures of permethrin with methyl parathion, chlorpyrifos (0,0-diethyl 0-[3,5,6 trichloro-2-pyridyl] phosphorothioate), and malathion for synergistic activity against O. nubialis. Synergism was exhibited in all tested mixtures of methyl parathion and chlorpyrifos with permethrin. No synergism was obtained by the mixture of malathion and permethrin.

Results from experiments on the combined use of insecticides and entomopathogenic bacteria have been contradictory. Reports by Genung (1960), Creighton, Kinard, and Allen (1961), and Hudon (1962; 1963) showed that the combined effects of Bt with an insecticide were no more effective than using the chemical insecticide alone against T. ni, TBW, and O. nubialis, respectively. On the other hand, field tests against T. ni by McEwen et al. (1960) with parathion (0, 0-diethyl 0-[p-nitrophenol], phosphorothioate) and carbaryl, as well as by Creighton et al. (1961) with naled (1, 2-dibromo-2,2-dichloroethyl dimethyl phosphate), indicated favorable activity when combined with Bt. Boric acid synergized the action of Bt in P. dispar at an almost nonlethal concentration (Doane and Wallis 1964). Creighton and McFadden (1974) obtained effective control of T. ni and the imported cabbage worm, Pieris rapae (L.), with sprays containing low rates of Bt and CDM hydrochloride on cole crops.

Benz (1971) stated that the chlorinated insecticides, particularly DDT, showed good potential in synergizing micro-organisms and viruses. These insecticides are known to stimulate the microsomal enzyme systems of living cells (O'Brien 1967). Moreover, insecticidal pyrethroids and DDT analogs, although chemically dissimilar, are known to be directly toxic to nerves and interact with related or identical target sites on the nerve membrance (Beeman 1982).

Pyrethrum (10^{-3}M) was highly synergistic to Bt (10^{-1}M) against third instar Halisidota argentata Packard (Morris 1972). The larch bud moth, Zeinaphera diniana (Guénee), is a primary pest of larch in subalpine forests of Europe. Benz (1975) reported that the action of Bt against this larch pest was enhanced by the addition of DDT.

Recently, it has been found that adequate control of insect pests can be achieved through combinations of Bt with low doses of pyrethroids. The effects of permethrin, Bt, or a mixture of Bt with a sub-lethal dose of permethrin on larvae of Operophtera brumata (L.) and Tortrix vividana L. were studied in an oak stand in Czechoslovakia. The combination resulted in greater mortality than when the bacteria were used alone. Although permethrin alone caused the highest mortality, it was considered unsatisfactory because of the adverse effects on the existing fauna (Svestka 1980). It was reported that larvae of T. ni infesting collards could be adequately controlled by a series of foliar sprays with the pyrethroid insecticide fenvalerate (Hofmaster and Francis 1979). However, an increase in control was noted when the spray application was a mixture of Bt with only one-tenth the recommended rate of fenvalerate.

Summary and Statement of Problem

Insecticides play an integral part in pest management. However, we have seen the TBW develop relatively high

levels of resistance to most insecticides available for its control. Pyrethroids are presently being used against Heliothis in many if not all of the cotton growing areas in the United States.

From the literature review it can be seen that resistance to pyrethroids has already become a problem in a small number of insect species. Recently, differences have been found in TBW susceptibility to the pyrethroids from various field strains and in laboratory versus field strains. This may be an indication that future control with these insecticides against this pest is limited. Therefore, alternate strategies and components must be found and ready for use in the event of resistance development.

The purpose of this study was to investigate the capacity of a field strain of TBW to develop resistance to permethrin. Coinciding with this task was the potential for delaying or preventing resistance by selecting with a one to one (wt:wt) mixture of permethrin and CDM. This test was terminated after twelve generations.

Within the twelfth generation, the following studies were conducted on the P_S strain: (1) the potential for cross-resistance to cypermethrin, (2) if CDM could synergize permethrin; and on the PC_S strain: susceptibility to permethrin alone. In addition, the microbial insecticide Bt was explored for its capacity to synergize pyrethroids.

MATERIALS AND METHODS

Rearing of H. virescens Cultures

A collection of approximately 500 H. virescens larvae from cotton fields in Maricopa County, Arizona, was made in the fall of 1980. These fields had been subjected to moderate and high insecticide usage, including pyrethroids. Larvae were returned to the Department of Entomology Cotton Insects Laboratory, Tucson, and reared on a modified lima-bean diet (Patana 1969). Newly-emerged moths were transferred to wide mouth 3.8 liter (1 gal.) glass jars, ca 30 moths per jar. To prevent serious dilution of the gene pool, at least six jars of moths were maintained at all times. A double layer of cheesecloth was used to cover the tops of the jars and to provide a surface for oviposition. Glass and polyethelene tubes, corked at one end and filled with a 5 percent sucrose solution, were inverted through the cheesecloth to serve as the adult food source.

Egg sheets were removed every other day once oviposition had begun. These were immediately surface-sterilized by washing briefly with a 5 percent sodium hyperchloride solution, then rinsed with a 10 percent sodium thiosulfate solution, and finally in tap water. Egg sheets were air-dried on the inner surfaces of paper toweling and

subsequently put into 474 ml (16 oz.) waxed cartons to complete egg incubation. Two or three neonate larvae were then transferred with a small camel's-hair brush to 29.6 ml (1 oz.) clear plastic cups. Each cup was half-filled with diet and capped with paper lids.

When large numbers of larvae were required for the dosage-mortality studies, the rearing method of Patana and McAda (1973) was slightly modified. Hot diet was poured into plastic storage boxes (31.8 x 17.1 x 9.5 cm) to a depth of ca 2 cm and after the diet had hardened the smooth surface was roughened with a spatula thus creating many grooves. These depressions in the diet formed a barrier between larvae while providing more feeding surfaces so that dried diet-flakes were not needed.

Pupae were collected from only the 29.6 ml cups and placed into 236.8 ml (8 oz.) paper cartons, one layer deep, for adult development. All TBW stages were maintained in a self-contained rearing room at 29 ± 1°C with a light:dark cycle of 16:8. No adjustments were made for humidity, which fluctuated between 30 and 40 percent.

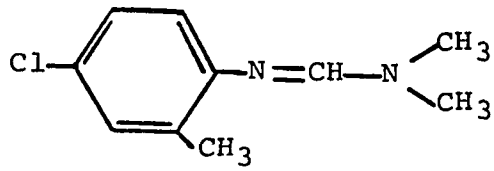
H. virescens Bioassays and Chemicals

Insecticide applications and mortality counts were by the standard method for detection of resistance in Heliothis (Anon. 1970). Third instar larvae weighing an average of 20 ± 3 mg were used in all bioassays (Mullins

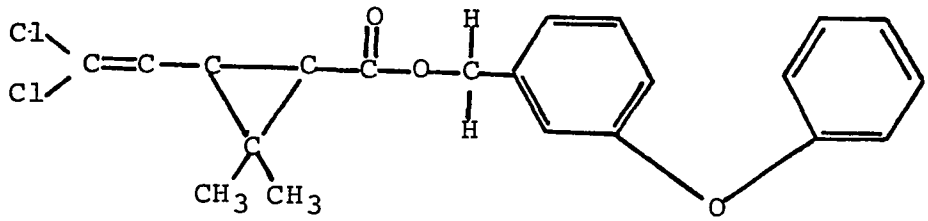
and Pieters, 1982). To determine the average weight, 15-20 percent of larvae designed for testing that day were weighed prior to treatment. Fifteen to thirty larvae per each of four replicates were used at each of 4-6 concentrations plus controls treated with acetone. Dosing was done by topical treatment of 1 µl of the insecticide solution to the dorsal thoracic surface of each larva with a motor driven micro-applicator. This was accomplished without removal of the larva from its diet cup. Treated larvae were held in these cups at 27 ± 1°C.

The 72-hour mortality count was used to compute dosage-mortality data. A larva was considered dead when it failed to respond to prodding with a blunt probe. Moribund larvae were considered dead after 72 hours so that mortality was based upon 72 hour moribund plus mortality counts.

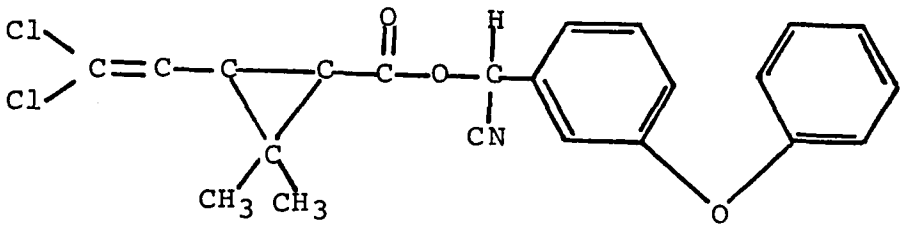
Insecticides employed for this study were technical grades of permethrin (Pounce®; FMC Corporation, Agricultural Chemical Division, Middleport, New York, 14105; 96.5%) cypermethrin (Cymbush®; ICI Incorporated, Biological Research Center, Goldsboro, North Carolina, 17530; 76.5%) and chlordimeform (Fundal®; NOR-AM Agricultural Products Incorporated, Schering, AG [West Germany] 99.6% [Figure 1]). All insecticides were dissolved in acetone and stored in a refrigerator at ca 5°C. The commercial preparation of B. thuringiensis used was Dipel®. This product is manufactured



Chlordimeform



Permethrin



Cypermethrin

Fig. 1. Structures of chlordimeform, permethrin, and cypermethrin. Chlordimeform is the active ingredient of Fundal®; permethrin is the active ingredient of Pounce® and Ambush®; cypermethrin is the active ingredient of Cymbush®.

by Abbott Laboratories, North Chicago, Illinois, and has a potency of 16,000 I.U./mg.

Selection for Permethrin Resistance

This experiment was conducted to investigate the potential for permethrin resistance in a field population of TBW. The F_1 generation of the permethrin-selected (P_s) strain was the progeny of the established parent culture (OM). This parent culture had been maintained in the laboratory for ca seven months prior to the initiation of any chemical treatment. Insecticide applications on larvae of the P_s strain were made by the method previously described. Development of resistance was monitored by calculating a dosage-mortality regression (d-mr) line at alternate (when possible) generations. Survivors from the highest dose were saved and used as parents for the next generation. In those generations where d-mr lines were not conducted, larvae were exposed to a LD_{80} dose to maintain pressure towards resistance. In this way, only those individuals surviving a dose giving approximately 70-90 percent mortality were used in the selection process. Slopes, LD_{50} and LD_{95} values, derived from the d-mr lines, were compared between generations to follow the development of resistance. This test was conducted for twelve generations, resulting in eleven generations of selection pressure.

Repression of Resistance by Chlordimeform

Combining CDM with permethrin to prevent or delay resistance to permethrin was examined. It was found in preliminary studies that CDM was without measurable toxicity to TBW third instar larvae, having an LD_{50} of > 7500 µg/g. The permethrin:chlordimeform selected ($P:C_S$) strain originated from the same parent stock as the P_S strain described earlier. CDM was mixed with permethrin in a 1:1 (wt:wt) standard acetone solution and used as the selection agent. The procedure for selection and measurement of resistance was done in parallel to the P_S strain as defined in the preceding section.

It was hypothesized that a population treated with the permethrin:chlordimeform combination would not develop resistance to permethrin as quickly as the permethrin-alone treatments. To challenge this hypothesis, a dm-r line was calculated for permethrin in the F_{12} generation in addition to the usual dm-r line determined with the permethrin: chlordimeform mixture. This test was conducted for twelve generations in which ten generations were subjected to selection pressure. In the F_6 generation, no selection was made due to an insufficient number of larvae.

Cross-Resistance to Cypermethrin

Since selection with a chemical can result in increased tolerance to related compounds, a test was performed

against the F_{12} generation of the P_s strain to investigate possible cross-resistance to cypermethrin. Dosing with cypermethrin and LD_{50} determinations were followed as previously described. Data were compared to LD_{50} values from a 1981 field population of TBW from Maricopa County, Arizona (Crowder and Watson, unpublished data).

Synergism of Permethrin by Chlordimeform

The synergistic capacity of CDM upon permethrin was investigated. This test was conducted against the TBW population before any insecticide pressure was initiated (F_1 generation) and again upon the P_s strain after eleven generations of selection pressure (F_{12} generation). Insecticide mixtures were the same as those used against the $P:C_s$ strain; applications were made as previously described. Measurement of synergism was done by synergistic ratios (sr) as described by Brattsten and Metcalf (1970):

$$\frac{LD_{50} \text{ of Permethrin}}{LD_{50} \text{ of Permethrin plus CDM}} = \text{SR of CDM.}$$

Synergism of Pyrethroids by B. thuringiensis

This study was done to explore possible synergistic activity between Bt and two pyrethroids, permethrin and cypermethrin. Third stage, 4-5 day old larvae (14 ± 3 mg) were placed in 19.6 ml media cups, two-thirds filled with lima-bean diet. The diet surface of each cup was treated with a suspension of water containing 80 µg of Dipel® in

100 ul, the estimated LD_{25} for a 24-hour feeding period. In preliminary studies the LD_{25} was also estimated for permethrin and cypermethrin since this was to be the only concentration required for this investigation. At 0, 12, and 24 hour post-exposure to Dipel®, larvae were selected at random and topically-treated with the estimated LD_{25} of permethrin and cypermethrin. All treatments were replicated 4-6 times with twenty-five larvae per replicate. Larvae were held at 27 ± 1°C and observed for mortality until termination of the test after five days.

The combined action of the different mixtures was represented as the co-toxicity factor according to the expression given by Mansour et al. (1966):

$$\frac{\text{observed \% mortality-expected \% mortality}}{\text{expected \% mortality}} \times 100.$$

A positive factor of 20 or more is considered synergism; a negative factor of 20 or more means antagonism; intermediate values that are more than -20 and less than +20 indicate additive effect. Since insecticide mixtures were prepared by adding two equal portions of doses each corresponding to the LC_{25} value, it was considered that the expected mortality should be approximately 50 percent.

Statistics

Statistical analysis for the selection study was computed by probit analysis (Finney 1952) through the

services of the Center for Quantitative Studies, University of Arizona. Analysis of Variance (ANOVA) was performed on dosage-mortality data and means separated by the Student-Newman-Keuls multiple range test.

RESULTS

Selection for Permethrin Resistance

Selection of TBW larvae with permethrin at the LD_{80} level produced strong tolerance towards this compound. After eleven generations of continuous selection pressure, the LD_{50} in the F_{12} generation had increased 36.8-fold compared to the LD_{50} of the F_1 generation (Table 1).

Within the course of this study, dm-r lines were calculated during six of the eleven generations under selection pressure, in order to follow the progression of tolerance development. These lines are presented in Figure 2.

The LD_{50} and LD_{95} levels of the F_1 generation were 4.8 and 36.8 µg/g, respectively, and the slope was 2.1. These values established a base-line or reference point by which future changes in permethrin susceptibility can be compared. The median lethal dose increased from 4.8 µg/g in the F_1 to 8.8 µg/g in the F_3 generation. The LD_{95} in the F_3 was 106.9 µg/g, a 2.9-fold increase compared to the LD_{95} of the F_1 (36.8 µg/g). The slope decreased from 2.1 in the F_1 to 1.5 in the F_3. The F_6 generation was found to be almost twice as susceptible to permethrin (LD_{50} 4.6, LD_{95} 42.3 µg/g, slope 1.7) than the F_3 (LD_{50} 8.8, LD_{95} 106.9 µg/g, slope 1.5) and had reverted to approximately the same level

Table 1. Dosage-Mortality Data[1/] of Permethrin and Permethrin:Chlordimeform on the Tobacco Budworm Subjected to LD_{80} Pressure for Several Generations

GENERATION	PERMETHRIN				PERMETHRIN:CHLORDIMEFORM (1:1)			
	LD_{50}	±SD	LD_{95}	SLOPE	LD_{50}	±SD	LD_{95}	SLOPE
1	4.8 A[2/]	.8	36.8	2.1	4.9 A	1.3	48.2	2.1
2	Pressure				Pressure			
3	8.8 A	4.0	106.9	1.5	7.2 A	2.3	122.7	2.1
4	Pressure				Pressure			
5	Pressure				Pressure			
6	4.6 A	1.2	42.3	1.7	No pressure[3/]			
7	Pressure				Pressure			
8	10.2 A	1.5	39.3	3.2	2.1 A	0.6	20.2	1.8
9	Pressure				Pressure			
10	104.3 B	20.0	1092.1	1.9	2.3 A	0.7	119.0	1.2
11	Pressure				Pressure			
12	176.9 C	28.4	1181.1	2.1	3.5 A	0.8	37.9	1.6

1/ Data analyzed by computer probit analysis (Finney 1952)

2/ LD_{50} values followed by the same letter are not significantly different at P = 0.05 as analyzed by Student-Newman-Keuls test

3/ Insufficient number of larvae

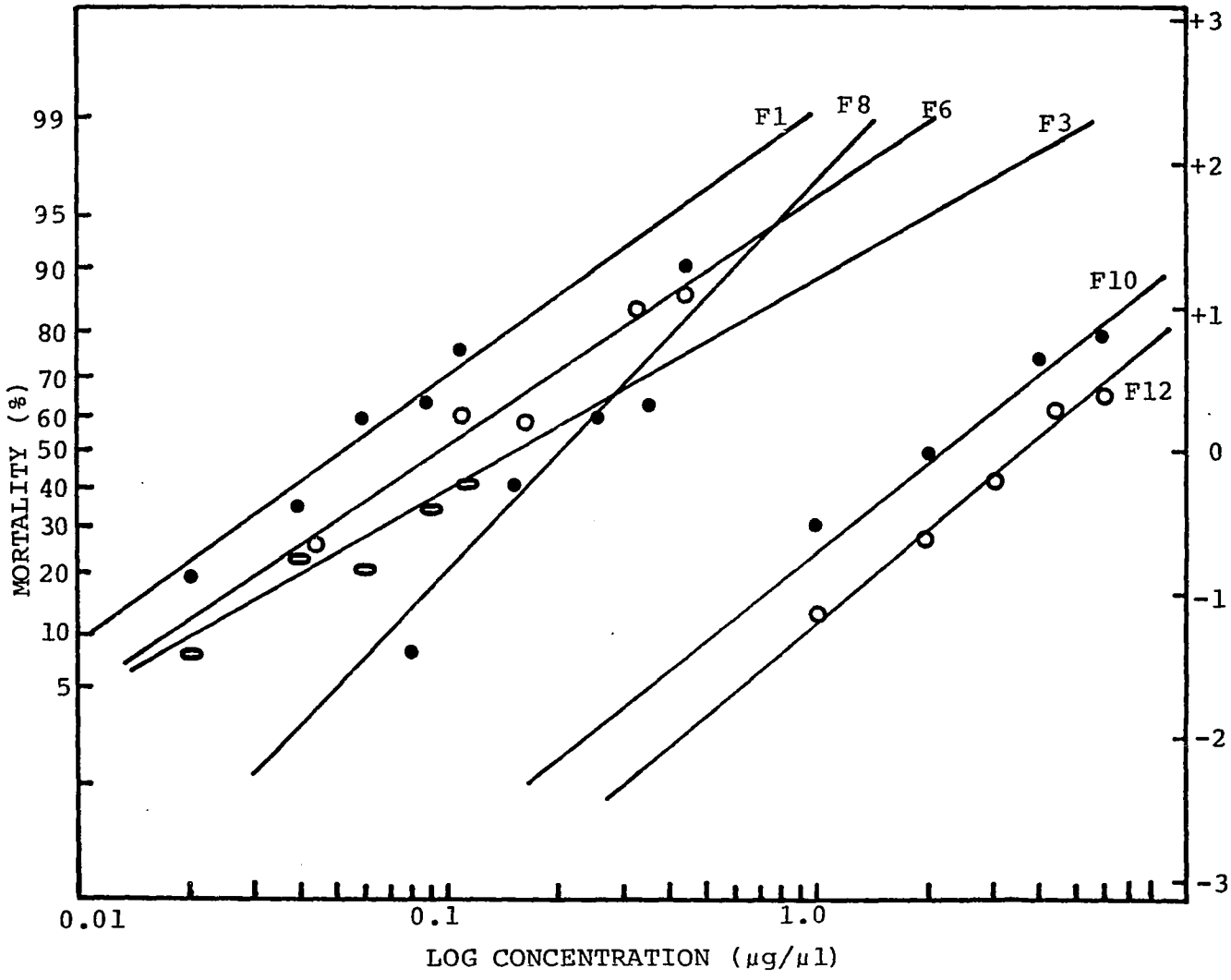


Fig. 2. Dosage mortality lines for permethrin on larvae of the tobacco budworm subjected to LD_{80} pressure for 11 generations

as the F_1 (LD_{50} 4.8, LD_{95} 36.8 μg/g, slope 2.1). The dm-r line for the F_8 generation exhibited a marked increase in slope (3.2) relative to prior generations. The LD_{95} for the F_8 (39.3 μg/g) had decreased slightly from the F_6 (42.3 μg/g), however, a LD_{50} of 10.2 μg/g was 2.2-fold greated compared to the LD_{50} of the F_6 generation (4.6 μg/g). These differences in LD_{50s} found throughout the first and eighth generations were not significant at the 0.05 level by the Student-Newman-Keuls test.

In the F_{10} generation, however, permethrin tolerance increased significantly. A median lethal dose of 104.3 μg/g represents a greater than 10-fold gain when compared to LD_{50s} of the previous generations. The LD_{95} of the F_{10} produced similar results. A 28-fold increase in LD_{95} (from 39.3 to 1092.1 μg/g) was found relative to the F_8. The slope decreased from 3.2 in the F_8 generation to 1.9.

The highest LD_{50} level occurred in generation 12, at the termination of the study. When compared to the F_1 generation, the LD_{50} rose from 4.8 to 176.9 μg/g, a 36.8-fold increase. In addition, this jump in LD_{50}, from the already high LD_{50} in the F_{10}, was found to be significant. Finally, the LD_{95} of the F_{12} (1181.7 μg/g) increased 32-fold from the LD_{95} of the F_1 (36.8 μg/g). The slopes for both F_1 and F_{12} generations were identical (2.1).

Repression of Resistance by Chlordimeform

TBW larvae were selected with a permethrin:CDM mixture at the LD_{80} level during ten of eleven generations. By the F_{12} generation, the degree of resistance to either permethrin or the mixture was scarcely different from levels established in the F_1. Table 1 shows the dosage-mortality data of P-C selection in the TBW for twelve generations. To follow the delay in resistance development, dm-r lines for several generations are given in Figure 3.

The LD_{50} and LD_{95} values for the F_1 were 4.9 and 48.2 μg/g, respectively, and the slope was 2.1. In the F_3, a slight increase in tolerance was noted (LD_{50} 7.2, LD_{95} 122.7 μg/g, slope 2.1) compared to the base-line level (F_1). The degree of tolerance recorded in the F_3 generation represents the highest level achieved during the course of this study. In contrast, the F_8 generation (LD_{50} 2.1, LD_{95} 20.2 μg/g, slope 1.8) demonstrated the greatest susceptibility to the mixture. The LD_{50} for the F_{10} was also very low (2.3 μg/g), however, the LD_{95} increased 6-fold (119.0 μg/g) when compared to the F_8 generation. The slope of the line was 2.1.

When dm-r lines were calculated for the F_{12}, larvae were found to be more susceptible to permethrin + CDM (LD_{50} 3.5, LD_{95} 37.9 μg/g, slope 1.6) than the F_1 (LD_{50} 4.9, LD_{95} 48.2 μg/g, slope 2.1). The tolerance level to permethrin alone was also computed in the F_{12} (Table 2). After ten

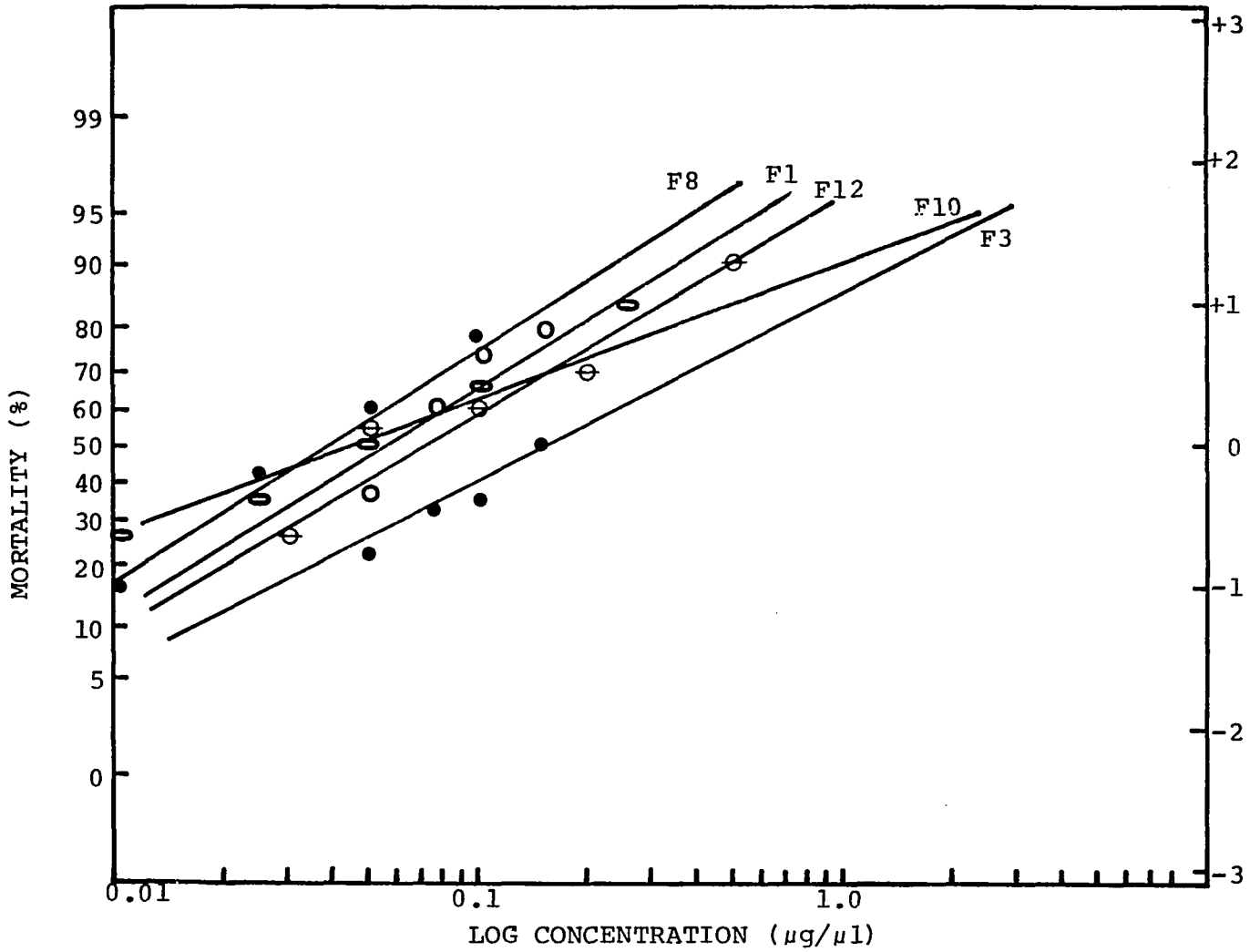


Fig. 3. Dosage-mortality lines for permethrin:chlordimeform (1:1) on larvae of the tobacco budworm subjected to LD_{80} pressure for 10 generations.

Table 2. Differences in Susceptibility to Permethrin in Permethrin:Chlordimeform ($P:C_S$)[1/] and Permethrin (P_S)[2/] Selected Strains of the Tobacco Budworm

	GENERATION					
	F_1			F_{12}		
STRAIN	LD_{50}	LD_{95}	SLOPE	LD_{50}	LD_{95}	SLOPE
PERMETHRIN: CHLORDIMEFORM (1:1)	4.8 A	36.8	2.1	4.5 A	29.6	2.3
PERMETHRIN	4.8 A[3/]	36.8	2.1	176.9 B	1181.1	2.1

1/ Selected at LD_{80} pressure during 10 of 12 generations

2/ Selected at LD_{80} pressure during 11 of 12 generations

3/ Values followed by the same letter are not significantly different at P = 0.05 level, ANOVA

generations of selection with permethrin + CDM, the susceptibility to permethrin in this strain had not changed statistically (LD_{50} 4.5, LD_{95} 29.6 µg/g, slope 2.3) when compared to base-line levels established by the F_1 of the OM strain (LD_{50} 4.8, LD_{95} 36.8 µg/g, slope 2.1).

Cross-Resistance to Cypermethrin

The extent of cross-resistance to cypermethrin in the P_S strain of TBW is given in Table 4. This strain had previously undergone eleven continuous generations of selection pressure with permethrin at the LD_{80} level. The degree of tolerance to cypermethrin was compared to a 1981 field population of TBW, collected from Maricopa County, Arizona.

The average median lethal doses for the P_S and field strains were 63.0 and 8.0 µg/g, respectively. This represents a 7.9-fold difference in LD_{50S} and suggests that the P_S strain has developed strong cross-resistance to cypermethrin. This is further evidenced when comparing the 95 percent mortality data. The LD_{95} values for the P_S and field strains were 1,554.7 and 31.0 µg/g, respectively, a difference of 50-fold.

Synergism of Permethrin by Chlordimeform

The ability of CDM to synergize permethrin against the TBW is shown in Table 3. It was found that if the TBW population was susceptible to permethrin, no synergism

Table 3. Relative Cross-Resistance to Cypermethrin in Permethrin-Selected (P_S) Larvae of the Tobacco Budworm

Dosage Level (μg/g)	STRAIN		
	Susceptible[2/]	P_S	RF[3/]
LD_{50}	8.0[4/]	63.0	7.9
LD_{95}	31.0	1554.7	50.0

1/ Selected at LD_{80} pressure during 11 of 12 generations

2/ Crowder et al. (1981 field strain; unpublished data)

3/ RF = Resistance Factor = (LD_{50} for R strain)/(LD_{50} for S strain)

Table 4. Synergistic Ratios (sr)[1/] for a Permethrin: Chlordimeform Mixture against Larvae of a Permethrin-Selected (P_S) Strain[2/] of the Tobacco Budworm

	GENERATION							
	F_1				F_{12}			
TREATMENT	LD_{50}	LD_{95}	SLOPE	sr	LD_{50}	LD_{95}	SLOPE	sr
PERMETHRIN	4.8	36.8	2.1	--	176.9 A[3/]	1181.1	2.1	--
PERMETHRIN + CHLORDIMEFORM (1:1)	4.9	48.2	2.1	0	51.4 B	203.3	2.8	3.4

1/ $\frac{LD_{50} \text{ of Permethrin}}{LD_{50} \text{ of Permethrin + Chlordimeform}} = sr$

2/ Selected at LD_{80} pressure during 1 of 12 generations

3/ Values followed by the same letter are not significantly different at P = 0.05 level, ANOVA

occurred. However, when the insecticide mixture (permethrin + CDM) was applied to a highly tolerant population of TBW, synergism was noted.

In the F_1 generation, the LD_{50s} for permethrin and permethrin + CDM were 4.8 and 4.9 µg/g, respectively, producing a sr of less than one. Dosage-mortality lines showed that LD_{95} was also higher for the combination of insecticides, 36.8 and 48.2 µg/g, for permethrin and permethrin + CDM, respectively. In the F_{12} generation, the LD_{50} values for permethrin and permethrin + CDM were 176.9 and 51.4 µg/g, respectively, demonstrating a sr of 3.4. The difference at the 95 percent mortality level was even more striking. LD_{95} values for permethrin and permethrin + CDM were 1,181.1 and 203.3 µg/g, respectively, yielding a 5.8-fold sr.

Synergism of Pyrethroids by B. thuringinesis

Table 5 gives the response of TBW larvae at 0, 12, and 24h post-infestion with Bt to permethrin and cypermethrin. The combined action of the different mixtures was expressed as a co-toxicity factor. The approximate LC_{25} dose of Bt, at 24h, resulted in a 21.5 percent mortality, whereas at 12h, gave 12.4 percent mortality. The LC_{25} dose of permethrin and cypermethrin resulted in 17.3 and 19.4 percent mortality, respectively.

Table 5. Response of Tobacco Budworm Larvae at 0, 12, and 24 Hours Post-Infection with *Bacillus thuringiensis* to Permethrin and Cypermethrin Expressed as the Co-Toxicity Factor[1/]

	HOURS		
TREATMENT	0	12	24
PERMETHRIN	-3	+117	+113
CYPERMETHRIN	-9	+ 16	+ 72

1/ $\frac{\text{Observed \% Mortality - Expected \% Mortality}}{\text{Expected \% Mortality}} \times 100$

In the 0h treatment, dosed larvae were immediately placed on Bt-diet. After the prescribed time period, no synergism was found as indicated by co-toxicity factors of -3 and -9 for permethrin and cypermethrin, respectively. With a 12h pretreatment period, the co-toxicity factors for permethrin and cypermethrin were +117 and +16, respectively, which demonstrated that synergism occurred with permethrin but not cypermethrin. Twenty-four h feeding on Bt resulted in synergism of both pyrethroids. For permethrin, the mortality of the mixture was 83.2 percent compared to the additive effect expected of 38.7 percent. This gave a co-toxicity factor of +113. In the case of cypermethrin + Bt, the observed mortality was 70.1 percent compared to the expected of 40.8 percent, resulting in a co-toxicity factor of +72.

DISCUSSION

Selection of Permethrin Resistance

The response by TBW larvae to permethrin selection progressed through a series of changes that closely resemble a population demonstrating resistance to the selecting agent. A LD_{50} of 4.8 $\mu g/g$ in the F_1 generation is typical of LD_{50s} for field-collected TBW in Arizona since 1978 (Crowder and Watson, unpublished data). The F_3 generation showed a 2-fold increase in tolerance to permethrin, although a LD_{50} of 8.8 $\mu g/g$ is still well within the realm of susceptibility. Brown and Pal (1971) stated that at the beginning of the selection process, slight increases in LD_{50s} may be independent of specific genes for resistance. The term "vigor tolerance" has been applied to this phenomenon by Hoskins and Gordon (1956). This expression implies that weaker individuals become eliminated in the early generations of selection while the stronger individuals, being more fit and showing increased vigor, survive. This effect (vigor tolerance) was exhibited by the F_3 and was especially evident at the LD_{95} level.

Since the slope of the dm-r line measures the variance in response to the toxin (Hoskins 1960), a steep slope is the reaction from a homogeneous population, whereas a

shallow or flat slope shows a broad range in response, or heterogeneity. The latter effect was seen in the F_3 and to a lesser degree in the F_6 generation. The F_8 showed a sharp increase towards homogeneity as the slope doubled compared to the F_3 and F_6 generations. Concurrently, there was a 2-fold increase in the LD_{50}, as evidenced by a marked shift of the dm-r line to the right. These changes in LD_{50}s and slopes may be indicative of a population developing specific resistance. When this occurs, dm-r lines become flatter as the LD_{50} increases (exhibited by the F_3), then becoming steeper as the population becomes homogeneous for resistant individuals (Hoskins and Gordon 1956). The steep slope in the F_8 generation probably represents the elimination of the very susceptible insects.

When the population becomes more homogeneous for resistant individuals, the LD_{50} level should increase. It was noted that during selection pressure in the F_9, new dilutions containing higher concentrations of permethrin were needed to attain 80 percent mortality. This was manifest in the F_{10} generation when the LD_{50} was quite unexpected, although it has been a common occurrence in other resistance studies (Brown and Pal 1971). Georghiou and Taylor (1976) stated that in most cases of laboratory selection, resistance evolves gradually at first,

then subsequently at a faster rate, and is dependent on the phenotypic expression of the R gene(s) in the resistant homozygote.

Although the slope in the F_{10} had decreased, the population showed that the development of resistance to permethrin was occurring. An explanation for the flattened slope may be that the population has not become pure for the resistance factor, thus more selection was needed. This is evidenced by the significant increase in the LD_{50} demonstrated by the F_{12} generation compared to the F_{10}. The level of tolerance has now increased 36.8-fold from the base-line level established by the F_1 generation.

Pyrethroid resistance in the TBW is usually monitored from year to year in many areas of the United States. Since there are no reported cases of "true" resistance to pyrethroids, these field populations are typically compared to "susceptible" laboratory colonies (see for review: Sparks 1981; Wolfenbarger, Bodegas, and Flores (1981). If the P_s strain from the present study (LD_{50} 176.9 $\mu g/g$) is compared to a standard susceptible laboratory strain (LD_{50} 0.28 $\mu g/g$) from Tucson, Arizona (Crowder et al. 1979), the difference in LD_{50s} for permethrin is > 600-fold. This further demonstrates the high level of resistance to permethrin presently found in the P_s strain, and provides evidence that the mechanism for resistance is a heritable one.

The reason why the TBW developed resistance to permethrin is unknown. This is due, in part, from the fact that the specific mode of action of pyrethroids has not been determined. However, resistance to pyrethroids, and to DDT, has been linked in insects possessing "target site insensitivity" or Kdr-like mechanisms (see for review: Plapp 1976, Elliott et al. 1978, Beeman 1982). In contrast, the resistance mechanisms to many insecticides in the TBW has been associated with increased metabolism (Bull 1981, Sparks 1981).

There appears to be a good correlation between DDT resistance and pyrethroid cross-resistance (Plapp 1976, Elliott et al. 1978), and has recently been demonstrated in several species of mosquito (Omer et al. 1980, Priester and Georghiou 1980, Priester et al. 1981). The levels of pyrethroid resistance presently found in field-collected TBW probably expresses some degree of innate tolerance which might be attributed to earlier chemical treatments, for example by DDT and its analogs, which eventually produced resistance to those compounds. Elliott et al. (1978) stated that pyrethrum resistance in Danish house flies was the result of widespread use of DDT. They speculated that a similar situation may be found elsewhere if pyrethroids were used extensively against other pests that had previously been resistant to DDT. If a similar correlation can be made

with the TBW, then resistance to pyrethroids may occur in the near future as demonstrated by the increased tolerance levels brought about by this study.

Repression of Resistance by Chlordimeform

This investigation demonstrated that induction of permethrin resistance in the TBW through selection might be delayed or prevented by the addition of CDM to the selecting agent. The reason why the P:C_S strain did not develop any tolerance to permethrin or to permethrin + CDM is not known, although three hypotheses can be drawn from the results.

In the companion study, selection at the LD_{80} level with permethrin alone caused 36.8-fold permethrin resistance. This indicates that the alleles responsible for resistance should have been in the parent population and occurred at a certain frequency. Georghiou and Taylor (1976) speculated that in the absence of insecticides, the relative incidence of resistant (R) alleles would be expected to be from 0.01 to 0.0001. Since the parent population was obtained from fields that were subjected to insecticide treatment, the frequency of R alleles would presumedly be higher, possibly from 0.1 to 0.001. However, selection pressure on the two test strains began approximately seven months after their acquisition from the field. Thus, many of the R alleles could have been lost during the six generations of rearing

prior to the initiation of selection pressure. When the parent culture was divided in half at the onset of the experiment, the distribution of the remaining R alleles could have all gone to the P_S strain, leaving nothing but susceptible insects in the P-C_S strain. Thus, insects having the genetic capability for resistance, within the P-C_S strain, may have been absent, along with their alleles, at the start of the selection process itself.

The second argument can be drawn from the fact that the specific modes of action of both permethrin and CDM are not known. Pyrethroids affect the peripheral and central nervous system resulting in rapid paralysis, and nerve excitation occurs as a result of changes in nerve membrance permeabilities to sodium and potassium ions (Narahashi 1971, 1976, see for review; Beeman 1982). A wide variety of biochemical and pharmacological actions of formamidines have been reported including mitochondrial uncoupling, monoamine oxidase inhibition and neuromuscular effects (see for review: Lund et al. 1979, Beeman 1982). Also, recent studies suggest that the formamidines in invertebrates may be destructive towards aminergic, nervous, or endocrine functions. Since it appears that these two insecticides possess dissimilar modes of action, CDM may be acting against the pyrethroid resistance mechanism and preventing it from functioning properly.

Furthermore, Brown and Pal (1971) stated that the speed of resistance development and the intensity it attains are greater with organochlorine insecticides than with organophosphate and carbamate compounds. If CDM acts in a similar manner by not affording sudden and extreme resistance as the organophosphates and carbamates, the selection of the mechanism for pyrethroid resistance may have been retarded.

Cross-Resistance to Cypermethrin

Cross-resistance to cypermethrin was induced in TBW larvae by selection in the laboratory with permethrin for eleven generations, to a level of 36.8-fold permethrin resistance. When compared to a susceptible field strain, the difference in tolerance to cypermethrin was 7.9-fold (at LD_{50}) and 50-fold (at LD_{95}) (Table 4).

The degree of susceptibility to cypermethrin in TBW larvae from Arizona was found to be comparable to levels from other states. For example, the LD_{50} of a field strain collected in North Carolina was 2.4 µg/g (Brown 1982). Plapp (1981), using a contact method, found that TBW from Texas was susceptible to cypermethrin having a LC_{50} of 1.7 µg/20 ml glass vial. These values in other areas are actually several times lower than the LD_{50} in TBW from Arizona. From this, it seems fair to assume that an LD_{50} of 63.0 µg/g for cypermethrin, although not as high as the selecting agent permethrin (176.9 µg/g), constitutes cross-

resistance to cypermethrin. Quraishi (1977) stated that maximum resistance often develops to the inducing compound while cross resistance to other compounds is less marked. Thus, the difference in resistance levels in the P_S strain to permethrin and cypermethrin are typical of insects exhibiting cross resistance patterns.

The TBW has been a major control problem for many years, in part, because of its ability to develop cross-resistance (see for review: Sparks 1981). Recent studies with house flies and mosquitoes have demonstrated that selection for resistance with one pyrethroid confers at least partial resistance to many other pyrethroids (Farnham 1973, 1976; Priester and Georghiou 1980). DeVries (1979) found that a trans-permethrin-selected strain of house fly, which became 140-fold resistant, also developed cross-resistance to all twenty-six pyrethroids tested against it. The level of cross-resistance was high (44 and 48-fold) for (+)-trans permethrin and (+)-cis permethrin, respectively, compared to the susceptible strain. Priester and Georghiou (1980) reported that selection of southern house mosquito larvae with either (1R)-cis permethrin or (1R)-trans permethrin produced high resistance levels in each strain. These strains, when tested, were found to be cross-resistant to all thirty pyrethroids. Cross resistance to (1R)-cis and (1R)-trans

cypermethrin was 94 and 432-fold greater, respectively, in the (1R)-trans permethrin strain compared to the susceptible strain.

The extent of cross-resistance to other pyrethroids in the TBW is not known. However, if TBW possesses similar mechanisms for the development of cross-resistance as in the house fly and southern house mosquito, it is likely that once it has acquired resistance to one pyrethroid, it would eventually show resistance to others.

Synergism of Permethrin by Chlordimeform

The results showed that synergism was found only after the P_S strain had undergone a change in status from a susceptible to resistant population. The F_{12} generation was 36.8-fold resistant to permethrin compared to the F_1, however, the difference between LD_{50S} was only 10.7-fold when the F_{12} was challenged with the permethrin + CDM mixture.

All et al. (1977) reported that a preparation of ten parts methyl parathion to one part permethrin was highly synergistic against the TBW. Crowder et al. (unpublished data) found that a combination of the same insecticides, but in a one to one ratio, against the TBW resulted in independent action. Differences in response from these two investigations may be due to two factors. All et al. (1977) used a 10:1 mixture and tested against a very susceptible strain whereas Crowder et al. employed a 1:1 mixture against

a relatively tolerant strain. Conversely, it was found in the present study that synergism occurred only in tolerant TBW.

Plapp (1979) reported synergism with a 1:1 mixture of permethrin + CDM against a laboratory strain of TBW. Synergism at the LC_{50} and LC_{90} levels was 1.8 and 1.9-fold, respectively. This report is in partial contradiction to the results from this study in that permethrin + CDM were not synergistic against the F_1 generation but showed a sr of 3.4 in the F_{12}. One possible explanation for the difference between the present study and Plapp's may be due to the different bioassay methods employed. Earlier, Plapp (1976) had observed that CDM was synergistic to many different types of insecticides against the TBW and was greatest with those compounds that had the least inherent toxicity. This is in agreement with the results from the present study, in that, when the effectiveness of permethrin declines (increase in LD_{50}) synergism was exhibited.

It has been suggested that CDM may compete with insecticides for insecticide-metabolizing enzymes such as the microsomal mixed-function oxidases (Plapp 1976, 1979). If CDM competes with permethrin for mixed function oxidase enzymes, a susceptible insect, that doesn't have an efficient mixed-function oxidase system necessary to overcome toxicity, when tested with the mixture might not exhibit

synergism. But in a resistant insect, whose mixed-function oxidase level may be high, the interaction by CDM with these enzymes could account for the 3.4-fold synergism noted in the present study.

Synergism of Pyrethroids by B. thuringiensis

Results from this study found that under certain conditions, a combination of either permethrin or cypermethrin with Bt resulted in greater mortality than the addition of each component separately (i.e. synergism). This finding confirms recent observations that low or sub-lethal doses of pyrethroids, mixed with Bt, enhanced the action of the latter product (Svestka 1980, Hofmaster and Francis 1979).

At the Oh treatment, synergism was not noted. Benz (1971) stated that antagonism between a microbial and chemical insecticide combination may result from the prevention of food uptake. If a low dose of insecticide has a strong knockdown effect, as with the pyrethroids, test-insects may be prevented from taking up an effective dose of the microorganism. This response is evident in the Bt + cypermethrin treatment. At Oh, the co-toxicity factor was -9. If allowed to feed on Bt-diet for 12h prior to being dosed with cypermethrin, the co-toxicity factor was +16. After spending 24h on Bt-diet, the co-toxicity factor increased to +72.

Synergism by permethrin + Bt was greater than that of cypermethrin + Bt. Several researchers also have reported dissimilarities between these two pyrethroids concerning penetration (Holden 1979), knockdown (Clements and May 1977) and nerve blockage (Gammon 1980). This development can possibly be justified by structural differences between these two compounds and may explain the superior synergistic ability of the permethrin + Bt combination.

The present study was conducted as a preliminary investigation, therefore, the explanations for synergism are purely speculative. Pyrethroids affect the peripheral and central nervous system resulting in rapid paralysis, and nerve excitation occurs as a result of changes in nerve membrane permeabilities to sodium and potassium ions (Narahashi 1971, 1976, see for review: Beeman 1982). In many Lepidoptera, the crystal endotoxin of Bt causes rapid paralysis of the midgut as a result of a disruption of the selective permeability of the midget epithelium to sodium, potassium, and calcium ions (Fast and Angus 1965; Ramakrishnan 1968; Griego, Moffett, and Spence 1979; Smirnoff and Valero 1980). Cooksey et al. (1969) reported that the digested crystal protein blocked nerve conduction in a preparation from the American cockroach. Furthermore, detrimental effects have been observed in isolated mitochondria (Travers and Faust 1976) and upon proteins such as cytochrome c (Faust 1968)

which are basic to the functions and maintenance of viable cells. The stress placed on the TBW by a combination of bacterial toxin and pyrethroid must be considerable and may account for the synergizing effects demonstrated between these two components.

Significance of Results

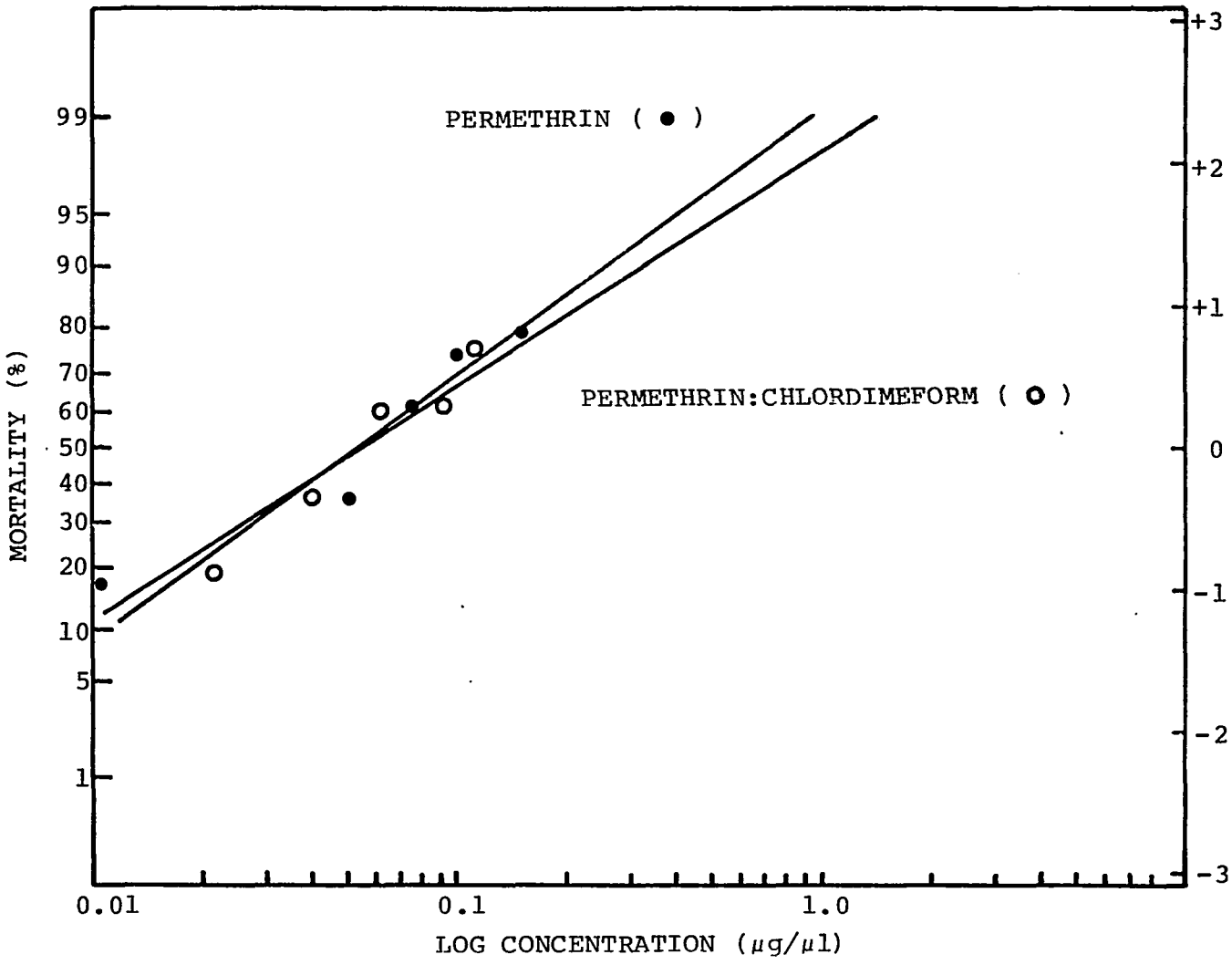
The TBW can cause major economic loss to a variety of cultivated crops. It has been shown at one time or another to be resistant to most insecticides that have been used against it. Presently, the synthetic pyrethroids are being employed to control this pest. This investigation demonstrates that TBW has the ability to become resistant to pyrethroids in a relatively short time provided the population is exposed to a high degree of selection pressure. A 36.8-fold increase in tolerance to permethrin was developed after eleven generations when compared to the level established by the first generation. Cross-resistance to cypermethrin was also found in this strain. From this, it is probable that field selection with any one synthetic pyrethroid could ultimately lead to resistance to all compounds in this group. Therefore, treatment regimes, such as alternating chemicals or the use of chemical mixtures, might be useful in minimizing the rate of resistance development.

A selection study using a 1:1 mixture of permethrin: CDM showed promise in that resistance to permethrin could be

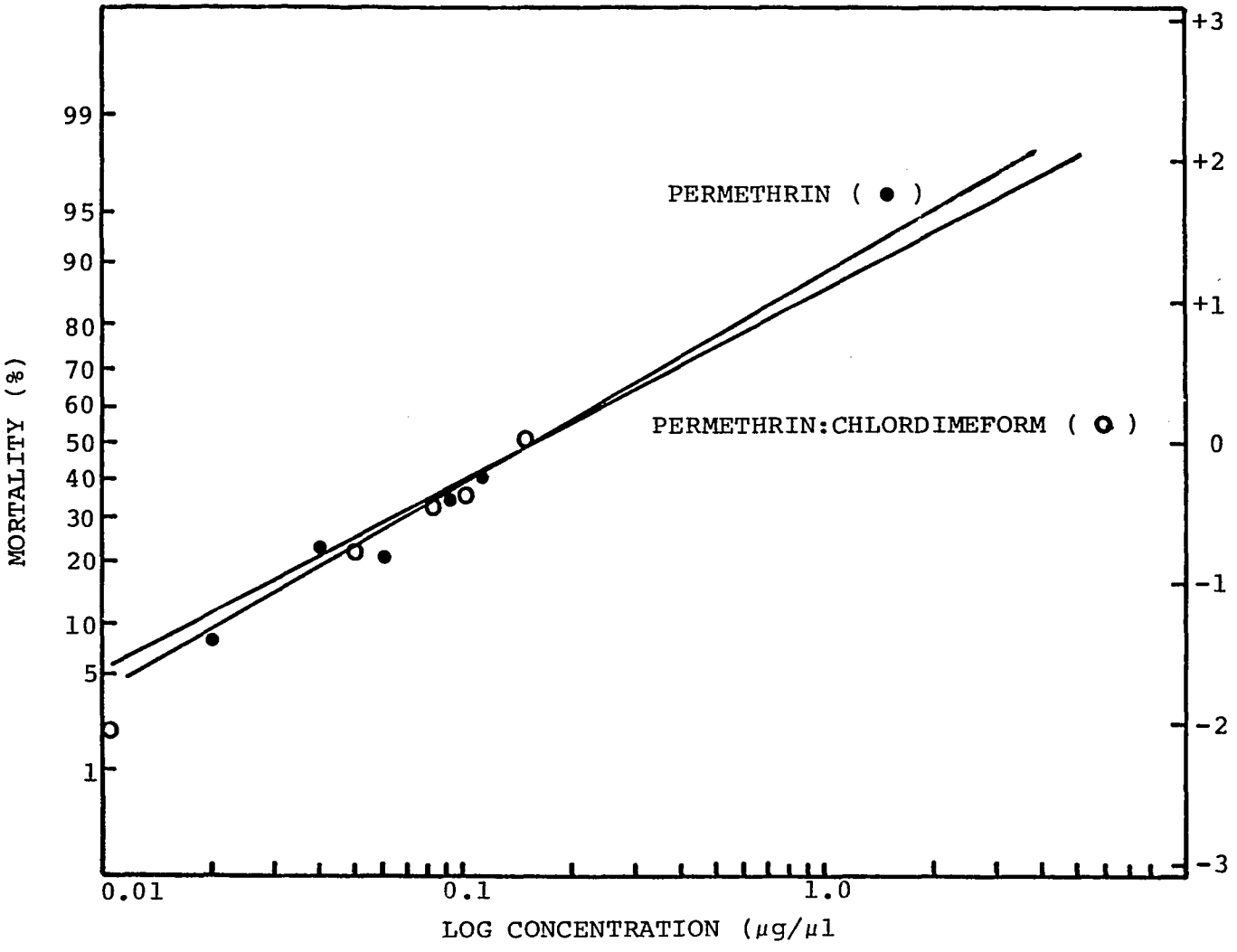
prevented or delayed. The susceptibility to these compounds in the twelfth generation is comparable to what it was in the first generation. However, since selection pressure was not applied to the sixth generation, some further selection will be required before this result is conclusive.

In addition to its possible role in preventing permethrin resistance, CDM was found to synergize permethrin from 3 to 4-fold. However, this effect only occurred after TBW larvae became tolerant to permethrin. No synergism was noted upon a permethrin-susceptible population.

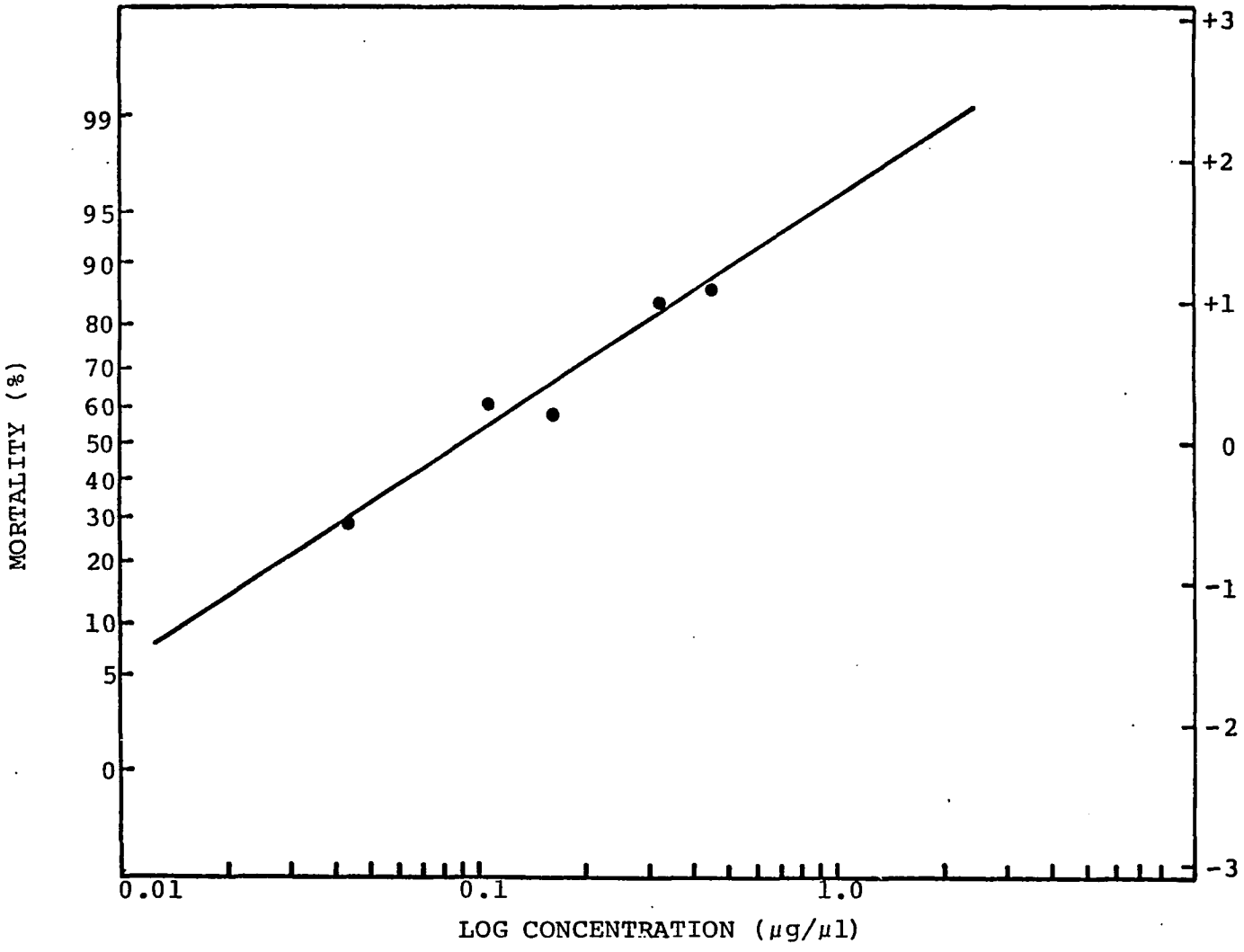
The synergism of pyrethroids by Bt also holds some potential for future control of TBW. The mortality from the combinations was increased approximately 2-fold from the expected additive mortality.



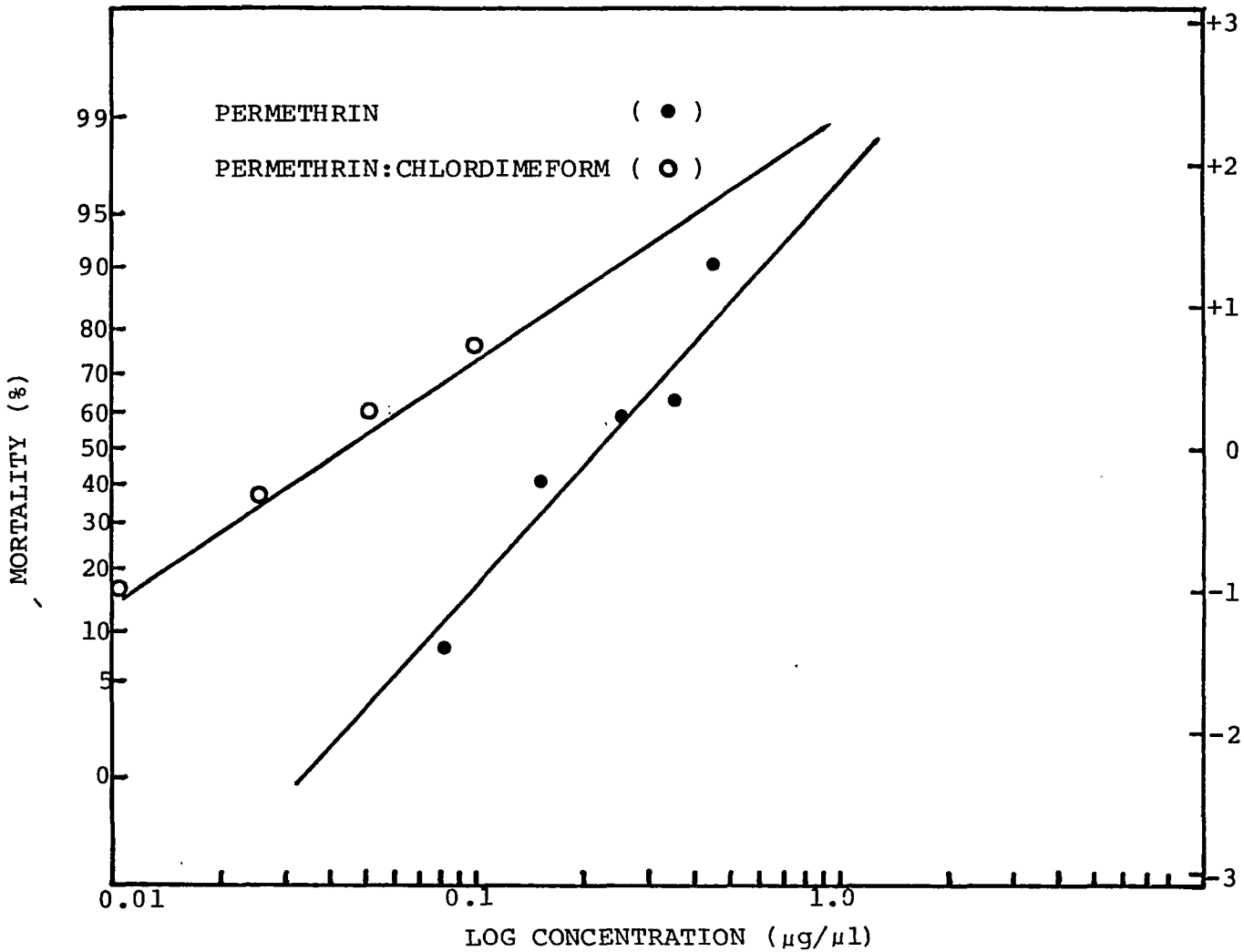
APPENDIX A: DOSAGE-MORTALITY LINES FOR PERMETHRIN AND PERMETHRIN: CHLORDIMEFORM (1:1) ON LARVAE OF THE TOBACCO BUDWORM IN ARIZONA



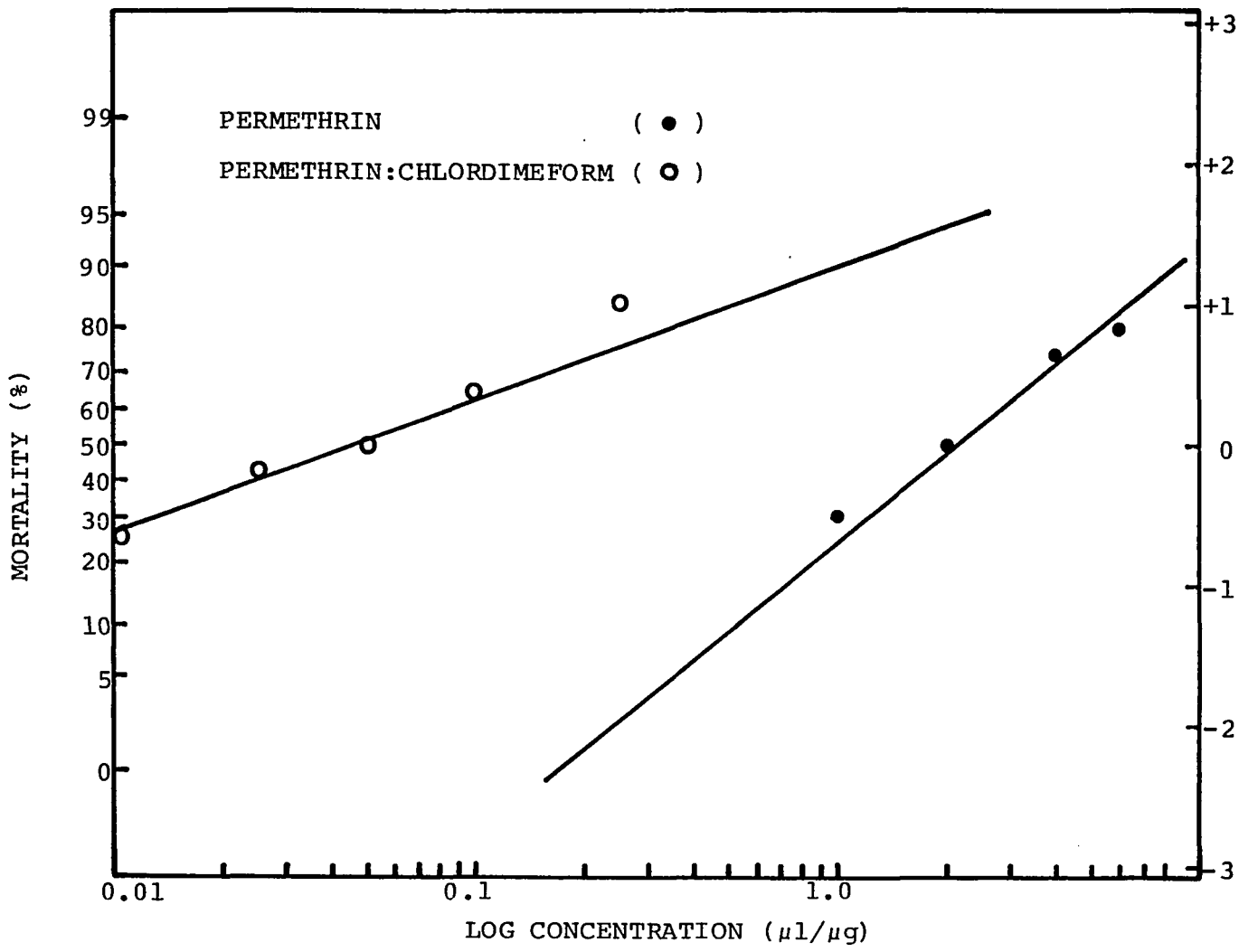
APPENDIX B: DOSAGE-MORTALITY LINES FOR PERMETHRIN AND PERMETHRIN:CHLORDIMEFORM (1:1) SELECTED STRAINS OF THE TOBACCO BUDWORM. LARVAE WERE SUBJECTED TO LD_{80} PRESSURE FOR 2 GENERATIONS.



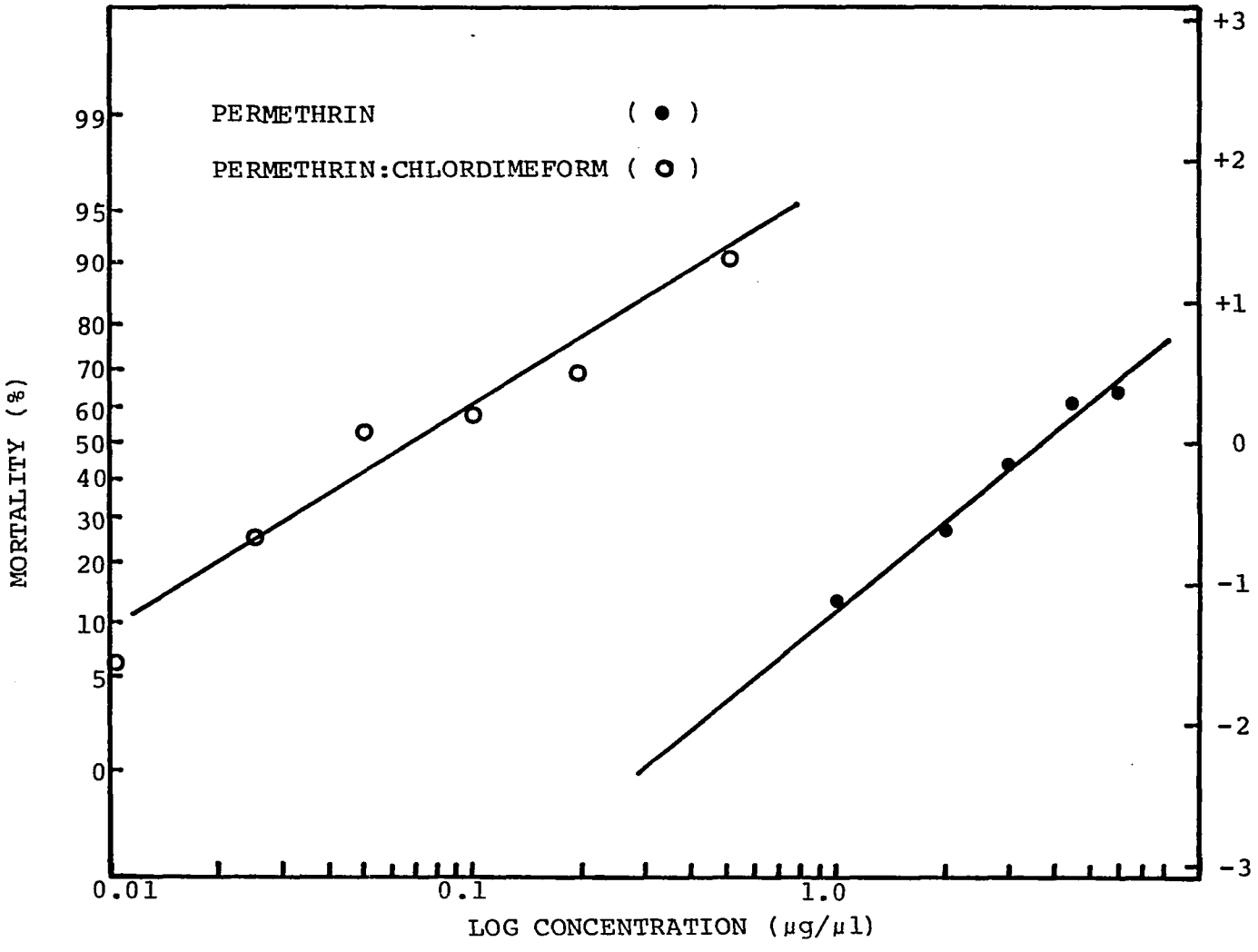
APPENDIX C: DOSAGE-MORTALITY LINE FOR A PERMETHRIN SELECTED STRAIN OF TOBACCO BUDWORM. LARVAE WERE SUBJECTED TO LD_{80} PRESSURE FOR 5 GENERATIONS.



APPENDIX D: DOSAGE-MORTALITY LINES FOR PERMETHRIN AND PERMETHRIN:CHLORDIMEFORM (1:1) SELECTED STRAINS OF THE TOBACCO BUDWORM. LARVAE WERE SUBJECTED TO LD_{80} PRESSURE FOR 7 AND 6 GENERATIONS, RESPECTIVELY.



APPENDIX E: DOSAGE-MORTALITY LINES FOR PERMETHRIN AND PERMETHRIN:CHLORDIMEFORM (1:1) SELECTED STRAINS OF THE TOBACCO BUDWORM. LARVAE WERE SUBJECTED TO LD_{80} PRESSURE FOR 9 AND 8 GENERATIONS, RESPECTIVELY.



APPENDIX F: DOSAGE-MORTALITY LINES FOR PERMETHRIN AND PERMETHRIN:CHLORDIMEFORM (1:1) SELECTED STRAINS OF THE TOBACCO BUDWORM. LARVAE WERE SUBJECTED TO LD_{80} PRESSURE FOR 11 AND 10 GENERATIONS, RESPECTIVELY.

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