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Synergism of permethrin by formamidines in resistant and susceptible strains of *Heliothis virescens*: A comparison of chlordimeform, amitraz, and a metabolite

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SYNERGISM OF PERMETHRIN BY FORMAMIDINES IN RESISTANT AND SUSCEPTIBLE STRAINS OF HELIOTHIS VIRESCENS: A COMPARISON OF CHLORDIMEFORM, AMITRAZ, AND A METABOLITE

by

Pharoah Opelo Pedro Mosupi

A Thesis Submitted to the Faculty of the

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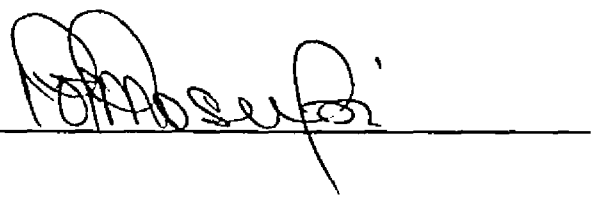
SIGNED: 

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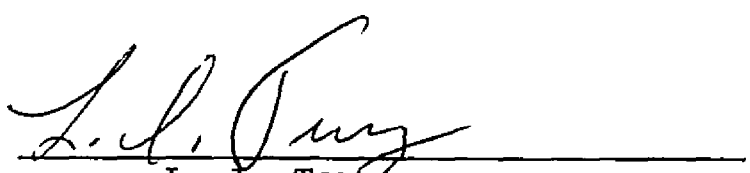

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TABLE OF CONTENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

ABSTRACT

Laboratory tests conducted to evaluate the ability of three formamidines (chlordimeform, amitraz, and N′ -(2,4-dimethylphenyl)-N-methylformamidine SN-49844, a metabolite of amitraz) to synergize permethrin in tobacco budworm (TBW) showed no synergism in susceptible larvae, but did show synergism in resistant larvae. Chlordimeform (CDF) and N′-(2,4-dimethylphenyl)-N-methylformamidine SN-49844 showed higher degrees of synergism than amitraz. Selection of cross-bred larvae of tobacco budworm using permethrin ± formamidine mixtures at LD_{80} for four generations produced strong tolerance to all agents used in the selection. After four generations of continuous selection pressure, the LD_{50} in the F_5 had increased by 100-fold with permethrin plus amitraz, and 180-fold with permethrin plus SN-49844 as compared to the F_1 generation. A test for cross-resistance to cypermethrin and to methyl parathion in a permethrin-selected strain of tobacco budworm showed resistance levels of 58-fold, and 48-fold, respectively, compared with susceptible TBW.

INTRODUCTION

Heliothis Species Biology

Of the genus Heliothis (Lepidoptera: Noctuidae), three species, H. armigera, H. virescens, and H. zea are important pests of pre-harvest crops. The bollworm, H. armigera is found in Australia, Africa and Asia and is a highly polyphagous pest that feeds on cereals, legumes, vegetables and cotton. The TBW, H. virescens, is a major pest of cotton in the USA and in Central and South America. It has caused disastrous cotton production losses in Mexico, Peru, and Nicaragua (Smith and van den Bosch 1967, Adkisson 1964, Metcalf 1980). The cotton bollworm, H. zea is an important pest of sweet corn, tomatoes, cotton and several other crops (Davidson and Lyon, 1979).

The larvae of Heliothis spp. cause crop damage and the majority of our chemical control measures is aimed at this stage, although ovicides are also important. The larvae are not highly mobile and are therefore largely restricted to the food supply selected by the adult female. In contrast, the adults are highly mobile (Hardwick 1965, Callahan et al. 1972, Schaefer 1976, Phillips 1979, Hendricks et al. 1973, Haile et al. 1975, Raulston 1979, Sparks 1979, Lingren and Wolf 1982). The physical displacement of Heliothis spp. over relatively long distances has been referred to by several investigators.

Snow and Copeland (1971) surveyed The Cooperative Economic Insect Reports in the USDA for a 19-year period, from 1951 to 1969, and observed that both in the eastern and western parts of the country, H. zea had been reported as overwintering roughly up to 45°N. Sparks (1972) released marked laboratory-reared H. zea adults near Tifton, Georgia, and captured moths at a 25-km distance after one night, and a distance of over 72km over a period of 1 to 4 days. Hendricks et al.(1973) studied the dispersal of laboratory reared TBW adults with pheromone traps in Texas, and demonstrated their movement up to 112km downwind of the prevailing wind pattern. The females of Heliothis spp. oviposit on a wide range of host plants (Brazzel et al. 1953, Hardwick 1965, Neunzig 1969, Pretorious 1976). This allows feliological survival of Heliothis spp. individuals over a broad range of conditions, and making the control of these polyphagous pests very difficult. One outstanding aspect of the biology of H. zea and H. virescens is that a female of either species is capable of producing approximately 1000 eggs (Brazzel et al. 1953). However, there is a great deal of variation in the numbers of eggs produced per female depending on the species (Brazzel et al. 1953), individual (Proshold et al. 1982), and the larval food source (Lukefahr and Martin 1964, Neunzig, 1969).

The adults of Heliothis spp. are primarily active at

night and very little is known about their nocturnal behavior. H.virescens (Lingren et al. 1982) and H. zea (Callahan, 1958) adults emerge from the pupal stage at night. H. virescens do not mate on the night of emergence, but both sexes are capable of mating the following night (Lingren et al. 1982). Following an initial mating, females generally do not mate again for 2 or 3 days (Raulston et al. 1975); however, males can mate every night. So as an adult population ages, an excess male mating potential is created. In other words, at some period during each generation cycle, males are available and searching for mates, but few females are receptive to mating (Lingren et al. 1982).

The larvae of the TBW leave the cotton fruit during the night and move to the top of the plants to molt (Lingren and Wolf 1982). After molting, they eat their cast skins and remain on the tops of the plants until their exoskeletons are cured. This exposes the larvae to direct contact from sprays applied at night.

The Tobacco Budworm

Description and life history

TBW adults are about 2.0 cm long. They have three oblique dark bands on the front wings and are usually olive-green (Werner et al. 1979). Females lay their eggs singly on the terminals of cotton plants. The eggs are pearly white and they

assume a dark gray color prior to hatching (Werner et al. 1979). The eggs begin to hatch in 2 days and the young larvae are yellowish or reddish, with large black bumps or pinnaculae on the body. Later instars develop tiny spines extending onto the first, second, and eighth abdominal segments. This characteristic distinguishes the TBW from the bollworm larvae which are spineless. An even more positive distinguishing character between these two Heliothis spp. is the presence of a tooth-like process on the inner face of the mandible of the TBW which is lacking in the bollworm (Werner et al. 1979).

Fye and Mc Ada (1972) reported that the developmental rate of egg, larva, and pupa in the TBW increases with temperature from 18 - 34°C. Larvae pass through 5 or 6 larval instars in as little as 12 days (Werner et al. 1979). Pupation takes place in the soil at an approximate depth of 5 cm (Potter 1979). The pupal period lasts for 9 days or longer. A complete life cycle requires as little as 25 days in mid-summer (Werner et al. 1979). There may be 6-8 generations in a season with the last entering the pupal stage for overwintering.

Insecticide resistance

The TBW has become one of the most serious pests of cotton in the USA (King et al. 1988). Despite attempts to

utilize non chemical control methods such as sterile male release and host plant resistance, the use of conventional insecticides remains the most efficient and cost effective means of TBW control. However, the TBW has developed resistance to most insecticides used extensively against it so far (Adkisson 1968 , Plapp 1971, Plapp 1972). The first reported case of resistance in the TBW was by Brazzel (1963),who, investigating suspected cases of DDT resistant bollworms, found only tobacco budworms, not bollworms. By 1969 (Nemec and Adkisson 1969) resistance in TBW had extended to organophosphates. Twine and Reynolds (1980) monitored the development of a 13.7-fold resistance to methyl parathion in field populations of TBW in California. Crowder et al. (1979) reported high levels of methyl parathion resistance in TBW from Arizona cotton fields.

The introduction of the pyrethroids in 1977 offered an opportunity to manage insecticide resistance because they have a broad spectrum of control and proved to be highly effective at very low dosages. Their usage increased rapidly and this has resulted in intense selection pressure on numerous pest populations to pyrethroids (Georghiou and Mellon 1983, Georghiou 1985). Georghiou (1985) reported the number of arthropod species resistant to pyrethroids to be more than 30. The pyrethroids were first used for the control of Heliothis spp. in cotton in 1978. Pyrethroid insecticides comprise up to

90% of the insecticides applied for control of Heliothis spp. on cotton in some areas (Bacheler 1985). When one considers the ability of TBW to develop resistance to most insecticides applied for their control (Sparks, 1981), the development of resistance to synthetic pyrethroids was inevitable. Increasing tolerance in TBW to pyrethroid insecticides was well documented in the early 1980's (Brown et al. 1982, Martinez - Carrillo and Reynolds 1983, Crowder et al. 1984). Miller (1984, 1986) reported extremely high LD_{50} values from the Imperial Valley of California in 1983. The first serious control problems with pyrethroids were identified in 1985 in Texas (Plapp and Campanhola, 1986). The authors observed an approximately 16-fold resistance in first instar larvae of a TBW field population. More recently, TBW resistance to the pyrethroid insecticides has been documented in the southern USA (Plapp et al. 1987, Leonard et al. 1987, Luttrell et al. 1987, Graves et al. 1988) at levels ranging from 2 - 74 fold resistance.

Resistance management

In spite of the recent increase in resistance to pyrethroids in TBW, these pesticides are still the most efficacious and economical for the control of this insect. Therefore, every effort must be made to extend the useful life

of pyrethroids by using them in a judicious manner. Strategies to circumvent resistance problems and/or delay resistance development to pyrethroids in cotton pests should be developed for each major cotton producing area. These strategies must involve all phases of cotton production, i.e. variety selection, planting date, fertilization rates, irrigation, etc. In general, practices which expedite development and maturity of the host will aid in reducing the severity of TBW problems (Clower 1987). For example, the use of early maturing varieties, synchronized planting dates, and the use of fertilizers to increase the growth rate. These cultural control tactics can help reduce the need for insecticides both directly and indirectly (Adkisson et al. 1982). For example, Adkisson et al.(1982) reported that if early sprays are avoided, bollworms and tobacco budworms are often controlled by their natural enemies.

The primary goal of insecticide resistance management is to prevent the development of resistance in insects to insecticides by reducing the number of insecticide applications during a growing season. The time to delay or prevent resistance from developing is when the frequency of the R-genes is still very low and resistance has not been detected under field conditions. This strategy is based on laboratory and field surveys using knowledge of biochemical, physiological and genetic mechanisms of action of the

pesticide being used as well as mechanisms and genetics of resistance.

CDF has been used to enhance the toxicity of pyrethroids against the tobacco budworm. Clower (1987), reported that the addition of chlordimeform to larvicides such as pyrethroids during periods of heavy oviposition appeared to increase the suppression of TBW especially in areas where resistance had developed.

The continuous employment of synergists may not be useful when controlling already resistant pests, but to prevent the increase in frequency of the R-genes in the population. Worldwide, synergists are not extensively used in pest control management for preventing the development of resistance because of the costs of using two compounds at the same time.

Chemical Insecticides

Pyrethroids Insecticides

Pyrethrum, the dried flower of Chrysanthemum cinerariaefolium, or its solvent extract has been used for centuries to kill insects (Matsui and Yamamoto 1971). The active substances of pyrethrum (rethrins) are pyrethrin I, pyrethrin II, cinerin I, cinerin II, jasmolin I, and jasmolin II.

Although possessed of excellent insecticidal properties,

the substances present in natural pyrethrum, are sensitive to light and oxygen. Therefore it was necessary to prepare synthetic analogs which would be cheaper, less photosensitive and stable for field applications. Schechter et al. (1949) synthesized the first pyrethrin analog (±)-3-allyl-2-methyl-4-oxo-cyclopent-2-en-1-yl ester of (±) - (E, Z)-chrysanthemic acid which became known as allethrin. Subsequently, much synthesis progress was made by a British team and by researchers of Sumimoto Company in Japan (Elliott et al. 1973). Though most of the first synthetic pyrethroids (e.g. resmethrin, furethrin, cyclethrin and allethrin) were more active toxicants and were simpler in synthesis than the natural pyrethrins, their photosensitivity prevented their adoption in agriculture for insect control. In 1973, Elliott et al.(1973) synthesized a new series of pyrethroids which were of major interest for agricultural insecticides. The new pyrethroids, typified by permethrin, showed greatly increased photo-stability while retaining favorable properties of high insect and low mammalian toxicity. Also these pyrethroids showed no adverse effects on soil microflora and microfauna and rapid degradation in the soil (Elliott, 1977).

Pyrethroid mode of action in insects.

Despite numerous studies of the structure-toxicity relationships, the toxic action of pyrethroids is not fully

known, but their point of attack is the nervous system. Pyrethroids act in a manner resembling DDT, which is kwown to be a peripheral neurotoxin in insects (Vinson and Kearns, 1952). In insects, pyrethroids initially have a stimulating effect on the nerve cells and nerve fibres, and this is then followed by a paralyzing effect (Miller and Adams, 1982). The most fundamental studies on pyrethroid mode of action have been those of Narahashi in 1962. Narahashi (1962, 1965, 1976), worked with giant fibre preparations of Periplaneta americana and he concluded that pyrethroids modify axonal conduction within the central nervous system of insects, altering the permeability of the nerve membrane to sodium and potassium ions. These findings were subsequently confirmed by Burt and Goodchild, (1977); Clements and May,(1977) in Periplaneta americana. Later on, Adams and Miller (1980), working with the housefly, reported that pyrethroids cause uncoupling of flight muscles motor pattern as a result of some direct action of the ganglionic pattern generator.

Structure - activity correlation studies by Briggs et al. (1976), and Lee, (1976) have shown that polarity is not important for the toxicity of pyrethroids, while receptor - substrate interactions due to molecular size and shape are important. This also correlated with bioactivity values. The activities of both DDT and pyrethroids exhibit a negative dependance on temperature, being more toxic at lower

temperature (Adams and Miller, 1980)

Pyrethroid resistance in insects can be the result of one of several mechanisms, including reduced penetration, knockdown resistance (kdr) and increased metabolism (Sawicki 1985). All of these mechanisms have been identified in larvae of the TBW (Nicholson and Miller 1985, Dowd et al. 1987, Little et al. 1988, Sparks et al. 1988). In addition to these observed resistance mechanisms, larvae of the resistant TBW have exhibited a modified behavior (Sparks et al. 1988, Sparks et al. 1990).

The effect of synergists such as piperonyl butoxide, in mixtures with pyrethroids is based on their inhibition of oxidases and esterases, thus increasing the insecticidal efficacy by preventing metabolism and the duration of action of the pyrethroids (Metcalf, 1967; Hewlett, 1960; Jao and Casida, 1974)

Formamidine Insecticides and their toxic and behavioral effects

Formamidines are used commercially to control certain insects and acarines including mites and ticks (Hollingworth 1976, Lund et al. 1979). Besides having toxic effects on adult and larval mites, formamidines have shown strong ovicidal effects against insects and mites. In addition to their acaricidal and ovicidal activities, the formamidines cause a

variety of behavioral effects in lepidopterans and mites (Hollingworth and Lund 1982, Knowles 1982). Recent studies with larvae of the TBW have shown that CDF increases larval movement on plants and reduces feeding (Treacy et al. 1987).

Dittrich (1966) discovered that CDF synergized the toxicity of dichlorvos to carmine spider mite, Tetranychus cinnabarinus (Boisd.). When CDF was applied in combination with dichlorvos, the additive toxicity was higher than either toxicant alone. Since this discovery, formamidines have been reported to synergize the toxicity to insects and acarine members of several different classes of insecticides-acaricides including the pyrethroids (Knowles 1982). Laboratory data indicate that the formamidines are toxic only to the egg stage at most dosage levels employed (Wolfenbarger et al. 1974). Several previous reports have dealt with CDF-insecticide interactions. Creighton and McFadden (1974) reported "complementary action" of an unspecified nature of CDF-Bacillus thuringiensis var. alesti (Ber.) against caterpillars on cole crops. Their experiment showed that the mixture performed more effectively than either toxicant applied separately. Neither ingredient alone was efficacious at the low rates tested, but each ingredient in the mixture complemented the other so well that the mixture was highly efficacious at very low rates. An alternative explanation for CDF action was suggested by Doane and Dunbar (1973) on two

lepidopterous forest defoliators, the gypsy moth, Porthetria dispar (L.), and the elm spanworm Ennomos subsignarius (Hubner). They reported that CDF acted as a repellent and an antifeedant rather than a toxicant in these insects. Subsequent studies have shown synergistic activity when insects and mites were exposed to binary mixtures of insecticides or acaricides with formamidines (Knowles 1982, El-Sayed and Knowles 1984a, b). Enhanced toxicity has been especially apparent when formamidines were tested in mixtures with pyrethroids (Plapp 1976, 1979, Dittrich et al. 1981, El-Guindy et al. 1981, Rajakulendran and Plapp 1982, El-Sayed and Knowles 1984a, b, Crowder et al. 1984). Earlier work (Crowder et al. 1984) has shown that the use of CDF in combination with pyrethroids may prevent the development of resistance in TBW. Crowder et al. (1984) selected for resistant TBW larvae with permethrin or permethrin:CDF mixture at the LD_{80} level for 10 generations. By the F_{12} generation, the degree of resistance to the permethrin:CDF mixture was scarcely different from the levels established in the F_1. By contrast, selection with permethrin only during 11 generations raised the LD_{50} 37-fold compared with that of the F_1.

Like CDF, amitraz appears to function, in part, as a pro-insecticide because it is activated during metabolism and probably owes much of its biological activity to one of its

primary metabolites, N'(2,4-dimethylphenyl)-N-methyl formamidine (SN-49844) (Knowles and Roulston 1973, Knowles 1982). SN-49844 has been identified as an amitraz metabolite found in insects and mites (Knowles and Gayen 1983, Franklin and Knowles 1984a, Knowles and Hamed 1989). In many bioassays SN-49844 is highly active against the twospotted mites (Chang and Knowles 1977, Knowles 1982, Franklin and Knowles 1984b).

Formamidines Mode of Action in Insects

The mode of action of formamidines is complex, as indicated by the previously mentioned dose-dependent lethal and sublethal effects (Beeman and Matsumura, 1978; Lund et al. 1979; Lund et al. 1979). In a number of model systems, including the firefly light organ (Hollingworth and Murdock, 1980) and the locust extensor - tibiae muscles (Evans and Gee, 1980), the formamidines activated octopamine receptors. Hollingworth and Murdock (1980) found that N-methylchlordimeform was an extremely potent and long lasting agonist for octopamine receptors in the firefly lantern. It stimulated octopamine - dependant adenylate cyclase (an indication of octopamine receptor activation) in the lantern (Hollingworth and Murdock, 1980) and caused the organ to glow brightly. Evans (1980) observed that formamidines mimick the modulatory effect of octopamine on neurally evoked contraction and relaxation of the locust leg muscle. He did this by

superfusing myogenic bundles of a locust leg with an isotonic saline solution and then introducing chlordimeform or N-dimethylchlordimeform into the superfactant at threshold ~ 10^{-6}M and 10^{-7}M respectively. The aminergic receptors in this tissue has previously been shown to be specific for octopamine, since they did not cross-react with dopamine, serotonin or several other related amines (O'Shea and Evans, 1979).

Hollingworth and Murdock (1980) and Evans and Gee (1980), by using these well defined systems, have shown that formamidines mimic the physiological action of octopamine. These systems (discussed above) provide clear evidence for a novel mode of action of formamidines, identifying at least one site of action as octopamine receptors. Possible explanation for the formamidines mode of action on the octopamine receptors can be drawn from the fact that the structures of formamidines, especially chlordimeform, N-dimethylchlordimeform and SN-49844, resemble that of octopamine. Therefore they can possibly fit in the octopamine receptors. Clearly, additional evidence is required to confirm and extend these ideas in lepidopteran larvae because octopamine has been studied only as a peripheral modulator of motor activity, whereas the stimulation of moth larvae by chlordimeform is mediated by synapses in the central nervous

system (Beeman, 1982).

Synergism

Synergism occurs when a mixture of two or more insecticides gives more than additive toxic or pharmacological action of the two substances. When used in reference to insecticide action, synergism has generally been regarded as applying only where one component of a mixture, the synergist, is inactive at the dosage employed and where the mixture is appreciably more active than the other component alone (Metcalf, 1967). The first discovery of insecticidal synergism was made by Eagelson in 1938. He found that the insecticidal activity of pyrethrum against mosquitoes and flies was considerably increased in the presence of sesame oil which by itself is non-toxic. Following this discovery, several synthetic analogs were developed, for example piperonyl butoxide and sesamex. More work on synergism has shown that the effect of synergism in mixtures with pyrethroids is based on their inhibition of oxidases and esterases, which speeds up the breakdown of pyrethroids in insects. This inhibition increases the insecticidal efficacy of the pyrethroids and the duration of their action (Metcalf, 1967, Hewlett 1960, Casida et al. 1974, Jao and Casida 1974).

Experimental results of Franklin (1974) indicate that during oxidative metabolism, the synergistic compounds,

piperonyl butoxide and sesamex, form an intermediate which strongly binds to cytochrome P-450 and thereby prevents further participation of the enzyme in oxidative metabolism.

Formamidines have been reported to synergize the toxicity of pyrethroids (Plapp 1976, 1979, Dittrich et al. 1981, El Guindy et al. 1981, Rajakulendran and Plapp 1982, Crowder et al. 1984, EL-Sayed and Knowles 1984a, b). Although little definitive data are available on the mechanisms associated with the synergism of pyrethroid activity by formamidines, studies by Kamel and Knowles (personal communication) suggest that events in both pharmacodynamic and pharmacokinetic phases of pyrethroid action may be affected. They observed that several formamidines synergized the activity of permethrin and cypermethrin in a susceptible and two resistant strains of the housefly, Musca domestica (L.). The resistance mechanisms in one strain were nerve insensitivity and enhanced detoxification. In biochemical studies Bodnaryk (1982) reported that synergism interaction between chlordimeform and permethrin in the moth Mamestra configurata IW.), resulted from pertubation of octopaminergic transmission by the formamidine and cholinergic transmission by the pyrethroid which might increase susceptibility of the insect to the pyrethroid. Bodnaryk (1982) also concluded that the synergistic action on mortality of a combined dose of formamidines and permethrin may result from simultaneous

disruption of cholinergic nervous system function by permethrin and of endocrine function by formamidines. cAMP mediates the action of several peptide hormones in insects, including the adipokinetic hormone (Gade, 1979) regulating water balance (Springs and Phillips 1980, Berridge 1975), and the neuroregulatory and neurohormonal actions of octopamine (Orchard and Loughton 1981, Candy 1978). Therefore, formamidines, by greatly enhancing cAMP - mediated metabolic effects such as glycogen or fat metabolism, may induce a physiological state in the insect equivalent to starvation and/or water deprivation. Starvation and water deprivation are known to render insects more susceptible to the effect of pesticides (Sun, 1960) and contribute therefore, to "conditional synergism" (Mousta, 1980). Chang and Plapp (1983) stated that the synergistic interaction between these two compounds in the tobacco budworm, Heliothis virescens (F.), resulted from the fact that chlordimeform increased the binding of permethrin to the presumed target receptors which are sodium channels of the nerve membrane. Chlordimeform has also been shown to inhibit parathion metabolism by microsomal preparations from house flies (Gagne 1980).

The classical explanation for synergism of insecticides by other xenobiotics is that the synergist exerts its effects in the pharmacokinetic phase, usually by inhibiting degradation of the insecticide (Raffa and Priester 1985). This

conventional mechanism of synergistic action is unlikely to apply to formamidines because they have been observed to synergize a variety of structurally unrelated pesticides; for example , organophosphates, carbamates, organochlorines, insect growth regulators, Bt's and pyrethroids (Dittrich, 1966; Plapp, 1976; 1979; 1981; EL-Guindy et al. 1981; Creighton and McFadden, 1974).

Lastly, the use of formamidines may affect the development of selection for resistance in the TBW to pyrethroids as reported in earlier work by Crowder et al. (1984).

Summary of Statements

The use of conventional insecticides remains the most efficient and cost effective means of pest control in most commercial crops and particularly cotton. However insecticide resistance is becoming a greater concern in cotton growing areas around the world. Currently new pesticides are not being introduced at the same rate as in the past (Andaloro 1989). The advent of the synthetic pyrethroids, which were introduced to agriculture worldwide in the late 1970's, appeared to be a long-term solution to problems posed on a number of crops by key insect pests. The indiscriminate and increased use of the pyrethroids has led to increasing tolerance in several of these key pests. Since the number of insecticides being

developed for the control of pest insects is dwindling, it is of paramount importance to extend the commercial life of efficacious larvicides such as the pyrethroids. One way by which the utility of these chemicals can be prolonged is with the use of synergists to increase toxicity. In Arizona, the recommended application rate for permethrin is 0.1-0.2 lbs.ai/A, and for CDF it was 0.25 lbs.ai/A in the field. This gives an approximate ratio of 1:1 ai/ai of permethrin:CDF, and this is the ratio we used in our study.

Formamidines, especially CDF, have been reported to synergize the toxicity of pyrethroids. Unfortunately CDF has been shown to cause cancer in laboratory rats and has been taken off the market. It is not known whether other formamidines can also produce the same effects as CDF, such as synergism and delay resistance development.

Therefore, the purpose of this study was to test for synergistic abilities of amitraz and a metabolite, SN-49844 compared with that of CDF in both susceptible (S) and resistant (R) laboratory strains of TBW, and also an Arizona field strain. Synergistic abilities of amitraz and SN-49844 were also compared using a cross-bred strain (S x R strains) of TBW. Since earlier studies by Crowder et al. (1984) indicated that selection for resistance could be delayed in susceptible populations by selecting with CDF plus permethrin, a similar study was initiated to determine the potential of

formamidine synergists in combination with permethrin to either delay or enhance resistance once resistant genes are in high frequency. This companion study investigated selection for resistance by selecting with 1:1 (wt/wt) mixtures of permethrin plus amitraz, permethrin plus SN-49844 and a comparative selection with permethrin alone in the cross-bred strain. Additionally, a study was conducted to determine the potential for cross-resistance to cypermethrin and to methyl parathion in the F_{77} generation of the permethrin selected strain of TBW.

MATERIALS AND METHODS

Tobacco budworm strains

Four strains of TBW were included in the bioassays. The susceptible strain (USDA(S)), a laboratory strain maintained in the lab for more than 220 generations with no exposure to insecticides) was obtained from the USDA Agricultural Research Service Honeybee Biology and Biological Control Laboratory in Tucson, Arizona. A field strain (FS) was collected from Laveen, Arizona in 1988 and reared for 5 - 7 generations in the laboratory, had a resistance level of 8-fold when compared to the USDA, but it was still considered susceptible in the field. A resistant strain (PSHI(R)) (selected for permethrin resistance for over 70 generations) was obtained from the University of Arizona Campus Agricultural Center, Department of Entomology. A cross-bred strain (PSDA) was developed by reciprocal crossing both sexes of the susceptible and the resistant laboratory strains. The PSDA (F_1) was then divided into four sub-strains each of which was selected using a different selecting agent(s) (Fig. 1).

Chemicals Used

The insecticides used in this study were technical grade permethrin [3-(phenoxyphenyl) methyl (t/-)-cis,trans-3-(2,2-dichloroet..,l-2,2-dimethylcyclopropane carboxylate; (Pounce

F.M.C. Corp. Agricultural Chemical Division; Middleport, NY 14105; 96.5%], cypermethrin [alpha-cyano-3-phenoxybenzyl 2,2-dimethyl-3-(2,2-dichlorovinyl) cychlopropane carboxylate; Cymbush, ICI Incorporated, Biological Research Center, Goldsboro, North Carolina, 17530; 76.5%], and methyl parathion (O,O-dimethyl-o-p-nitrophenyl phosphorothioate). The formamidines used were technical grades of chlordimeform [N'-(4-chloro-otolyl)-N,N-dimethyl formamidine, (CDF)], amitraz [N-methyl-N'-2,4-xylyl-N-(N-2,4-xylylformamimidoyl) formamidine] and SN-49844 [N-(2,4-dimethylphenyl-N-methylformamidine, a metabolite of amitraz], all obtained from NOR/AM Chemical Company, (3509 Silverside Rd., Wilmington, DE 19803) The structures of all these chemicals are depicted in Fig. 2.

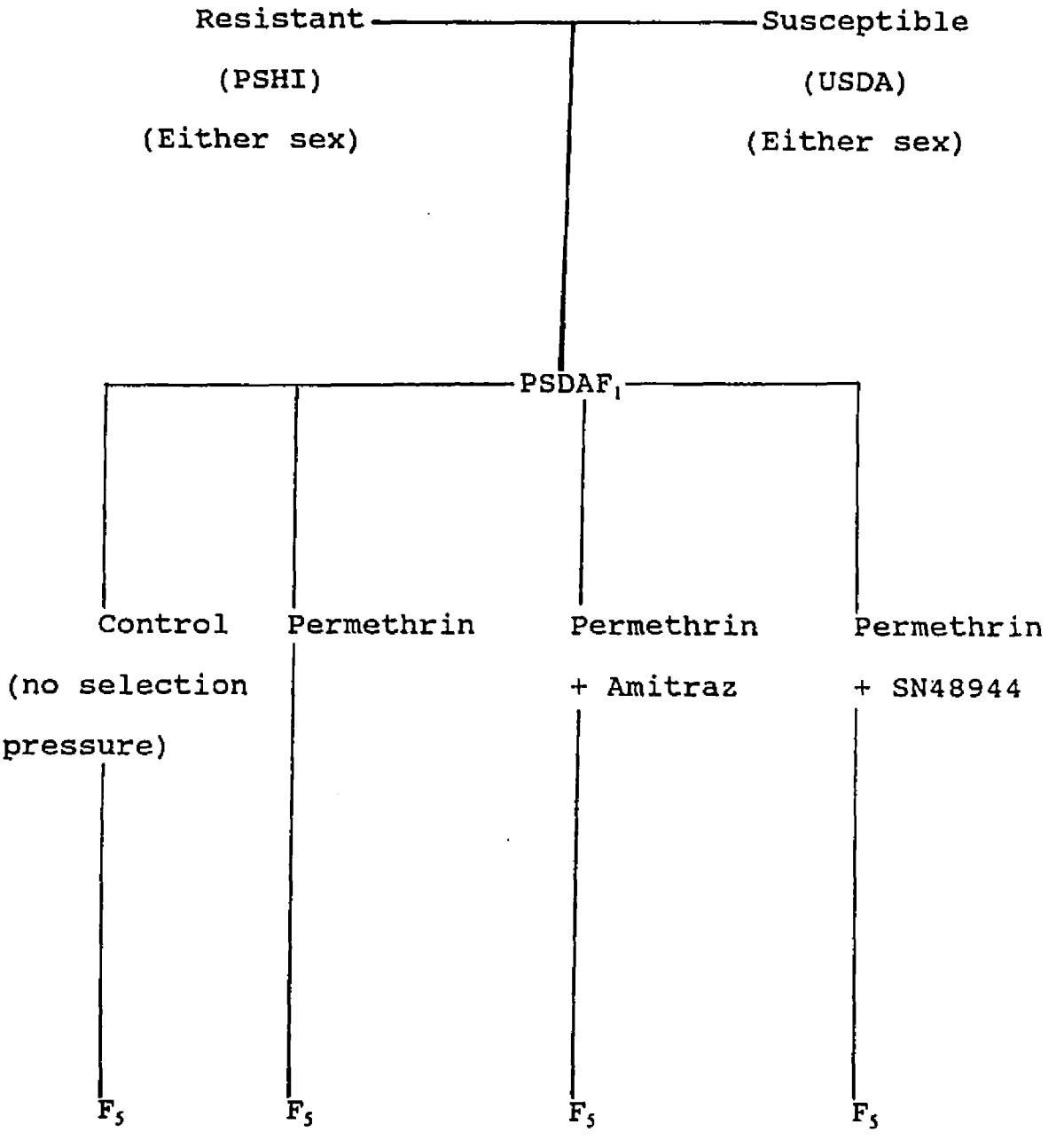
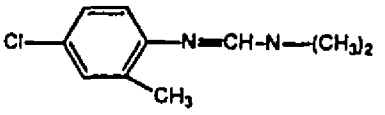
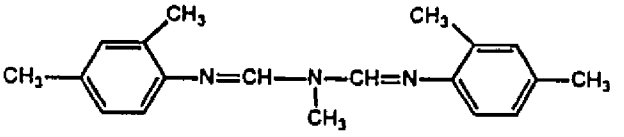


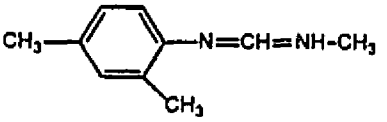
FIG 1. Schematic diagram of splits of TBW cross-bred strain (PSDA).



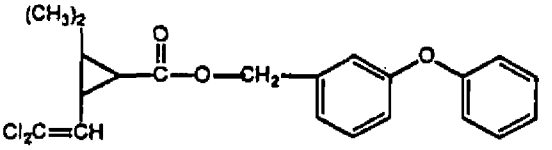
CHLORDIMEFORM



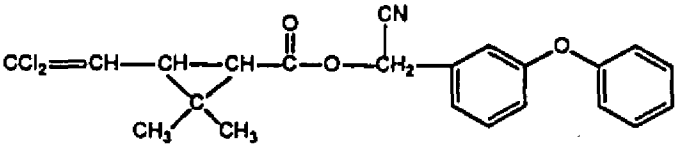
AMITRAZ



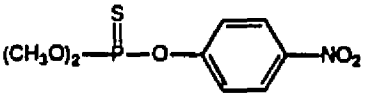
SN-49844



PERMETHRIN



CYPERMETHRIN



METHYL PAPATHION

FIG 2. Structures of chemicals used in the study.

Rearing of Heliothis virescens Strains

The rearing procedures for Heliothis virescens were as described by Jensen (1983) with certain modifications. Newly emerged moths were placed into 3.8 liter (1 gallon) wide mouth glass jars at room temperature which was maintained at 27 ± 2°C. About thirty moths were placed in each jar and fed with five percent sucrose solution contained in glass and polyethylene tubes corked at one end. The feeding tubes were inverted through a cheese cloth which covered the mouth of the jars and served as an oviposition substrate. The sugar solution was changed every other day to prevent the spread of a bacterial infection (Pseudomonous spp.). Egg sheets were also changed every other day once oviposition began. These were surface sterilized by washing with 5% sodium hypochlorite solution, rinsed with a 10% sodium thiosulphate solution and then rinsed with tap water. The egg sheets were air-dried on paper toweling and then put into 474 ml (16 oz) plastic cartons where eggs completed development and hatched.

Newly hatched larvae were transferred with camel's hair brush to 29.6 ml (1.0 oz) plastic creamer cups (2-3 larvae/cup), which were half filled with modified lima bean diet (Patana 1969). Cups with larvae were then stored in a 30°C temperature cabinet for three-five days. The cups with larvae were then transferred to a rearing room kept at 27 ±

2°C to pupate. The pupae were then collected from the diet cups and placed into 236.8 ml (8 oz) waxed cups until adult eclosion.

Bioassays

The test procedure used was similar to that recommended by the Entomological Society of America (Brazzel 1970). Technical grade materials were dissolved in acetone and one microliter (ul) of solution was applied to the dorsal surface of 22 ± 5 mg third-instar larvae using a motor driven micro-applicator. The average weight of larvae was determined from a sample of about 15% of the larvae to be treated.

Insecticide(s) + Synergists

Insecticides plus synergists were tested at a 1:1 (wt/wt) ratio. Four to five different concentrations were used for each insecticide or insecticide + formamidine combination in addition to the controls. Control larvae, to compare with the insecticide alone treatments, were treated with only acetone. For the permethrin plus formamidine treatments, control larvae were treated with the highest test concentration of formamidine used in the 1:1 ratio.

The treated larvae were maintained at 27 ± 2°C and mortality was recorded at 72 hours after treatment. Larvae

were classed as dead if they failed to respond to probing with a blunt probe. Moribund larvae were counted as dead after 72 hours. This data was used to compute the probit analysis (Finney 1971).

LD_{50} values were reported in micrograms (ug) of insecticides per gram (g) of larvae body weight to compensate for variation in the size of the larvae used.

Analysis of Data

Dosage-mortality studies

Results from the dosage-mortality experiments were used in the probit analysis (Finney 1971) which yielded an LD_{50} value, slope and confidence intervals. The 95% confidence intervals ($\pm$95% CI) were used for determining significant difference among the LD_{50} values. When LD_{50} $\pm$95% CI overlap there is no significant difference. The slopes were compared using the standard error of each slope value (b) calculated in the program. Additionally, slopes were compared by standard t-tests of differences in values using a pooled variance formula (Zar, 1983).

Control mortality was corrected for in the probit analysis with Abbott's (1925) formula.

$$\text{Corrected mortality \%} = \frac{\text{Test mortality} - \text{Control mortality \%} \times 100}{100 - \text{Control mortality}}$$

Resistance

The resistance levels were calculated by dividing the LD_{50} of the resistant strain by the LD_{50} of the susceptible strain that were treated with the same insecticide.

$$\text{Resistance Factor (RF)} = \frac{LD_{50} \text{ of resistant strain}}{LD_{50} \text{ of scusceptible strain}}$$

Synergism

The synergism ratios (SR) were calculated by dividing the LD_{50} of the insecticide by the LD_{50} of the insecticide with synergist.

$$\text{Synergism Ratio (SR)} = \frac{LD_{50} \text{ of insecticide}}{LD_{50} \text{ of insecticide + Synergist}}$$

RESULTS

Dosage-mortality of Permethrin on Tobacco Budworm

The dosage-mortality data of permethrin on the four strains are shown in Table 1. Mortality of the controls in all tests ranged from 1-3%. The regression lines for the susceptible (USDA), the resistant (PSHI) and the field strain (FS) are shown in Fig. 3. There is no significant difference between the LD_{50} value ($\pm$95% CI) of the FS and that of the cross-bred strain (PSDA). However, the slopes are significantly different , FS = 2.2 and PSDA F_1 = 1.1. This indicates that the FS is a more homogenous strain than the PSDA F_1 in terms of their response to treatment with permethrin.

The hybrid of the crosses between the S X R strains gave an LD_{50} of 5.79 ug/g, which is significantly different from the LD_{50} values of both the susceptible (0.67 ug/g) and the resistant (3933 ug/g) parents. The slope of the F_1 of the cross is not significantly different from that of the susceptible parent, but the resistant parent has a much steeper slope which indicates a more homogenous population as compared to the F_1 (Fig.4). These results might be showing contamination of the USDA(S) strain by resistant insects since the strain now shows higher LD_{50} values than previous studies

Table 1. Dosage-Mortality Data of Permethrin on Different Strains of TBW.

Strain[1]	n[2]	LD_{50}[3] (95% CI)	LD_{95} (95% CI)	Slope (SE)[4]	RF[5]
USDA (S)	875	0.67 (0.36-1.06)	23 (9.9-120)	1.1 (0.08)	1.0
FS	600	5.42 (4.76-6.18)	30 (21-40.5)	2.2 (0.17)	8.1
PSHI (R)	750	3933 (3384-4571)	27000 (21000-35000)	2.0 (0.15)	5900
PSDA F_1	710	5.79 (4.63-7.25)	158 (94-266)	1.1 (0.11)	8.6

1 USDA(S) = susceptible lab strain, FS = field strain, PSHI(R) = permethrin resistant strain, PSDA = cross-bred strain between USDA (S) and PSHI (R).

[2] n = Number of larvae tested excluding controls.

[3] LD_{50} and LD_{95} are expressed in ug of toxicant/g of larvae

[4] SE = Standard error of the slope.

[5] RF = Resistance Factor =(LD_{50} of Strain)/(LD_{50} of USDA(S))

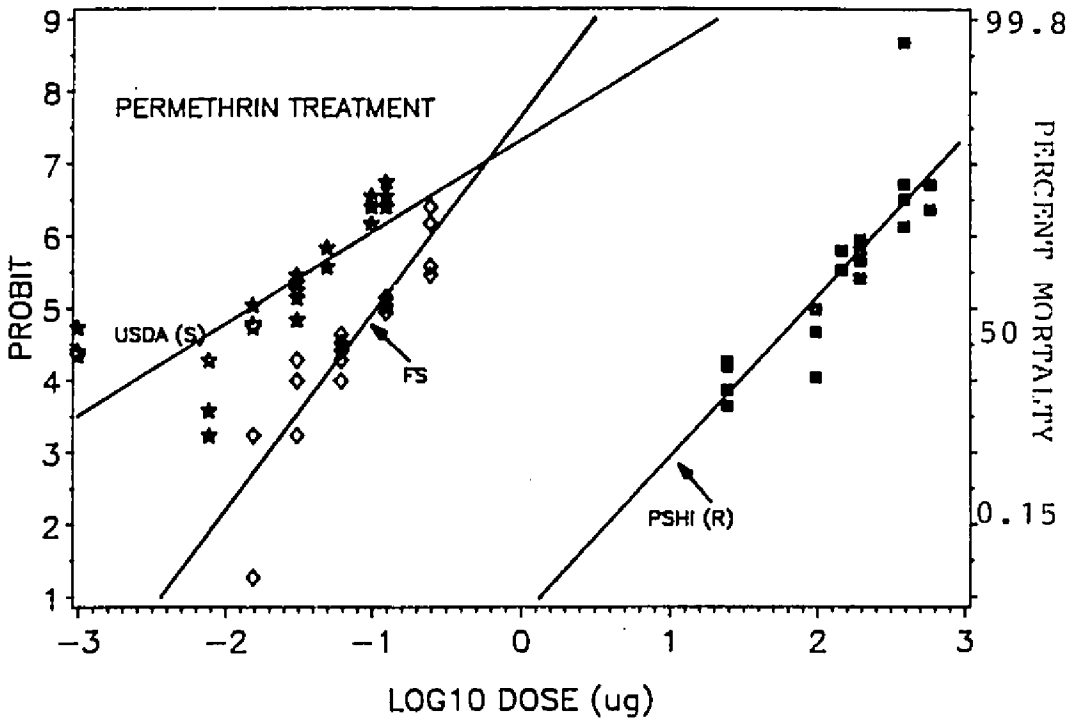


FIG 3. Dose-regression lines for susceptible (USDA(S)), the resistant (PSHI(R)) and the field(FS) third instar larvae of tobacco budworm.

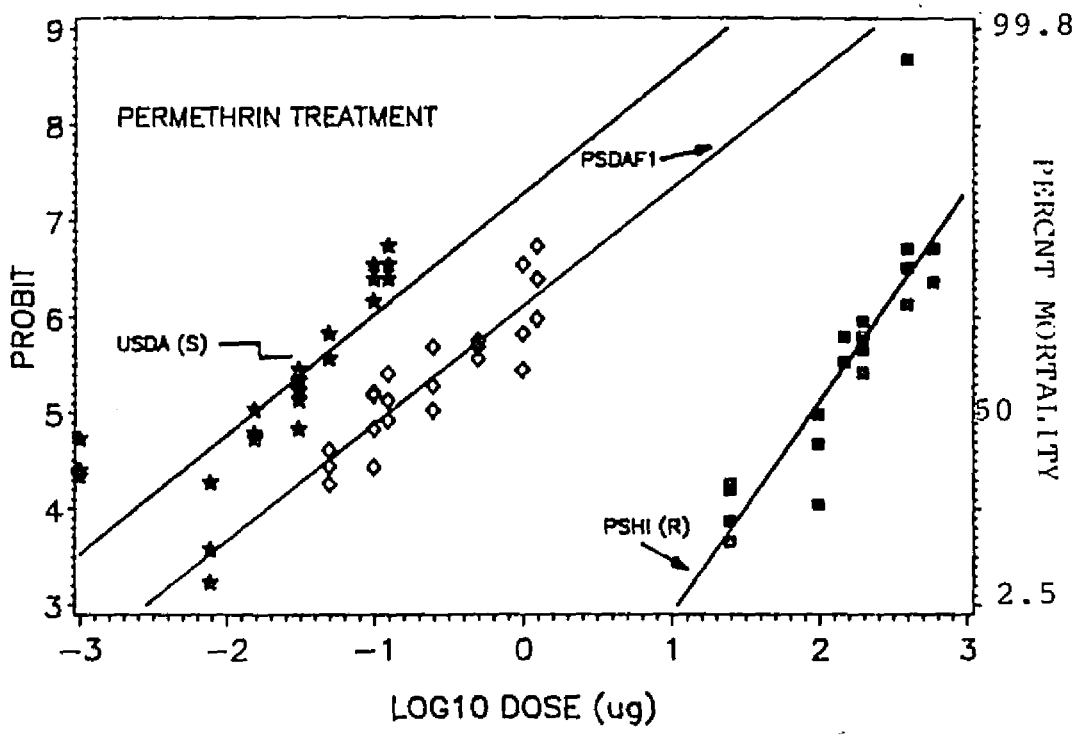


FIG 4. Dose-mortality regression lines for the susceptible (USDA(S)), resistant(PSHI(R)) and the F1 (PSDA) of their crossing.

on the same strain. THe USDA(S) also showed a significantly lower slope value (1.1) as compared to that of the PSHI(R) (2.0). This is another indication that the USDA(S) population used in the study was less homogeneous for the susceptible trait.

The PSHI showed very high levels of resistance to permethrin with a resistance factor of 5900-fold as compared with the susceptible USDA strain (Table 1).

Cross-Resistance to Cypermethrin and Methyl Parathion

The extent of cross-resistance to cypermethrin and methyl parathion in the permethrin-selected strain of TBW is given in Table 2. This strain has previously undergone seventy-six continuous generations of selection pressure with permethrin. The degree of tolerance of this strain to cypermethrin was compared with data on a 1980 field population of TBW, collected from Maricopa, AZ. (Watson and Kelly, 1990). Similarly, the degree of tolerance to methyl parathion was compared with a 1977 laboratory strain (Watson et al. 1986). The permethrin selected strain showed 58-fold and 48-fold resistance levels to cypermethrin and methyl parathion, respectively.

Table 2. Relative Cross-Resistance to Cypermethrin and Methyl Parathion in Permethrin-Selected (PSHI(R)) Larvae of the Tobacco Budworm.

Insecticide	Susceptible		PSHI (R)[1]		RF[2]
	LD_{50}[3] (95% CI)	Slope	LD_{50} (95% CI)	Slope (SE)[4]	
Permethrin	0.67 (0.40-1.10)	1.1	4681 (3625-5774)	1.9 (0.17)	7000
Cypermethrin	8.0[5] (7.73-9.49)	3.1	463 (351-613)	1.1 (0.11)	58
Methyl-Parathion	11.5 (9.4-11.7)[6]	2.5	555 (447-689)	1.2 (0.11)	48

[1] PSHI = Permethrin high resistant strain

[2] RF = Resistance Factor = (LD_{50} of Resistant Strain)/ (LD_{50} of susceptible strain)

[3] LD_{50} = ug toxicant/g of larva

[4] SE = Standard error of the slope.

[5] Crowder (1981 Field strain; unpublished data)

[6] Data from Watson et al. (1986)

Synergism of Permethrin by Formamidines

The synergistic levels of the three formamidines on permethrin against different strains of TBW are shown in Table 3. The 1:1 (wt/wt) mixtures of permethrin plus formamidines showed increased toxicity in the permethrin resistant strain of the TBW (Fig.5). Compared with CDF and SN-49844, amitraz showed a lower degree of synergistic ability. There was no synergism obtained when permethrin plus any formamidine were used against the laboratory susceptible strain. Synergism values for the field and the cross-bred strains ranged from 1.1 - 1.7 and <1 - 1.8 respectively with all the formamidines.

Selection for Resistance by Formamidines

Selection of the cross-bred TBW larvae with permethrin ± formamidine mixtures at LD_{80} for four generations produced strong tolerance to all mixtures used. After four generations of continuous pressure, the LD_{50} values of the strains at the F_5 had increased by 100-fold with permethrin alone, 122-fold with permethrin plus amitraz and 180-fold with permethrin plus SN- 49844 compared to the LD_{50} value of the F_1 generation (Table 4 and Fig 6). The control population of the cross-bred strain, which was not exposed to insecticides showed a significant decrease in the LD_{50} value of the F_5 as compared to the F_1. In addition, the slope of the the F_5 (2.4) was much

higher than that of the F_1 (1.1) which is an indication of homogeneity of the F_5 population. The permethrin plus formamidine combinations showed a significant increase in LD_{50} values as well as an increase in the slope.

Table 3. Synergism Ratios (SR) for Permethrin:Formamidine Mixtures Against Different Strains of Tobacco Budworm.

Treatment	USDA (S)			Field strain			PSHI (R)			PSDA		
	LD_{50}[1]	Slope	SR	LD_{50}	Slope	SR[2]	LD_{50}	Slope	SR	LD_{50}	Slope	SR
Permethrin	0.67	1.1	1.0	5.42	2.2	1.0	3933	2.0	1.0	5.79	1.1	1.0
Permethrin +CDF	1.51	2.6	0.44	3.20	1.5	1.7	415	1.8	10	-	-	-
Permethrin + Amitraz	1.62	2.2	0.41	4.77	2.5	1.1	2870	1.4	1.4	9.64	1.1	0.6
Permethrin + SN49844	1.50	2.4	0.45	4.79	2.0	1.1	337	1.8	12	3.21	1.4	1.8

[1] LD_{50} = in ug of toxicant/g of larvae

[2] SR = Synergism Ratio = LD_{50} of Permethrin/(LD_{50} of Permethrin + Formamidine)

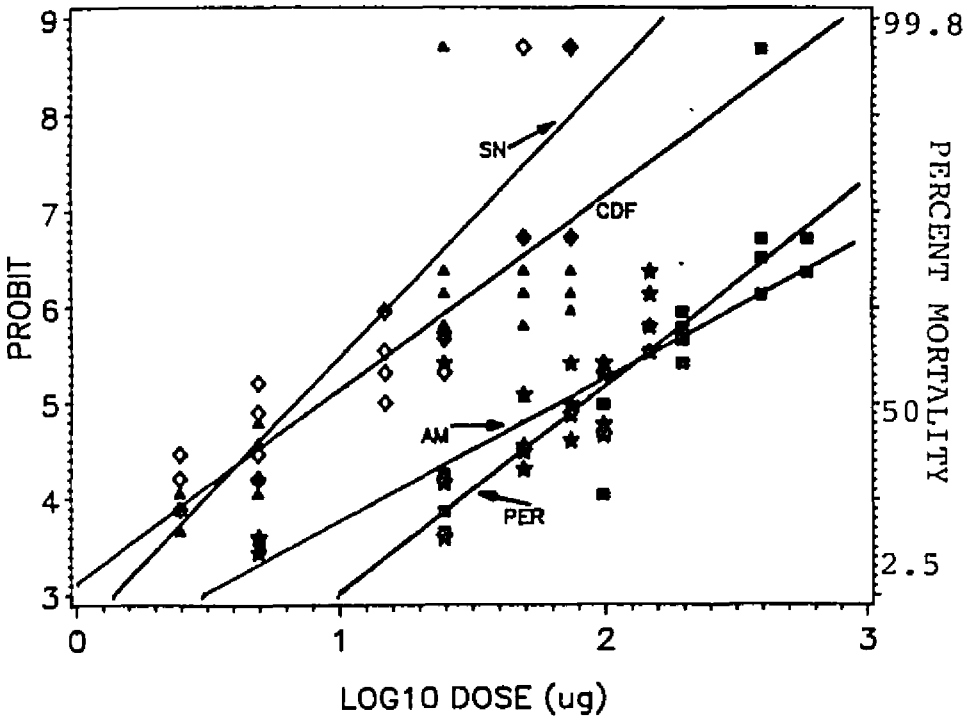


FIG 5. Dose-mortality lines for permethrin (PER) and permethrin plus synergists (AM= amitraz, SN=SN-49844, andCDF=chlordimeform) on permethrin resistant third instar larvae.

Table 4. Dosage-Mortality of Permethrin on the Cross-bred (PSDA) Strain of Tobacco Budworm Subjected to LD_{80} Pressure from Generation 1 - 4 Using Different Selecting Agents.

	SELECTING AGENTS											
F(x)[1]	Control			Permethrin			Permethrin +Amitraz			Permethrin + SN 49844		
	LD_{50}[2]	slope	RF[3]	LD_{50}	slope	RF	LD_{50}	slope	RF	LD_{50}	slope	RF
1	5.79	1.1	1.0	5.79	1.1	1[4]	5.79	1.1	1[4]	5.79	1.1	1[4]
3	2.82	1.3	0.5	25.6	1.1	4.4	34.9	1.4	6	19.6	1.0	3.4
5	2.57	2.4	0.4	632	0.9	109	717	1.4	124	1097	1.4	190

[1] F(x) = Generation where dose mortality regressions were completed

[2] LD_{50} is expressed in ug of toxicant/g of larvae

[3] RF = Resistance Factor = (LD_{50} of Fx)/(LD_{50} of PSDA F_1))

[4] Results of permethrin on PSDA F_1 larvae

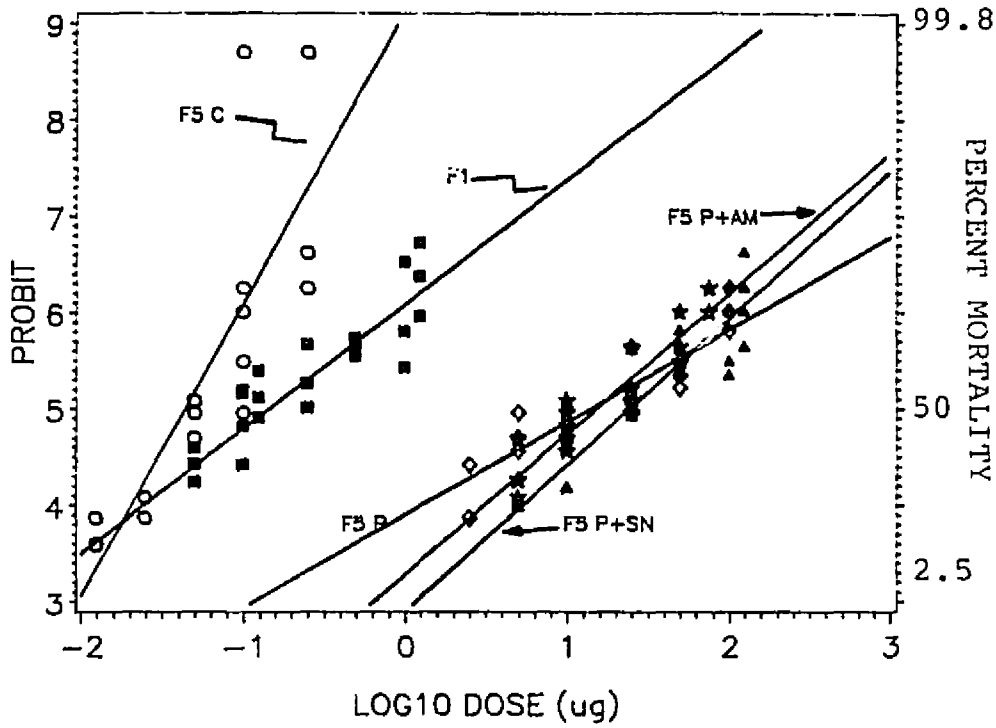


FIG 6. Dosage-mortality regression lines of permethrin on the cross-bred strain (PSDA) subjected to LD80 pressure for four generations using different selecting agents.

DISCUSSION

Dosage-Mortality of Permethrin on TBW

The dosage-mortality data of permethrin on the PSHI (R) show that an Arizona field collected TBW strain could be selected for very high permethrin-resistance in the laboratory. This strain has a resistance factor of 5900 - fold compared with the susceptible laboratory strain. The strain collected from the field in 1980 (Crowder et al. 1984) and then continually subjected to permethrin insecticide and selected for resistant individuals now at F_{71} showing a resistance factor of 800-fold as compared to the LD_{50} value of the F_1 (4.8 ug/g) (Jensen et al. 1984). The field strain of 1988 has shown an LD_{50} value of 5.42 ug/g when compared with the USDA, and a slope of 2.2. This slope indicates a homogeneously susceptible population. When compared to the field strain of 1980, which had an LD_{50} of 4.8 ug/g and a slope value of 2.1 (Jensen et al. 1984), there is no significant difference between the LD_{50} and the slope values of the two strains. This is an indication that the TBW field populations in Arizona have not increased their resistance to pyrethroids.

Although no failures to control field populations with pyrethroids have occurred in Arizona, failures have already been documented in some areas in the US where pyrethroids have been extensively used. Plapp and Campanhola (1986) diagnosed

permethrin resistance of 16-fold in populations of TBW from west Texas where control failures had occurred. Allen et al. (1987) reported more field failures in Texas during the 1985/86 season. Roush and Luttrell (1987) reported control failures at several locations in Mississippi during the 1986 season. Laboratory assays showed 5 to 23-fold resistance levels to pyrethroids in larvae from three of eight areas. The other five strains were brought from areas where resistance has not been reported and did not show resistance to pyrethroids. Leonard et al. (1987) tested field strains of tobacco budworms collected in Louisiana, Texas, Arizona, and Mississippi during 1985 and 1986 and these insects exhibited moderate to high levels of resistance to fenvalerate (2 to 35-fold), permethrin (1 to 74-fold) and cypermethrin (2 to 9-fold). Except for these cases, the performance of synthetic pyrethroids on many cotton insect pests has been outstanding since their introduction into commercial pest management in 1977. Therefore, every effort must be made to extend their useful life by using them in a judicious manner. Further results of this study also indicate that synergists could be used to enhance mortality results only in slightly resistant populations. A caveat is necessary in that when resistant phenotypes appear, selection for resistance may be enhanced when using synergists, rather than delayed as shown by Crowder et al. (1984).

Cross - resistance to cypermethrin and methyl parathion

Cross-resistance to cypermethrin and to methyl parathion in the permethrin selected strain of TBW was found to be high. In the F_{77} of this strain, LD_{50} values were 58-fold for cypermethrin, and 48-fold for methyl parathion as compared to a field collected susceptible strain of 1980 and a laboratory susceptible strain, respectively. Crowder et al. (1984) working with the F_{12} of the same permethrin resistant-selected strain (37-fold), showed a 7.9-fold cross resistance to cypermethrin compared with a field strain.

Earlier studies by Crowder et al. (1979) reported low level cross-resistance to pyrethroids in TBW populations resistant to methyl parathion in Arizona. Similar results were obtained by Twine and Reynolds (1980) and Brown et al. (1982) in California and South Carolina, respectively. Priester and Georghiou (1980) have demonstrated in their studies with mosquitoes that selection for resistance with one pyrethroid confers at least partial resistance to many other pyrethroids. Our results confirm these earlier observations in that the permethrin selected strain demonstrated resistance to another pyrethroid and to an organophosphate. Additionally, increasing levels of resistance to permethrin appears to accompany increasing resistance to cypermethrin although at a lower rate.

Inheritance of permethrin resistance in the TBW

The dosage-mortality regressions for the cross-bred strain show a large decrease in the LD_{50} value. The F_1 regression line lies closer to the susceptible than the resistant parent (Fig. 3). This indicates that the resistance gene(s) of the permethrin selected strain are incompletely recessive. Studies by Watson and Kelly (1990) involving crosses of the same strains from F^{37} to F^{44} have indicated a similar decrease in the LD_{50} value. Their results from the F_2 generation and from the back-crosses with both the resistant and the susceptible parents suggest that more than one gene might be involved, probably one major gene and one or more minor genes.

Only a few previous studies have analyzed the genetics of resistance factors in Lepidoptera. Liu et al. (1981), demonstrated that fenvalerate resistance in diamond back moth, Plutella cylostella was partially recessive and conferred by more than one autosomal gene. Past studies with TBW have addressed the inheritance patterns of resistance to methyl parathion (Whitten 1978) and methomyl (Roush and Wolfenbarger 1985). In both cases the authors concluded that resistance was conferred by a single autosomal gene of incomplete dominance. Payne et al. (1988) working with TBW demonstrated that permethrin resistance in the TBW was inherited as an

incompletely recessive, autosomal gene that segregates as a single major factor. The authors made various reciprocal crosses between the S X R, and they concluded that susceptibility in the TBW was autosomal and incompletely dominant. Hybrid populations exhibited permethrin resistance levels from 5-to 28-fold compared with the F_1,s of the crosses. The authors backcrossed the F_1 to a resistant parental strain and the results indicated that a single gene was responsible for resistance. In our study, the hybrid of the crosses between the S X R gave a dosage mortality line closer to the susceptible parent. In the absence of permethrin selection for four generations, permethrin resistance in the hybrid had reverted 2.3-fold as compared with the F_1. These observations suggest that resistance to permethrin was not homozygous in the parental strain and that resistance is incompletely recessive.

Synergism of Permethrin by Formamidines

The results indicate no synergism activity of permethrin by formamidine on the USDA(S) susceptible lab strain. However, some degree of synergism was observed on FS, PSDA, and the PSHI strain. Plapp (1979) reported in an earlier study a 1.8-fold synergism with a 1:1 mixture of permethrin plus CDF against a laboratory susceptible strain of TBW using the vial

test. His lab strain had an LC_{50} of 0.29ug/ml. This synergism might have been attributed by the behavioral effect of CDF. Increased movements of larvae within the vial allowed more contact with the insecticide and thus increased mortality, which is therefore a "behavioral synergism".

Our studies also indicate that SN-49844 is a more promising synergist than amitraz and is equally effective as CDF against the resistant strain of TBW. Amitraz poorly synergized permethrin compared with its metabolite and CDF. Previous studies have shown that mixtures of pyrethroids and formamidines (CDF, amitraz) enhance toxicity to arthropods (Heliothis virescens, Spodoptera littoralis, Memostra configurata, Chrysopa carnea,and Tetranychus spp.) (Plapp 1976, 1979, Dittrich et al. 1981, EL-Guindy et al. 1981, Bodnaryk 1982, Rajakulendran and Plapp 1982, EL-Sayed and Knowles 1984a,b).

The physiological mechanism by which formamidines synergize other insecticides has not yet been determined. EL-Badry et al. (1987) suggested that the mechanism associated with the synergism of pyrethroid activity by formamidines affects events in both pharmacodynamic and pharmacokinetic phases of pyrethroid actions. For example, EL-Badry and Knowles (personal communication) observed that several formamidines synergized activity to a susceptible and to two

resistant strains of the housefly. The resistance mechanism in one strain was nerve insensitivity (kdr) and the other was mixed as both nerve insensitivity and enhanced detoxification. Results of a biochemical study indicated that the synergistic interaction between CDF and permethrin on the moth Mamestra configurata (Bodnaryk 1982) resulted from perturbation of octopaminergic transmission by the formamidine and cholinergic transmission by the pyrethroid. Chang and Plapp (1983) stated that the synergistic interaction of these two compounds in the TBW resulted from the fact that CDF increased the binding of permethrin to the presumed target receptors (sodium channels of the nerve membrane) involved in nerve insensitivity. Gagne (1980) working with CDF in combination with carbamates and organophosphates found that CDF interfered with insecticide detoxification by TBW. The classical explanation of synergism of insecticides by other xenobiotics (e.g. piperonyl butoxide, PBO) remains that the synergist exerts its effect in the pharmacokinetic phase, usually by inhibiting degradation of the insecticide (Raffa and Priester 1985). More research work needs to be done to comprehensively elucidate the physiological mechanism by which formamidines synergize the activity of other insecticides. This can be done by conducting more biochemical studies on the neurotoxicology of the pyrethroids as well as the formamidines.

Selection for resistance by formamidines

This investigation demonstrated that selection of permethrin resistance in the TBW might be enhanced (rather than repressed) by the addition of formamidines to the selecting agent when the resistant allele is present in high frequency. The F_1 of the cross between either sexes of PSHI (R) and USDA (S) had a resistance of 9-fold when compared with the susceptible parent. This strain, when subjected to continuous selection (starting at F_1) with permethrin plus formamidines at LD_{80} for four generations, became resistant to permethrin at 122 and 180-fold for permethrin plus amitraz and permethrin plus SN-49844, respectively compared with LD_{50} of the cross. Selection at the LD_{80} level with permethrin alone caused a 100-fold increase in resistance. Comparatively, a strain from the cross, that was not exposed to insecticides for four generations, showed only a 2.3-fold decrease in resistance in the F_5 when compared with the F1. These results indicate that the alleles responsible for resistance were present in relatively high frequencies in the cross-bred strain. In the original selection studies described earlier in this manuscript, Crowder et al.(1984) observed rapid development of resistance after the ninth generation when selecting with permethrin alone on a susceptible population of the TBW. But the permethrin plus CDF inhibited development of

resistsance when genes for resistance were at low frequency. After eleven generations of selection with permethrin + CDF at the LD_{80}, LD_{50} of the F_{12} was 3.5 ug as compared with 4.9 ug/g in the F_1. The source of the population used in this study was from insecticide-treated fields, there was no indication of resistance in the field yet. The R alleles were probably at an extremely low frequency after rearing them under laboratory conditions in the absence of selection for 5 - 7 generations. Their results suggest that synergists plus permethrin may be effective tools for resistance management. The use of this as a tool has a caveat based on the results of the current study, however.

Our results suggest that when signs of resistance are observed in a population, resistance levels may increase rapidly when under strong selection pressure, especially in the presence of formamidine synergists. The best thing to do may be to switch to a different insecticide chemistry and refrain from using synergists.

Significance of Results

The performance of synthetic pyrethroids on many cotton insect pests has been outstanding since their introduction into commercial pest management in 1977. However, failures to control field populations of TBW with pyrethroids has been

reported in some areas and suggests that widespread and frequent use of pyrethroids may lead to resistant populations. This investigation demonstrated that field populations can develop extremely high resistance to pyrethroids under constant exposure. A field-collected strain subjected to high selection pressure with permethrin for 70 generations in the laboratory showed a 5900-fold increase in tolerance when compared with the susceptible laboratory strain. Cross-resistance to cypermethrin and to methyl parathion was also found in this strain. From this, it is probable that field selection with any one synthetic pyrethroid could ultimately lead to some level of resistance to all compounds in this group as well as resistance to organophosphorous compounds. This investigation demonstrated that increasing levels of resistance to permethrin appears to accompany increasing resistance to cypermethrin as well as methyl parathion. Therefore, a possible management approach is to use another chemistry and avoid the use of organophosphates when pyrethroids have failed. Pyrethroids and organophosphates have some common elements in insect resistance. These include mechanisms such as: hydrolases and MFO (which would oxidize and degrade both toxicant classes), and reduced penetration by thicker cuticle which should offer resistance to many toxins. Therefore selection directed for pyrethroid resistance will definitely select for alleles that will be resistant in some

ways to organophosphates. This explains why there is some degree of cross-resistance between permethrin and methyl parathion.

Since the factor for pyrethroid resistance in tobacco budworm appears to be a recessive character, the resistance will tend to decrease when resistant individuals breed with susceptible budworms. Therefore every effort should be made to expose the smallest possible portion of the total tobacco budworm population to pyrethroids throughout the year.

In Arizona there are no field failures at this time possibly due to the factor discussed above. The TBW is a late season pest of cotton in Arizona. It appears in large numbers sometimes in late summer and fall. These populations are therefore, subjected to insecticidal pressure for a relatively short period of time. In addition, Rathman and Watson (1985) indicated that TBW populations in Arizona develop initially on a number of wild hosts which are never treated with pesticides, thereby providing susceptible popultions that mate with those subjected to insecticidal pressure.

Formamidines were shown to synergize permethrin in resistant larvae of TBW. This study demonstrated that SN-49844, a metabolite of amitraz is equally effective as CDF. Such effective synergists can be used in management strategies in the TBW and can help to extend the useful life of synthetic pyrethroids when resistant alleles are at low frequency.

However, selection of pyrethroid resistance in TBW might be enhanced by the use of these synergists when the resistant allele is present in a population at high frequency. This study suggests that when signs of resistance to pyrethroids are observed in a population, the best thing to do is to refrain from using synergists such as formamidines. Extension agents must use testing techniques such as the "vial testing technique" or other biochemical or immunological approaches to monitor susceptibility differences of TBW populations in their areas.

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