RESISTANCE TO PYRETHROID INSECTICIDES IN THE TOBACCO BUDWORM

CLAYTON CAMPANHOLA

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RESISTANCE TO PYRETHROID INSECTICIDES IN THE TOBACCO BUDWORM (LEPIDOPTERA: NOCTUIDAE)

A Dissertation

by

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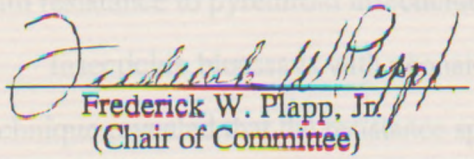

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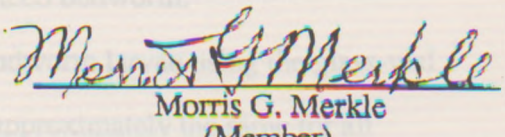

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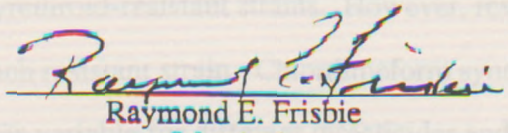

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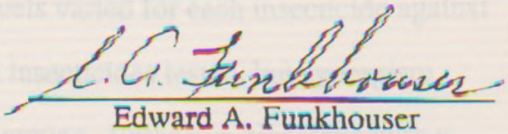

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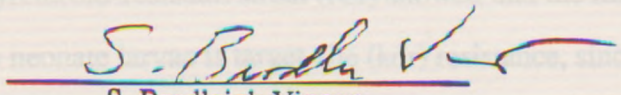

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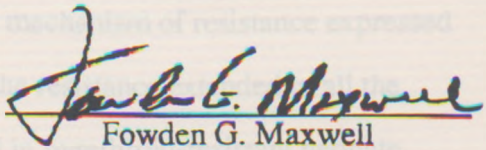

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ABSTRACT

Resistance to Pyrethroid Insecticides in the Tobacco Budworm (Lepidoptera: Noctuidae). (December 1988)

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Chair of Advisory Committee: Dr. Frederick William Plapp, Jr.

The objectives of the present study were to evaluate the resistance spectra of different pyrethroid-resistant tobacco-budworm strains, to determine alternate insecticides for controlling these insects, to evaluate synergism of insecticides by chlordimeform, and to identify the mechanisms of resistance and possible biological deficiencies associated with resistance to pyrethroid insecticides in the tobacco budworm.

Insecticide bioassays with neonate tobacco-budworm larvae using the glass-vial technique revealed that the resistance spectra were approximately the same for all pyrethroid-resistant strains. However, resistance levels varied for each insecticide against each resistant strain. Chlordimeform synergized all insecticides tested, but synergism was variable for different insecticides and different strains. Extensive bioassays with a pyrethroid-resistant strain (ICI) showed that the main mechanism of resistance expressed in neonate larvae is target-site (kdr) resistance, since the resistance extended to all the pyrethroids tested. No cross resistance was observed in pyrethroid-resistant neonate larvae to the organophosphates monocrotophos, methyl parathion, profenofos, sulprofos, and acephate, the oxime carbamates methomyl and thiodicarb (only one resistant strain, Uvalde, was probably resistant to this insecticide), and the cyclodiene endosulfan.

Bioassays with Stoneville (susceptible) and ICI (resistant) third instars showed resistance to methyl parathion, but no resistance to another phosphorothionate

chlorpyrifos, the S-alkyl phosphorothiolates profenofos, sulprofos, and acephate, the carbamates methomyl and thiodicarb, and endosulfan. Chordimeform synergized most insecticides against both tobacco budworm strains. The high level of resistance to cypermethrin at this stage, the resistance to methyl parathion, and the high level of cypermethrin synergism by piperonyl butoxide are evidence for the presence of metabolic resistance in the ICI strain in addition to target site resistance. Based on the synergism data, increased mixed-function oxidase activity seems to be the main factor responsible for resistance in third instars.

The resistance spectra were similar for adults and neonate larvae. Cypermethrin resistance was present in both adult sexes and there were no significant differences between sexes. Results were similar to those obtained with neonate larvae, indicating either life stage can be tested to determine the presence of pyrethroid resistance in the tobacco budworm. Resistance to methyl parathion and thiodicarb (only in the Hearne strain) was also present in adult males but no resistance to acephate was found.

Biological differences were found between the Stoneville and ICI strains. Differences include longer developmental period, reduced egg production, and lower number of females producing offspring in the resistant (ICI) strain. Based on these differences and on the results of bioassays, a general approach for managing pyrethroid resistance in the tobacco budworm using insecticides is proposed.

This dissertation is dedicated to my wife, Silvia

and to our children, Andréa and Felipe

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CHAPTER I

INTRODUCTION

The discovery of chemical insecticides promoted a great interest in the control of pest insects. Insecticides have been used extensively in the recent years because they are among the cheapest and most efficient approaches to control agronomic pests. Therefore, the use of chemicals, in spite of all their adverse effects, has played and probably will continue to play an important role in pest control for many years.

Insects have evolved defensive mechanisms to protect themselves against insecticides. It appears that the gene pool of most pest species already contains genes that enable the pests to degrade enzymatically or otherwise circumvent the toxic effect of many types of chemicals (Georghiou 1986). Thus, the development of resistance to insecticides is dependent on genetic variability already present in a population, or arising during the period of selection.

Resistance to pesticides by insects and other arthropod pests has become a serious problem for agriculture and public health. Resistance to one or more insecticides has been reported in at least 447 species of insects and mites (Georghiou 1986). Of those species, 59% are of agricultural importance, 38% are of medical or veterinary importance, and 3% are beneficial parasites or predators. Resistance is most frequently seen in the Diptera (156 spp.), reflecting the strong chemical selection pressure that has been applied against disease-vector mosquitoes throughout the world. Arthropod orders of agricultural importance that have developed resistance to insecticides include Lepidoptera (67 spp.), Coleoptera (66 spp.), Acarina (58 spp.), Homoptera (46 spp.), and Heteroptera (20 spp.).

This dissertation follows the format and style of the *Journal of Economic Entomology*.

Georghiou (1986) has also gathered data regarding the chemical groups to which resistance has been observed. Cyclodiene insecticide resistance is found in 62% of the reported species and DDT resistance in 52%. Organophosphate resistance is reported in 47% of the resistant species.

Low percentages are reported for the more recently introduced pyrethroid insecticides. At least 23 species are known to be resistant to pyrethroids. Most of those species are very important pests such as the Colorado potato beetle (*Leptinotarsa decemlineata* (Say)), the flour beetle (*Tribolium castaneum* (Herbst)), the malaria vectors (*Anopheles albimanus* Wiedemann, *A. sacharovi* Favre), the house fly (*Musca domestica* L.), the horn fly (*Haematobia irritans* (L.)), the white fly (*Bemisia tabaci* (Gennadius)), the virus-vector aphid (*Myzus persicae* (Sulzer)), the diamondback moth (*Plutella xylostella* (L.)), the armyworms (*Spodoptera* spp.), and *Heliothis* spp. (Georghiou 1986).

For the past two decades the tobacco budworm (TBW), *Heliothis virescens* (F.), has been a major pest of field crops in North, Central, and South America (Wolfenbarger et al. 1981). The first difficulties in field control of *Heliothis* spp. with DDT were noticed in the 1950's (Ivy & Scales 1954, Graves et al. 1963, 1967). Based on journal articles and reports appearing in the Annual Beltwide Cotton Production and Research Conference Reports, by 1970 DDT resistance had been reported for bollworm, *H. zea* (Boddie), and TBW in 12 and 8 states, respectively (Anonymous 1963-1980, cited by Sparks 1981). Concomitantly, resistance to other organochlorine insecticides was also detected.

The strategy adopted was to shift to the organophosphate insecticides. These gave adequate control of *Heliothis* spp. However, resistance to methyl parathion was first noticed in the TBW in Texas in the late 1960's (Whitten & Bull 1970, Wolfenbarger & McGarr 1970, Wolfenbarger et al. 1973, Nemec & Adkisson 1973) and continued to

increase in the following years. Resistance to methyl parathion was then observed in virtually every state of the cotton belt. TBW resistance to organophosphate insecticides was determined to be primarily the result of increased detoxification (Whitten & Bull 1970, 1974, Bull 1981). No organophosphate resistance has occurred in the bollworm in North America, although Wolfenbarger et al. (1981) reported it from Central America.

Several pyrethroids were shown to be highly active insecticides against *Heliothis* spp. attacking cotton (Harding et al. 1977). In addition to their high toxicity, their light stability and low toxicity to mammals (Elliott 1977) contributed to their widespread use in cotton for bollworm and TBW controls.

Chlordimeform, a formamidine, is used as an ovicide to control many lepidopteran pests. In addition, chlordimeform synergizes different classes of insecticides against susceptible and organophosphate-resistant populations of TBW (Plapp 1976a, Plapp 1979, Rajakulendran & Plapp 1982). Therefore, the synergistic effect of chlordimeform can increase the effectiveness of insecticides against resistant TBW and decrease the amount of insecticides required to control resistant TBW populations. Accordingly, the costs of insecticides applications are lowered since chlordimeform is cheaper than most insecticides.

The first report on *Heliothis armigera* (Hübner) resistance to pyrethroids was made by Gunning et al. (1984) in Australia. In the United States, problems of TBW control with pyrethroids have been reported for several years, most notably in California (Twine & Reynolds 1980, Martinez-Carrillo & Reynolds 1983). The first serious control problems with pyrethroids in Texas occurred in the Uvalde, St. Lawrence, and Fort Stockton areas in 1985 (Plapp & Campanhola 1986).

In the 1986 season, problems of TBW control with pyrethroids occurred in several cotton production areas of the United States. An adult monitoring program (Plapp et al. 1987) performed during that year confirmed the existence of TBW resistance in Texas

(Allen et al. 1987, Plapp et al. 1987), Arkansas (J. R. Phillips, personal communication), Mississippi (Roush & Luttrell 1987), and Louisiana (Leonard et al. 1987).

Resistance seems to extend to all pyrethroids. Field strains of TBW collected in Louisiana, Texas, Arizona, and Mississippi during 1985 and 1986 exhibited moderate to high levels of resistance to fenvalerate (2-35 X), permethrin (1-74 X), and cypermethrin (2-9 X) (Leonard et al. 1987). The mechanism of target site resistance to DDT (kdr) appears to confer pyrethroid resistance (Plapp 1976b, Miller et al. 1979, Osborne & Hart 1979, Nicholson et al. 1980). Thus, the kdr gene being already present in the populations favored the relatively rapid selection for resistance to pyrethroids.

The development of insecticide resistance by insects represents a serious risk to agribusiness. With the onset of resistance, growers tend to increase the amount of insecticides used as well as the number of applications. This represents an increase in the costs of production. Also, more chemicals are released into the environment, and many adverse and secondary effects can be aggravated. Nevertheless, the level of pest control promoted by insecticides may not prevent economic damage in crops which depend mostly on chemical control of pests, such as cotton. Consequently, a decrease in profitability may be observed.

The objectives of the present study were: i) to determine alternate insecticides or combinations of insecticides to control pyrethroid resistant TBWs in cotton; ii) to evaluate the resistance spectra for different resistant populations of the TBW; iii) to evaluate chlordimeform synergism of insecticides and insecticide combinations against pyrethroid-resistant TBWs; iv) to determine and relate the resistance levels to pyrethroids in different life stages of the TBW for different resistant populations to identify the mechanism(s) of resistance involved; and v) to evaluate possible biological differences between susceptible and resistant strains of the TBW.

CHAPTER II

REVIEW OF LITERATURE

Mechanisms of Resistance

There are three known physiological mechanisms of insecticide resistance in insects. These include alterations at the site of action, increased detoxification, and reduced penetration. In addition, behavioral resistance may often be present, but at this time it is not well understood (Lockwood et al. 1984). In this chapter we review what is known about these resistance mechanisms.

One case of alteration at the site of action involves the reduced sensitivity of acetylcholinesterase (AChE), the target site of organophosphate and carbamate insecticides. This mechanism was first observed in resistant spider mites, *Tetranychus urticae* Koch, that showed a decrease in sensitivity of AChE to organophosphates (Smissaert 1964). Thereafter, at least one mutant AChE with reduced sensitivity to insecticides has been found in *T. pacificus* McGregor (Zon & Helle 1966), *Boophilus microplus* (Canestrini) (Lee & Batham 1966), *Nephotettix cincticeps* Uhler (Hama & Iwata 1971), *Musca domestica* (Tripathi & O'Brien 1973), *Anopheles albimanus* (Ayad & Georghiou 1975) and *Spodoptera littoralis* (Boisduval) (Voss 1980). In the cattle tick, *B. microplus*, two very different insensitive AChE's exist. There is also evidence that several different mutant AChE's occur in spider mites (Schulten 1968, Zahavi et al. 1971). The reduced AChE activity of resistant strains could be due to reduced amounts of enzyme, or to reduced catalytic activity. Nolan & Schnitzerling (1976) found that the latter was the case in the cattle tick.

Another mechanism of resistance involving alteration at the site of action is related to DDT and pyrethroids (Farnham 1977, Plapp 1976b, Elliott et al. 1978, Omer et al.

1980, Chang & Plapp 1983a). A similar mechanism seems also to be responsible for resistance to the hard-to-metabolize cyclodienes (Plapp 1986). Consequently, this type of resistance is not affected by synergists acting as metabolism inhibitors and confers no cross resistance to organophosphate and carbamate insecticides.

For years the biochemical bases of kdr and cyclodiene resistance have remained unclear. However, several recent findings may serve as clues to elucidate the mechanism of those types of resistance. These include less Ca-ATPase inhibition by DDT in cockroaches, *Blattella germanica* (L.), with kdr-type resistance (Ghiasuddin et al. 1981), different characteristics of phospholipids from nerves of resistant and susceptible house flies (Chialiang & Devonshire 1982), a reduced number of pyrethroid receptors in kdr flies (Chang & Plapp 1983a), a reduced number of receptors for picrotoxinin (a plant-derived neurotoxicant) and cyclodiene insecticides in cyclodiene-resistant cockroaches (Kadous et al. 1983, Tanaka et al. 1984), or a reduced pyrethroid sensitivity of sodium channels in the kdr insect nerve (Kasbekar & Hall 1988).

Another mechanism of resistance, probably the most widespread one, is based on an increased capacity to degrade insecticides. This mechanism of resistance is most important with biodegradable insecticides such as organophosphates and carbamates. Sometimes it may also be important with pyrethroids (Plapp & Wang 1983).

Animals possess many enzymes which enable them to defend against the many harmful products that they encounter in their environment. The various detoxifying enzymes, mixed-function oxidases, glutathione S-transferases, hydrolases, and DDT-ases, may well constitute an integrated system for degradation of xenobiotics present as the result of a long evolutionary history, common to vertebrates and invertebrates (Oppenoorth 1985).

In the house fly several strains resistant to parathion, diazinon, and other organophosphate compounds have hydrolytic enzymes which act as phosphatases on the

organophosphate analogues such as paraoxon (Welling et al. 1971). Resistance to malathion and some related compounds forms a special case in which hydrolysis is of greater importance since the carboxylester groups in the molecule can be attacked rapidly. In the house fly, two carboxylesterases have been found (Welling & Blaakmeer 1971). A soluble enzyme is present in susceptible as well as in resistant strains, and an additional, much more active one, in the microsomes of resistant strains. In the Indian meal moth, *Plodia interpunctella* (Hübner), high monogenic resistance to malathion was due to 33 times as much carboxylesterase activity as in the susceptible strain (Beeman & Schmidt 1982). In the sheep blowfly, *Lucilia cuprina* (Wiedemann), parathion resistance has been found to be mainly due to hydrolysis of paraoxon, and additional oxidation of parathion (Hughes & Devonshire 1982).

An increased esterase activity associated with resistance to several pyrethroids has been reported in *Spodoptera littoralis* (Riskallah 1983). Evidence for hydrolases as cause of pyrethroid resistance has also been found in a strain of cattle tick in which these enzymes are probably not the only factor (Schnitzerling et al. 1983). However, as pointed out by Dauterman (1983), hydrolases appear to play a smaller role in resistance than might be expected and typically are associated with other enzymatic resistance mechanisms.

It is well established that an increase in mixed-function oxidase (MFO) activity is one of the most common mechanisms of resistance to a great variety of insecticides. It has been assumed that these microsomal enzymes have evolved as a protective mechanism against naturally occurring toxicants (Wilkinson 1983). A general characteristic of MFO's is their wide range of substrates, which results in cross resistance patterns that are not restricted to particular groups of insecticides. The MFO activity is dependent on a complex system, the activity of which is determined by a reductase, one or more cytochrome P-450's and the concentration of NADPH. They exhibit an unusual

degree of nonspecificity and a predilection for fat-soluble compounds, which they metabolize through reactions involving numerous functional groups (Wilkinson 1983). Among these reactions are aromatic, alicyclic and aliphatic hydroxylation, dealkylation of ethers and substituted amines, oxidation of thioethers to sulfoxides and sulfones, epoxidation of aromatic and olefinic double bonds, and desulfuration (Testa & Jenner 1976, Nakatsugawa & Morelli 1976).

In view of the lack of substrate specificity for MFO enzymes, it is not surprising to find increases in oxidation of many insecticides in resistant strains which result in some degree of cross resistance. Carbamate resistance due to increased oxidase has been found in *Culex pipiens fatigans* Wiedemann (Shrivastava et al. 1970) and *Trichoplusia ni* (Hübner) (Kuhr 1971), whereas *Sitophilus granarius* (L.) can oxidize pyrethroids (Lloyd & Ruczkowski 1980). MFO's have been shown to be of importance in the resistance of various strains of insects to DDT (Oppenoorth 1965, Sawicki 1973), pyrethrins (Farnham 1973), carbamates (Georghiou et al. 1961, Metcalf & Fukuto 1965), several organophosphate compounds (Wilkinson 1971), and some of the new groups of compounds such as the juvenile hormone analogue methoprene (Hammock et al. 1977) and the chitin synthetase inhibitor diflubenzuron (Pimprikar & Georghiou 1979).

The blockage or reduction of insecticide resistance by the action of methylenedioxyphenyl synergists such as piperonyl butoxide and sesamex constitutes a useful indicator of the extent of MFO's involvement in insect resistance. The ability of those chemicals to inhibit microsomal oxidation was clearly established as the primary mechanism through which they exert their synergistic effect (Casida 1970, Hodgson 1976, Wilkinson 1976a, Hodgson & Philpot 1974).

Another group of enzymes recognized as important in insecticide resistance is the glutathione S-transferases (GST). There are no specific inhibitors that would enable the study of their importance in the presence of other detoxification mechanisms (Oppenoorth

1985). Some differences have been found in the amount of GST's present in resistant flies (Saleh et al. 1978, Ottea & Plapp 1984), which were attributed as the cause of resistance. Demethylation by the GST's is the only mechanism responsible for azinphosmethyl resistance in the predacious mite, *Neoseiulus fallaris* (Garman) (Motoyama et al. 1977). A two-fold elevated GST content has also been reported as a cause of two-fold resistance of the granary weevil to methyl bromide (Starrat & Bond 1981).

DDT-dehydrochlorinase is involved in resistance to DDT in house flies. This enzyme degrades DDT to the non-toxic DDE and hydrogen chloride and is not found in susceptible strains (Oppenoorth 1985). This mechanism of resistance was most studied with house flies (Lipke & Kearns 1960). There are indications that in *Aedes aegypti* (L.) the enzyme is an important resistance factor in many strains (Kimura & Brown 1964). Still other DDT-ases have been demonstrated in *Culex fatigans* Wiedemann and *C. tarsalis* Coquillett (Kimura et al. 1965). For those species the larvae of resistant strains had about ten and four times as much DDT-ase activity as the susceptible ones, respectively.

A reduced rate of penetration of insecticides has been found in a number of resistant strains of insects. Compared with the other types of resistance, reduced absorption is of secondary importance. Plapp & Hoyer (1968) showed that a gene on chromosome III of house flies causes a reduction of 2 to 5 times in the rate of penetration of many insecticides. Sawicki & Lord (1970) showed that the rate of penetration is dependent upon the insecticide, dose, and solvent. However, this resistance type has a very pronounced effect as an enhancer of resistance by detoxification, where the magnifying factors range from 1.6 to 3 for parathion, to unmeasurably large for DDT (Sawicki & Lord 1970). With DDT the decreased penetration increases a 10-fold resistance due to oxidative degradation to near immunity. A difference in penetration has also been

reported to be a mechanism of resistance to dimethoate in citrus red mite, *Panonychus citri* (McGregor) (Hirai et al. 1973) and to pyrethroids in cattle ticks (Schnitzerling et al. 1983).

The existence of behavioral resistance has been questioned until recently (Muirhead-Thomson 1960, W. H. O. Expert Committee on Insecticides 1976). Lockwood et al. (1984) defined this resistance as "those actions, evolved in response to the selective pressures exerted by a toxicant, that enhance the ability of a population to avoid the lethal effects of that toxicant". Trapido (1954) was the first to recognize behavioral resistance and implied that it developed in the absence of physiological resistance. Other reports of an apparent inverse relationship between behavioral and physiological resistance were published. Such studies included *Anopheles sacharovi* (Zulueta 1959), *A. albimanus* (Rachou et al. 1973), *C. fatigans* (Busvine 1971), *A. aegypti* (Muirhead-Thomson 1960, Mukwaya 1974), *M. domestica* (Smythe & Roys 1955) and *Drosophila melanogaster* (Meigen) (Pluthero & Threlkeld 1981), among others.

Georghiou (1972) stated that behavioral (irritability) and physiological resistances are negatively correlated. However, behavioral resistance, in the form of repellency, was found to coexist with physiological resistance in house flies (Kilpatrick & Schoof 1958). More recently evidence for coexistence of behavioral and physiological resistances to pyrethroids in horn flies has been reported in Louisiana (Lockwood et al. 1985). These investigators observed that behavioral resistance can take either the form of hypersensitivity or a lowered threshold, or both.

Genetics of Resistance

The genetics of resistance to insecticides has been extensively investigated. Resistance to insecticides is almost invariably due to a single major gene (Milani 1960, Brown 1967, Georghiou 1969). Genetic studies with house flies showed that change at a

single genetic locus on chromosome II appears to control resistance associated with multiple detoxification enzymes (Plapp 1986). The gene on that chromosome appears to interact with minor genes on other chromosomes. In house flies there are at least two genes involved with resistance by increased oxidation, one on chromosome II and one on V (Oppenoorth 1967, Tsukamoto et al. 1968, Schonbrod et al. 1968, Plapp & Casida 1969, Khan et al. 1973, Tate et al. 1974). The resistance gene on chromosome II is common and is associated with increased oxidation of aldrin (Khan 1969, Georghiou 1971), carbamates (Shrivastava et al. 1969, Plapp & Casida 1969), organophosphates (Plapp & Casida 1969, Yang et al. 1971, Oppenoorth 1972), and pyrethrins (Plapp & Casida 1969). The gene on chromosome V is associated with oxidation of DDT, DDE, diazoxon and, no doubt, a number of other insecticides (Oppenoorth 1967, Oppenoorth & Houx 1968).

Possible allelism exists among genes for metabolic resistance to insecticides in other insect species as well. The gene RI located on chromosome II of *D. melanogaster* confers resistance to organophosphates, carbamates, and DDT (Kikkawa 1964 a,b). Major genes for metabolic resistance to diazinon and malathion were located on the same chromosome in different populations of *Lucilia cuprina* (Hughes et al. 1984). Conversely, Priester & Georghiou (1979) concluded that permethrin resistance in *Culex pipiens quinquefasciatus* (Say) is of polyfactorial origin. Likewise, Croft & Whalon (1983) reported a polygenic, recessive basis for resistance to permethrin in the predatory mite, *Amblyseius fallacis* (Garman).

In contrast to metabolic resistance there are several major genes for target site resistance, one for each type of insecticide. In *Culex quinquefasciatus* Say, permethrin resistance was inherited as a single, major gene of incomplete recessive expression (Halliday & Georghiou 1985). In the horn fly, *H. irritans* , cypermethrin resistance appeared to be inherited as a single, autosomal gene of incomplete recessivity (Roush et

al. 1986). In the house fly a pleiotropic effect associated with the recessive gene kdr-O was suggested. This gene confers resistance to DDT in the Orlando DDT fly strain and it was also found to confer resistance to DDT analogues, pyrethrins, and pyrethrins:piperonyl butoxide (Plapp & Hoyer 1968). In addition, they suggested that resistance to DDT and pyrethrins in *C. tarsalis* is controlled by a similar mechanism. Genetic crossing studies in both mosquitofish and insects have shown that cyclodiene resistance is conferred by a single, autosomal, intermediate (incompletely recessive) gene (Plapp 1976b, Yarbrough et al. 1986). Thus, both genes for target site resistance to organochlorines are incompletely recessive.

Decreases in numbers of target sites may be responsible for target-site resistance to both DDT/pyrethroids and cyclodienes (Chang & Plapp 1983a, Kadous et al. 1983). Recessive inheritance of resistance agrees with the idea of quantitative change (Plapp 1986). He pointed out that the specific mutations conferring resistance are probably in genes coding for proteins that determine the number of target-site proteins synthesized. The heterozygotes would have the normal number of receptors since the diffusible protein product of the wild-type regulatory gene would act on both structural genes. Only the resistant homozygotes, those with two mutant genes, would produce fewer target-site receptor proteins than normal.

Unlike kdr resistance, a single dominant gene seems to be responsible for the difference in AChE sensitivity and the resistance caused by it in spider mites (Helle 1962, Schulten 1968), cattle ticks (Stone et al. 1976), green rice leafhoppers (Hama & Iwata 1978), and houseflies (Plapp 1986). In this case the inheritance is intermediate, that is, hybrids have intermediate AChE sensitivity since half of the altered enzyme is produced compared with resistant homozygotes. This is consistent with the idea of codominant inheritance of an altered enzyme conferring resistance.

Most of the studies conducted so far were based on dipterans and the genetic basis

for resistance in lepidopterans remains to be elucidated. A study of the inheritance of pyrethroid resistance in a lepidopterous pest demonstrated that fenvalerate resistance in the diamondback moth, *P. xylostella* , was partially recessive and conferred by more than one autosomal gene (Liu et al. 1981). A recent study with the tobacco budworm based on segregation in back crosses showed that permethrin resistance was inherited as a single, major, incompletely recessive, autosomal factor (Payne et al. 1988). Thus, the resistance seems to be of kdr-type. Other studies with tobacco budworm resistance were performed with methyl parathion (Whitten 1978) and methomyl (Roush & Wolfenbarger 1985). In both cases, resistance was due to a single, autosomal gene of incomplete dominance.

A regulatory-gene hypothesis is a more likely model to account for resistance, particularly at the population or subspecific level (Plapp 1986). Two types of regulatory genes seem to be present, and they differ in inheritance and biochemistry. One type exhibits all-or-none inheritance (fully dominant or recessive) and appears to involve changes in the amount of protein (detoxifying enzyme) synthesized. The second shows codominant (intermediate) inheritance and involves changes in the nature of proteins synthesized. The first seems to be associated with target site resistance and the second with metabolic resistance.

Management of Resistance

Several factors are known to affect the rate at which insects can evolve resistance to pesticides (Brown 1971, Georghiou 1972, Georghiou & Taylor 1977 a,b). These include operational factors such as the type of insecticide, dose and timing of application, and genetic-biological factors such as the frequency of resistant alleles, migration rates, and the mechanisms of resistance (Taylor et al. 1983). The only factors that can be manipulated seem to be the operational ones (Wood & Bishop 1981, Georghiou 1983).

Georghiou (1983) listed three main modes of chemical strategies that can be used for the management of resistance. These are management by moderation, management by saturation, and management by multiple attack. The first two approaches involve the use of a single insecticide and the management is affected through factors such as control of effective dominance, preservation of 'refugia', and suppression of detoxification mechanisms by synergists. For the last mode, two subdivisions were given, namely, the use of mixtures of chemicals and the alternation of chemicals either in space or in time.

The simplest use of synergists in resistance management is by their direct application to resistant populations. This measure is particularly pertinent to cases where metabolic resistance is present, since synergists block detoxifying systems involved in resistance.

One of the most appealing prospects for the use of synergists is the prevention of resistance development (Raffa & Priester 1985). According to this view, exposing susceptible populations to an insecticide-synergist mixture would remove the selective advantage of certain metabolic alterations. This principle was successfully demonstrated by Moorefield (1960) who found that carbamate resistance in the house flies was 194 times higher after 20 generations of exposure to carbaryl than after the same period of treatment with carbaryl plus piperonyl butoxide. Selection of a population of *C. quinquefasciatus* over three generations with Kitazin P® (S-benzyl O,O-diisopropylphosphorothioate) plus malathion decreased the resistance gene frequency at a level similar to that of the unselected population (Hemingway & Georghiou 1984). In another study, selection by temephos plus DEF (S,S,S-tributyl phosphorotrithioate) of a temephos-resistant strain of *C. pipiens fatigans*, known to posses only limited MFO-detoxification abilities, virtually abolished esterase-based resistance while preventing the emergence of significant alternate resistance mechanisms (Ranasinghe & Georghiou 1979). Therefore, for synergists that block metabolism to be able to delay or overcome

resistance, the resistance mechanism should be limited to the detoxification pathway involving the enzyme system affected by the synergist. However, alternative resistance pathways may be selected if the genes conferring resistance are present in the population.

There are many other examples of synergists blocking or decreasing considerably the resistance levels (Dyte & Rowlands 1968, Hemingway 1982, Plapp et al. 1963, Riskallah 1983). On the other hand, alternate means of resistance may develop by different metabolic pathways (Cole & Clark 1961, Farnham 1971, Cochran 1973).

Piperonyl butoxide greatly enhances the toxicity of certain insecticides because it inhibits microsomal detoxification-enzymes (Casida 1970, Wilkinson 1976b, Georghiou 1980). For instance, the pyrethroids fenvalerate and permethrin are synergized by a number of chemicals, including piperonyl butoxide (Jao & Casida 1974, All et al. 1977, Plapp 1979, Roberts et al. 1980, Forgash 1981). Silcox et al. (1985) showed in the laboratory that Colorado potato beetle control by pyrethroid:piperonyl butoxide combinations depends on the amount of insecticide applied, the ratio or amount of piperonyl butoxide applied and the resistance level in the population. They observed that synergism is generally optimal at a 1:4 insecticide:piperonyl butoxide ratio, but the amount of piperonyl butoxide applied is probably the most important factor affecting synergism. However, photoinstability limits the piperonyl butoxide use in the field (Georghiou 1980).

Chordimeform, a formamidine, has been tested as insecticide synergist against susceptible and resistant insect species. This chemical has been used as ovicide and also demonstrates alteration in adult behavior (Etheridge 1972, Wolfenbarger et al. 1974, Streibert & Dittrich 1977). Synergism by chlordimeform was observed with monocrotophos and resmethrin against a resistant strain of *Spodoptera littoralis* (Dittrich et al. 1981). In another study, chlordimeform was included among the four best synergists out of 104 formamidines tested for synergism of pyrethroids against two

spotted spider mites (*Tetranychus urticae* Koch) (El-Sayed & Knowles 1984). In most cases, the ratio used was 2:1 formamidine:insecticide. With regard to the different pyrethroids, there was evidence that formamidine synergism was generally greatest with cypermethrin and deltamethrin, intermediate with fluvalinate, flucythrinate, and fenpropathrin, and least with fenvalerate and permethrin.

Chlordimeform was tested as a synergist of insecticides against third instars of susceptible and resistant strains of tobacco budworm. For an organophosphate-resistant population, chlordimeform synergized different classes of insecticides at a 1:1 ratio, with levels of synergism at the LC_{50} level varying from 2- to 3-fold with methyl parathion and monocrotophos to as much as 17-fold with less toxic chemicals such as pyrethrins and TH 6040 (Plapp 1976a). For susceptible and resistant populations of tobacco budworm, chlordimeform in combination with permethrin, fenvalerate, or decamethrin provided synergism ranging from 2- to 7-fold at the LC_{90} level (Plapp 1979). Also, a 1:10 insecticide:synergist ratio was more effective than a 1:1 ratio. In another study, five pyrethroids, flucythrinate, fluvalinate, tralomethrin, phenothrin, and cypermethrin, were tested alone and in combination with chlordimeform for toxicity to susceptible tobacco budworms. With a 1:10 insecticide:chlordimeform ratio, chlordimeform synergism varied from 7.4- to 63.4-fold at the LC_{90} level and highest synergism was observed with the least toxic insecticide (Rajakulendran & Plapp 1982).

The use of pyrethroid:formamidine mixtures may prove useful in preserving beneficial insects in cotton fields. In tests with adult male *Campoletis sonorensis* (Carlson), a parasite of the tobacco budworm, pyrethroid:chlordimeform mixtures were only slightly more toxic to the parasite than pyrethroids alone (Plapp 1979). Also in a study with a tobacco budworm predator, *Chrysopa carnea* (Stephens), pyrethroid-chlordimeform combinations were more toxic to tobacco budworms than to the predators, except for phenothrin plus chlordimeform (Rajakulendran & Plapp 1982).

The use of chlordimeform in combination with pyrethroids may prevent the development of resistance. After 10 generations of selection with a 1:1 permethrin: chlordimeform ratio at the LD_{80} level, the susceptibility to permethrin did not change in the population of tobacco budworms tested (Crowder et al. 1984). In contrast, selection with permethrin only during 11 generations raised the LD_{50} 37-fold compared with the LD_{50} of the F_1. Thus, based on all the results presented, chlordimeform seems to be a promising synergist for insecticides for controlling pyrethroid-resistant tobacco budworms.

Mechanisms thought to be involved in the synergism of pyrethroids by chlordimeform include inhibition of oxidation (Plapp 1979) and increased specific binding of the pyrethroid to the target site, *i. e.*, the receptor on nerve membranes (Chang & Plapp 1983b). Therefore, chlordimeform could be a target site synergist of DDT and pyrethroids against the tobacco budworm by increasing the specific binding of these insecticides to the target site. However, Treacy et al. (1987) suggested that under field conditions, chlordimeform may also enhance efficacy of pyrethroids against *Heliothis* spp. through behavioral mechanisms.

In the past, strategies to circumvent the problem of resistance emphasized the development of new insecticides. However, this approach has become less attractive for several reasons (Metcalf 1980). New insecticide molecules tend to be more sophisticated in chemical structure than those previously developed. In addition, developmental costs for pesticides have increased manyfold during the past 30 years due to inflation and to increasingly rigid requirements for registration.

A very controversial topic is the relative usefulness of insecticide mixtures or insecticide alternations to overcome or prevent the development of resistance. Insecticide mixtures can show synergistic effects against many species of susceptible and pesticide-resistant arthropods (Chapman & Penman 1980, Wolfenbarger & Cantu 1975, Robertson

& Smith 1984, Gaughan et al. 1980, Ozaki et al. 1984, Koziol & Witkowski 1982). All et al. (1977) observed synergism in topical experiments with tobacco budworm larvae especially with methyl parathion:permethrin at a 10:1 ratio. Synergism of methyl parathion:permethrin (9:1) was also suggested with *H. zea*, but not with methyl parathion:fenvalerate (9:1). However, toxicity of methomyl to *H. zea* was synergized by both permethrin and fenvalerate. In many other cases where mixtures have been applied, the results have been negative or inconclusive, apparently as a function of the components of the mixture (Georghiou 1980). With *H. armigera* and *H. punctigera* Wallengren, Kay (1981) observed no synergistic effect when methomyl plus fenvalerate or deltamethrin was tested against larvae.

Use of mixtures has successfully delayed the onset of resistance in insects and mites (Burden et al. 1960, Asquith 1961, Graves et al. 1967, Ozaki et al. 1973, Georghiou 1983, Brindley & Selim 1984). Pimentel & Bellotti (1976) observed that house flies evolved resistance to each of six insecticides when used alone, but were apparently unable to develop resistance to a mixture of the compounds. In another study (MacDonald et al. 1983), a very high and stable permethrin resistance developed in the house flies under continuous selection in laboratory. By alternating permethrin and dichlorvos selections, the stability of resistance to both insecticides was reduced. Likewise, selection with a 1:1 mixture of permethrin and dichlorvos resulted in even more substantial suppression of resistance development to permethrin and dichlorvos.

The idea of alternating insecticides to prevent or delay the development of resistance has been considered for many years. Brown (1981) referred to this approach as "a prophylactic countermeasure employing temporal reduction of selection pressure and taking full advantage of the principle of reversion of induced resistance". The Australian strategy to manage pyrethroid resistance in *H. armigera* in cotton is based on the restriction of pyrethroid use to only one generation of the insect per year (Sawicki 1985).

Any pyrethroid-resistant survivors are controlled by alternative chemicals. Pyrethroids were saved for use in the most vulnerable period of the cotton growth cycle to take advantage of the excellent insecticidal properties of these compounds. Toxicants of at least three chemical groups were suggested for use throughout the season to forestall resistance development to non-pyrethroid insecticides. For the brown planthopper (*Laodelphax striatellus* Fallen), on rice, good control was obtained with alternations of certain insecticides in Japan (Sasaki & Ozaki 1976, Ozaki 1983). However, when insecticide combinations were tested against house flies, bed bugs (*Cimex lectularius* L.), or cockroaches (*Blattella germanica* (L.)), the insects often developed resistance to both insecticides (Burden et al. 1960, Brown 1977).

Theoretical models have often been designed to evaluate the use of multiple insecticide strategies in the development of resistance. The use of mixtures, if the insecticides are rightly chosen, seems to be more promising than alternating insecticides (Curtis 1985). However, the exposure of an insect population to an insecticide mixture may promote rapid selection for double resistance. Nevertheless, in practice, this argument may be invalid because of non-uniform exposure of wild populations to insecticides (Curtis 1985) and immigration of susceptible individuals to the treated area (Curtis et al. 1978).

Other studies have shown the advantage of insecticide mixtures over single insecticides. It has been reported that the use of mixtures is always more effective in delaying the onset of resistance (Knipling 1979, Knipling & Klassen 1984, Mani 1985, Comins 1986). Furthermore, Knipling & Klassen (1984) showed in a theoretical study that there would be no advantage in alternating insecticides over the use of one insecticide until a highly resistant population develops followed by change to a second insecticide. Therefore, it seems that using appropriate mixtures of insecticides is more promising than alternating insecticides for delaying the development of resistance in the field.

In this dissertation I evaluated resistance to insecticides in the tobacco budworm and characterized it in relation to the literature reviewed above. I also studied the toxicity and potential usefulness of many insecticide combinations as tools for managing insecticide resistance. Again, the experiments were designed and evaluated in relation to the literature reviewed here.

CHAPTER III

III. NEONATE TOBACCO BUDWORM : INSECTICIDE TOXICITY AND SYNERGISM

Introduction

Insecticide bioassays with lepidopterous larvae in the laboratory have usually been done with third or fourth instars. I developed a technique where unfed neonate TBW larvae were exposed to films of insecticides in liquid-scintillation glass vials (Plapp & Campanhola 1986, Campanhola & Plapp 1987). These studies showed that it was possible to detect pyrethroid resistance in neonate TBW larvae.

In bioassays with late instars, only one larva can be tested per vial due to the cannibalistic habit of this species. However, five neonate larvae can be tested per vial without a cannibalism problem. Another advantage is that there is no need for rearing larvae to third or fourth instars before testing them. This aspect is particularly promising for insecticide-resistance monitoring in the field due to its quickness. Neonate larvae obtained from eggs collected in the field can be tested for resistance within two or three days of collecting the eggs (McCutchen & Plapp 1988). The only apparent limitation for this method is that it may not be accurate for metabolic resistance. First instars are poor metabolizers of xenobiotics and probably are less likely to express metabolic resistance than later instars.

I performed the present study to determine the resistance spectra of neonate larvae of different pyrethroid-resistant TBW strains and to discover possible alternate insecticides or insecticide combinations for controlling pyrethroid-resistant TBW populations in the field. I also evaluated the effects of the insecticide synergists chlordimeform and piperonyl butoxide in combination with the test insecticides.

Materials and Methods

The susceptible and resistant TBW strains for the bioassays were obtained from laboratory colonies maintained on artificial diet (Vanderzant et al. 1962). The susceptible strain (Stoneville) was provided by the Southern Field Crop Insect Management Laboratory, USDA, ARS, Stoneville, MS, where it has been reared for several years without exposure to insecticides. Three resistant strains designated ICI, Uvalde, and Hearne were studied. The ICI strain was prepared by ICI Americas, Goldsboro, NC, from a mixture of 10 different populations collected from cotton fields in different states. Resistance was developed by selection with permethrin and cypermethrin in the laboratory for several generations. In this study we used two samples of the ICI strain. The other two resistant TBW strains were brought to the laboratory from cotton fields where control failures with pyrethroids had been observed. The Uvalde strain was collected near Uvalde, TX by D. F. Clower, consultant for ICI Americas, in July, 1986. The Hearne strain was provided by V. V. Turner, a private consultant, and was collected near Hearne, TX in August, 1986.

The insecticides tested included the pyrethroids cypermethrin, fenvalerate, essfenvalerate, permethrin, deltamethrin, tralomethrin, biphenthrin, cyhalothrin, cyfluthrin, and fluvalinate; the organophosphates methyl parathion, chlorpyrifos, monocrotophos, profenofos, sulprofos, and acephate; the carbamates methomyl and thiodicarb; the cyclodiene endosulfan; the microbial product avermectin; and the cubé root extract rotenone. I also tested insecticide combinations such as cypermethrin plus other insecticides (methyl parathion, profenofos, acephate, methomyl, and thiodicarb) and chlorpyrifos plus sulprofos. I also evaluated the synergistic effects of chlordimeform and piperonyl butoxide when combined with insecticides. All were supplied by commercial sources as technical grade materials. The chemical names for the chemicals used in this

study are listed in Table 1.

Neonate TBW larvae were exposed to films of chemicals on the inner surfaces of 20-ml glass liquid scintillation vials (Plapp 1971). A piece of artificial diet and five larvae were placed in each vial. Thereafter, the vials were plugged with cotton. At least four replicates with five larvae each were tested per concentration of insecticide. All insecticides were tested with and without chlordimeform with susceptible and resistant TBW strains. Insecticide(s) with chlordimeform or piperonyl butoxide or both were tested at a 1:10 (wt:wt) ratio and insecticide combinations were tested at a 1:1 (wt:wt) ratio. The use of a 1:10 (insecticide:synergist) ratio was based on previous studies (MacDonald et al. 1983, Plapp 1976b, 1979, Rajakulendran & Plapp 1982). Four or five different concentrations were used for each insecticide or insecticide combination in addition to untreated controls (acetone only). During rearing and bioassays the insects were maintained in an incubator at 25 ± 1°C and a 14:10 (L:D) photoperiod. Percent response was determined at 24 h and probit regressions were estimated (SAS Institute 1982). Data from all tests were corrected for control mortality with Abbott's (1925) formula. The resistance level was determined by dividing the LC_{50} (or LC_{90}) of each toxicant for the resistant strain by the LC_{50} (or LC_{90}) for the susceptible strain. The synergism levels due to chlordimeform were calculated by dividing the LC_{50} (or LC_{90}) for the insecticide only by the LC_{50} (or LC_{90}) for the insecticide with chlordimeform. The synergistic effect of insecticide combinations was evaluated by cotoxicity coefficients (Sun & Johnson 1960).

Results and Discussion

Bioassays with a susceptible and different resistant strains of the TBW

The results of toxicity tests for the insecticides, alone and combined with chlordimeform, with neonate larvae of different TBW strains are presented in Table 2.

Table 1. Common and chemical names of the compounds studied[a]

Common Name	Chemical Name
Cypermethrin	(±)-(Cyano(3-phenoxyphenyl)methyl)*cis-trans*-(±)-3-(2,2-dichloroethenyl)-2,2 dimethylcyclopropanecarboxylate
Fenvalerate	Cyano(3-phenoxyphenyl)methyl 4-chloro-*alpha*-(1-methylethyl)benzeneacetate
Essfenvalerate	[S]-Cyano(3-phenoxyphenyl)methyl [S]-4-chloro-*alpha*-(1-methylethyl)benzeneacetate
Permethrin	3-Phenoxybenzyl (±) *cis-trans*-3-(2,2-dichlorovinyl)2,2-dimethylcyclopropanecarboxylate
Deltamethrin	(*S*)-(Cyano(3-phenoxyphenyl)methyl)*cis*-(+)-3-(2,2-dibromoethenyl)-2,2 dimethylcyclopropanecarboxylate
Tralomethrin	1*R*1(*S*)3(*RS*) 2,2-Dimethyl-3-(1,2,2,2-tetrabromoethyl) cyclopropanecarboxylate acid-cyano 3(3-phenoxyphenyl) methyl ester
Biphenthrin	2-Methyl-(1,1'-biphenyl)-3yl)methyl *cis*-3-(2-chloro-3,3,3-trifluoropropenyl)-2,2-dimethylcyclopropane carboxylate
Cyhalothrin	3-(2-Chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropanecarboxylate (*RS*)-(*RS*)-*cis-Z* isomers
Cyfluthrin	Cyano-(4-fluoro-3-phenoxyphenyl)methyl-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane carboxylate

Table 1. (continued)

Common Name	Chemical Name
Fluvalinate	*N*-[2-Chloro-4-(trifluoromethyl)phenyl]-*DL*-valine(*d*)-cyano(3-phenoxyphenyl)methyl ester
Methyl Parathion	*O*,*O*-Dimethyl-*O*-*p*-nitrophenyl phosphorothioate
Chlorpyrifos ethyl	*O*,*O*-Diethyl *S*-3,5,6 trichloro-2-pyridyl phosphorothioate
Monocrotophos	3-(Dimethylphosphinyloxy)-*N*-methylisocrotonamide
Profenofos	*O*-(4-Bromo-2-chlorophenyl) *O*-ethyl *S*-propyl phosphorothioate
Sulprofos	*O*-(4-Methylthiophenyl) *O*-ethyl *S*-propyl phosphorodithioate
Acephate	*O*,*S*-Dimethyl *N*-acetylphosphoramidothioate
Methomyl	*S*-Methyl *N*-(methylcarbomyl-oxy)thioacetimideate
Thiodicarb	Dimethyl ester of *N*,*N*'-(thio*bis* ((methylimino)carbonyloxy)) bisethanimidothioic acid
Endosulfan	6,7,8,9,10-Hexachloro-1,5,5a,6,9,9a hexahydro-6,9-methano-2,4,3-benzo [*E*]-dioxathiepin-3-oxide

Table 1. (continued)

Common Name	Chemical Name
Avermectin	(2a*E*,4*E*,5'*S*,6*S*,6'*R*,7*S*,8*E*,11*R*,13*R*,15*S*,17a*R*,20*R*,20a*R*,20b*S*)-6'-((*R*)-*sec*-Butyl)-7-((2,6-dideoxy-4-*O*-2,6-dideoxy-3-*O*-methyl- -*L*-*arabino*-hexopyranosyl)-3-*O*-methyl- -*L*-*arabino*-hexopyranosyl)oxy)-5',6,6',7,10,11,14,15,17a,20,20a,20*b*-dodecanydro-20,20b-dihydroxy-5,6,8,19-tetramethylspiro(11,16-methano-2H,13H,17H-furo(4,3,2-*pg*) (2,6)benzodioxacyclooctadecin-13,2'-(2H)pyran)-17-one
Rotenone[b]	6aβ, 12aβ-4'5'-tetrahydro-2,3-dimethoxy-5'β-isopropenyl-furano (3',2',8,9)-6H-rotoxen-12-one
Chlordimeform	N-(4-Chloro-*O*-tolyl)-*N*,*N*-dimethylformamidine
Piperonyl Butoxide	3,4-Methylenedioxy-6-propylbenzyl (heptyl)diethylene glycol ether

[a] Entomological Society of America (1987).
[b] Fukami (1985).

Table 2. Toxicity of different insecticides ± chlordimeform (CDF) to susceptible (S) and resistant (R) neonate TBW larvae

Insecticide	Strain	n[a]	LC_{50} (95% CL)[b]	LC_{90} (95% CL)[b]	Slope (± SD)
Cypermethrin					
	Stoneville(S)	189	0.15 (0.11-0.19)	0.66 (0.47-1.17)	1.96 (± 0.29)[c]
	ICI(R)	254	3.04 (2.15-4.31)	38.68 (21.64-94.70)	1.16 (± 0.14)
	Uvalde(R)	195	7.83 (4.06-12.99)	258.85 (86.46-4,379)	0.84 (± 0.20)
	Hearne(R)	365	1.63 (1.23-2.13)	17.71 (11.36-33.43)	1.24 (± 0.13)
Cyperm. + CDF					
	Stoneville(S)	260	0.008 (0.004-0.010)	0.39 (0.17-1.58)	0.75 (± 0.11)
	ICI(R)	247	0.19 (0.11-0.29)	4.63 (2.29-16.18)	0.92 (± 0.15)
	Uvalde(R)	164	0.20 (0.092-0.31)	3.11 (1.50-17.13)	1.07 (± 0.24)
	Hearne(R)	80	0.055 (0.0032-0.11)	0.29 (0.17-0.89)	1.78 (± 0.62)
Fenvalerate					
	Stoneville(S)	245	0.24 (0.17-0.34)	3.15 (1.58-10.97)	1.14 (± 0.18)
	ICI(R)	230	11.50 (7.74-16.51)	163.51 (83.35-552.26)	1.11 (± 0.18)[e]
	Uvalde(R)[d]	-	-	-	-
	Hearne(R)	210	2.17 (1.49-2.97)	19.10 (11.49-44.94)	1.36 (± 0.20)
Fenval. + CDF					
	Stoneville(S)	180	0.04 (0.028-0.060)	0.40 (0.18-2.43)	1.28 (± 0.27)
	ICI(R)	189	1.31 (0.90-1.78)	9.69 (5.97-22.78)	1.47 (± 0.24)
	Uvalde(R)[d]	-	-	-	-
	Hearne(R)	165	0.59 (0.42-0.74)	2.02 (1.52-3.41)	2.41 (± 0.44)

[a] Number of larvae tested excluding controls.

[b] Concentrations are expressed in micrograms of insecticide per vial.

[c] Slopes for insecticide and insecticide + CDF significantly different for the Stoneville strain ($P<0.05$; t-test).

[d] Data not available due to loss of strain.

[e] Mean slopes for insecticides and insecticides + CDF significantly different for resistant strains ($P<0.05$; t-test).

Table 2. (continued)

Insecticide	Strain	n[a]	LC_{50} (95% CL)[b]	LC_{90} (95% CL)[b]	Slope (± SD)
Profenofos					
	Stoneville(S)	148	0.084 (0.054-0.12)	0.39 (0.27-0.71)	1.93 (± 0.33)
	ICI(R)	133	0.21 (0.16-0.27)	0.73 (0.52-1.28)	2.31 (± 0.33)
	Uvalde(R)	264	0.15 (0.039-0.29)	1.04 (0.45-43.33)	1.51 (± 0.33)
	Hearne(R)	105	0.18 (0.12-0.27)	1.06 (0.55-8.56)	1.68 (± 0.47)
Profen. + CDF					
	Stoneville(S)	194	0.020 (0.015-0.028)	0.15 (0.090-0.33)	1.47 (± 0.19)
	ICI(R)	150	0.044 (0.030-0.070)	0.61 (0.30-2.53)	1.12 (± 0.21)
	Uvalde(R)	233	0.050 (0.036-0.064)	0.29 (0.20-0.53)	1.67 (± 0.24)
	Hearne(R)	170	0.079 (0.058-0.11)	0.50 (0.30-1.28)	1.59 (± 0.26)
Acephate					
	Stoneville(S)	185	4.66 (3.38-6.29)	29.25 (18.61-60.59)	1.61 (± 0.22)[c]
	ICI(R)	245	2.36 (0.13-6.55)	17.28 ($6.34\text{-}2.9\times10^{6}$)	1.48 (± 0.41)[e]
	Uvalde(R)	256	9.16 (5.38-19.32)	684.80 (150.48-29,291)	0.68 (± 0.15)
	Hearne(R)	150	6.26 (3.74-10.04)	94.99 (42.93-461.75)	1.09 (± 0.20)
Aceph. + CDF					
	Stoneville(S)	233	0.11 (0.033-0.22)	8.83 (3.47-56.51)	0.67 (± 0.13)
	ICI(R)	125	0.72 (0.50-0.99)	3.81 (2.28-11.15)	1.77 (± 0.35)
	Uvalde(R)	233	1.74 (1.26-2.69)	10.98 (5.67-44.98)	1.60 (± 0.32)
	Hearne(R)	125	0.57 (0.32-0.86)	4.87 (2.68-16.99)	1.38 (± 0.28)

[a] Number of larvae tested excluding controls.

[b] Concentrations are expressed in micrograms of insecticide per vial.

[c] Slopes for insecticide and insecticide + CDF significantly different for the Stoneville strain ($P<0.05$; t-test).

[e] Mean slopes for insecticides and insecticides + CDF significantly different for resistant strains ($P<0.05$; t-test).

Table 2. (continued)

Insecticide	Strain	n[a]	LC_{50} (95% CL)[b]	LC_{90} (95% CL)[b]	Slope (± SD)
M. Parathion					
	Stoneville(S)	200	0.16 (0.096-0.24)	2.92 (1.45-9.63)	1.01 (± 0.15)
	ICI(R)	145	0.29 (0.13-0.43)	2.20 (1.33-7.24)	1.45 (± 0.34)[e]
	Uvalde(R)	100	0.21 (0.13-0.29)	1.08 (0.62-4.43)	1.78 (± 0.43)
	Hearne(R)	130	0.065 (0.053-0.080)	0.17 (0.13-0.28)	3.06 (± 0.50)
M. Par. + CDF					
	Stoneville(S)	321	0.016 (0.090-0.025)	0.72 (0.35-2.12)	0.77 (± 0.092)
	ICI(R)	100	0.20 (0.071-0.32)	2.31 (0.99-49.76)	1.20 (± 0.36)
	Uvalde(R)	100	0.064 (0.020-0.10)	0.58 (0.31-3.78)	1.34 (± 0.37)
	Hearne(R)	100	0.032 (0.017-0.045)	0.14 (0.092-0.34)	2.00 (± 0.47)
Endosulfan					
	Stoneville(S)	290	1.22 (1.00-1.48)	5.28 (3.86-8.27)	2.01 (± 0.21)
	ICI(R)	185	0.53 (0.32-0.76)	5.35 (3.06-15.34)	1.28 (± 0.23)
	Uvalde(R)	150	3.47 (2.52-5.05)	21.62 (12.13-62.45)	1.61 (± 0.27)
	Hearne(R)[f]	120	1.11 (0.86-1.41)	3.29 (2.42-5.46)	2.72 (± 0.42)
Endos. + CDF					
	Stoneville(S)	101	0.069 (0.038-0.099)	0.32 (0.21-0.80)	1.91 (± 0.43)
	ICI(R)	200	0.12 (0.082-0.16)	0.71 (0.49-1.34)	1.66 (± 0.26)
	Uvalde(R)[d]	-	-	-	-
	Hearne(R)[f]	195	0.65 (0.46-0.91)	5.64 (3.19-15.03)	1.37 (± 0.21)

[a] Number of larvae tested excluding controls.
[b] Concentrations are expressed in micrograms of insecticide per vial.
[d] Data not available due to loss of strain.
[e] Mean slopes for insecticides and insecticides + CDF significantly different for resistant strains ($P<0.05$; t-test).
[f] Hearne strain after 10 generations in the lab (R ratio to cypermethrin = 3).

Table 2. (continued)

Insecticide	Strain	n[a]	LC_{50} (95% CL)[b]	LC_{90} (95% CL)[b]	Slope (± SD)
Thiodicarb					
	Stoneville(S)	198	1.95 (1.22-2.99)	34.07 (16.41-123.09)	1.03 (± 0.16)
	ICI(R)	315	0.74 (0.27-1.37)	195.01 (31.27-65,300)	0.53 (± 0.14)[e]
	Uvalde(R)	126	33.22 (18.64-90.61)	851.73 (209.41-84,098)	0.91 (± 0.25)
	Hearne(R)	125	1.01 (0.0001-3.28)	85.66 ($26.15\text{-}9\text{x}10^5$)	0.66 (± 0.27)
Thiod. + CDF					
	Stoneville(S)	285	0.035 (0.023-0.050)	0.74 (0.39-1.95)	0.97 (± 0.11)
	ICI(R)	100	0.17 (0.11-0.23)	0.66 (0.45-1.40)	2.17 (± 0.44)
	Uvalde(R)	269	0.11 (0.010-0.27)	4.14 (2.30-16.09)	0.82 (± 0.20)
	Hearne(R)	104	0.39 (0.24-0.52)	1.47 (1.01-3.23)	2.22 (± 0.49)
Cyp. + Thiod.					
	Stoneville(S)	90	0.085 (0.068-0.11)[g]	0.20 (0.15-0.33)[g]	3.54 (± 0.62)[c]
	ICI(R)	95	2.91 (1.15-5.90)[g]	56.93 (16.87-13,643)[g]	0.99 (± 0.33)
	Uvalde(R)	175	0.62 (0.12-1.22)[g]	9.61 (5.60-30.14)[g]	1.07 (± 0.26)
	Hearne(R)	150	0.45 (0.25-0.71)[g]	6.50 (3.16-26.60)[g]	1.11 (± 0.20)
Cyp.+Th.+CDF					
	Stoneville(S)	165	0.028 (0.020-0.038)[g]	0.18 (0.11-0.39)[g]	1.56 (± 0.22)
	ICI(R)	75	0.040 (0.001-0.090)[g]	0.71 (0.31-35.66)[g]	1.02 (± 0.38)
	Uvalde(R)	169	0.058 (0.023-0.20)[g]	1.21 (0.55-7.85)[g]	0.97 (± 0.22)
	Hearne(R)	125	0.093 (0.057-0.14)[g]	0.93 (0.44-4.70)[g]	1.28 (± 0.27)

[a] Number of larvae tested excluding controls.
[b] Concentrations are expressed in micrograms of insecticide per vial.
[c] Slopes for insecticide and insecticide + CDF significantly different for the Stoneville strain ($P<0.05$; t-test).
[e] Mean slopes for insecticides and insecticides + CDF significantly different for resistant strains ($P<0.05$; t-test).
[g] Concentration of each insecticide.

Control mortality never exceeded 10%. Chlordimeform alone was almost nontoxic compared with the insecticides tested. The LC_{50}'s for chlordimeform were 25.92 and 44.06 µg per vial for susceptible and ICI neonate larvae, respectively. Without chlordimeform, LC_{50}'s ranged from 0.084 to 1.95 µg insecticide per vial for the susceptible strain and from 0.065 to 33.02 µg insecticide per vial for the resistant strains. With chlordimeform, LC_{50}'s ranged from 0.008 to 0.11 µg insecticide per vial for the susceptible strain and from 0.032 to 1.74 µg insecticide per vial for the resistant strains.

Resistance ratios and chlordimeform synergism are presented in Tables 3 and 4. Tests with pyrethroids revealed resistance levels of 10.9- to 52.2-fold for cypermethrin and from 9- to 47.9-fold for fenvalerate at the LC_{50} level. At the LC_{90}, resistance ratios for cypermethrin were higher for all the resistant strains with as much as 392.2-fold being observed for the Uvalde strain. However, resistance to fenvalerate was not greater at the LC_{90} than at the LC_{50}. Resistance was greater in the ICI strain for fenvalerate than for cypermethrin. For the Hearne strain the resistance level was similar for both insecticides. Leonard et al. (1987) also found variations in resistance to these insecticides in third instar TBW from different resistant field populations.

Chlordimeform synergized both pyrethroids against all strains. Synergism was greater for cypermethrin than for fenvalerate. A synergism level as high as 39.2-fold at the LC_{50} level was observed for cypermethrin with chlordimeform against the Uvalde strain. Synergism occurred with both susceptible and resistant strains and no consistent pattern of synergism could be established for the different strains and the two pyrethroids tested. Even though chlordimeform did not completely block resistance to either pyrethroid (i. e., resistance was still observed when insecticide with chlordimeform toxicities were compared between susceptible and resistant strains), it increased toxicity of pyrethroids to a level such that the LC_{50} for the combination of an insecticide with chlordimeform for the resistant strains usually became nearly equal to the LC_{50} for the

Table 3. Resistance ratios[a] for insecticides ± chlordimeform (CDF) at the LC_{50} and LC_{90} levels for different strains of neonate TBW larvae

Insecticide(s) ± CDF	ICI		Uvalde		Hearne	
	LC_{50}	LC_{90}	LC_{50}	LC_{90}	LC_{50}	LC_{90}
Cypermethrin	20.3	60.1	52.2	392.2	10.9	26.8
Cyperm. + CDF	24.4	11.9	25.6	8.0	7.1	0.7
Fenvalerate	47.9	51.9	-	-	9.0	6.1
Fenval. + CDF	32.8	24.2	-	-	14.8	5.1
Profenofos	2.5	1.9	1.8	2.7	2.1	2.7
Profen. + CDF	2.2	4.1	2.5	1.9	3.9	3.3
Acephate	0.5	0.6	2.0	23.8	1.3	3.2
Aceph. + CDF	6.5	0.4	15.8	1.2	5.2	0.6
M. Parathion	1.8	0.8	1.3	0.4	0.4	0.1
M. Parat. + CDF	12.5	3.2	4.0	0.8	2.0	0.2
Endosulfan	0.4	1.0	2.8	4.1	0.9	0.6
Endos. + CDF	1.7	2.2	-	-	9.4	17.6
Thiodicarb	0.4	5.7	16.9	25.0	0.5	2.5
Thiod. + CDF	4.9	0.9	3.1	5.6	11.1	2.0
Cyp. + Thiod.	34.2	284.7	7.3	0.2	5.3	32.5
Cyp.+Th.+CDF	1.4	3.9	2.1	6.7	3.3	5.2

[a] Calculated by dividing the LC_{50} (or LC_{90}) for the resistant strain by the LC_{50} (or LC_{90}) for the susceptible strain.

Table 4. Synergism[a] by chlordimeform of insecticides at the LC_{50} and LC_{90} levels for different strains of neonate TBW larvae

Insecticide(s)	Stoneville		ICI		Uvalde		Hearne	
	LC_{50}	LC_{90}	LC_{50}	LC_{90}	LC_{50}	LC_{90}	LC_{50}	LC_{90}
Cypermethrin	18.8	1.7	16.0	8.4	39.2	83.2	29.6	61.1
Fenvalerate	6.0	7.9	8.8	16.9	-	-	3.7	9.5
Profenofos	4.2	2.6	4.8	1.2	3.0	3.6	2.3	2.1
Acephate	42.4	3.3	3.3	4.5	5.3	62.4	11.0	19.5
M. Parathion	10.0	4.1	1.5	1.0	3.3	1.9	2.0	1.2
Endosulfan	17.7	16.5	4.4	7.5	-	-	1.7	0.6
Thiodicarb	55.7	46.0	4.4	295.5	300.2	205.7	2.6	58.3
Cyp. + Thiodic.	3.0	1.1	72.8	80.2	10.7	7.9	4.8	7.0

[a] Calculated by dividing the LC_{50} (or LC_{90}) for the insecticide by the LC_{50} (or LC_{90}) for the insecticide + CDF.

insecticide only for the susceptible strain.

Neonate larvae showed low or no tolerance to the organophosphates profenofos, acephate, and methyl parathion. The resistance ratios for these chemicals at the LC_{50} level were 2.5-fold or less. Therefore, these chemicals are possible alternates for controlling first instars in instances where pyrethroid resistance is present.

For years methyl parathion was used for *Heliothis* spp. control in cotton. In IPM programs, methyl parathion is too disruptive of natural enemies and may cause pest outbreaks when used early in the season. Another restriction on the widespread use of methyl parathion as a pyrethroid-alternate insecticide is that TBW resistance to this compound was previously observed throughout the cotton belt (Wolfenbarger & McGarr 1970, Graves et al. 1973, Pieters & Boyette 1977, Crowder et al. 1979, Twine & Reynolds 1980) and selection pressure might easily select for resistance again. Therefore, among the organophosphates, the S-alkyl phosphorothiolates are possibly the best alternate insecticides to the pyrethroids where resistance to the latter is present. S-alkyl phosphorothiolates seem also to be safer on natural enemies than other insecticide types. Plapp & Vinson (1977) reported that these insecticides were relatively safer on *Campoletis sonorensis* , a parasite of the TBW, than other insecticides such as phosphorothionates, a carbamate, a formamidine, and several organochlorines.

Chlordimeform synergized all organophosphates against all strains. Profenofos synergism by chlordimeform was greatest against the susceptible and ICI strains. With acephate or methyl parathion, chlordimeform synergism was higher against the susceptible strain than against any of the resistant strains. As much as 42.4-fold synergism at the LC_{50} level was observed with acephate plus chlordimeform against the susceptible strain. Usually, resistance increased when chlordimeform was combined with organophosphates. Nevertheless, the addition of chlordimeform to these chemicals made them equally or more toxic to resistant larvae than the insecticides only to

susceptible larvae.

Uvalde neonate larvae showed substantial resistance to the oxime carbamate thiodicarb, but Hearne and ICI larvae were more susceptible to the insecticide than the susceptible strain. In previous tests with the ICI strain, a 120-fold resistance to thiodicarb was observed in first instar larvae (Campanhola & Plapp 1987). The tests reported in the present study were conducted with a different sample of the ICI strain. Reasons why the resistance level changed and if there is a cross resistance relationship for pyrethroids and oxime carbamates are not known. Also, the test used may not be appropriate for thiodicarb, an orally toxic chemical.

Chlordimeform synergism with thiodicarb was very high for the susceptible and Uvalde strains, with levels of 55.7- and 300.2-fold at the LC_{50}, respectively. High levels of synergism were also observed at the LC_{90}, 46.0- and 205.7-fold, respectively. For the other two resistant strains, ICI and Hearne, synergism was 4.4- and 2.6-fold at the LC_{50}, respectively, and 295.5- and 58.3-fold at the LC_{90}, respectively. Even though chlordimeform did not block resistance completely, it increased thiodicarb toxicity to resistant larvae to a level greater than that observed for the insecticide only for susceptible larvae.

Cotoxicity coefficients at the LC_{50} level for the combination of cypermethrin plus thiodicarb for the ICI, Uvalde, and Hearne strains were 0.2, 10.2, and 1.4, respectively, indicating synergistic interaction only with the Uvalde strain. Chlordimeform synergized this combination 72.8- and 10.7-fold at the LC_{50} against the ICI strain and the Uvalde strains, respectively , but only 3-fold against the susceptible strain. However, with the resistant strains, no significant changes were observed in the slope of the probit regression lines with the addition of chlordimeform.

Neonate TBW larvae showed almost no tolerance to endosulfan. The ICI strain was even more susceptible to that insecticide than the susceptible strain. Thus, there

seems to be no cross resistance between pyrethroids and endosulfan in the TBW. This insecticide seems to be appropriate for use in resistance management as in IPM programs. A study with a predator, *Chrysopa carnea*, showed that endosulfan was more toxic to the TBW than to the predator (Plapp & Bull 1978).

Chlordimeform synergism with endosulfan was higher against the susceptible than against resistant strains. Consequently, there seems to be not little advantage in combining chlordimeform with endosulfan for control of pyrethroid-resistant TBWs. However, when chlordimeform was combined with this toxicant, the LC_{50} for the resistant strains became lower than the LC_{50} for the insecticide only for the susceptible strain. Therefore, endosulfan alone or combined with chlordimeform can be another alternative for controlling pyrethroid-resistant TBWs.

The t-test was used for comparisons between the mean slopes of response lines to each insecticide or insecticide combination alone and combined with chlordimeform for resistant strains (Table 2). Chlordimeform increased the mean slopes for fenvalerate, acephate, and thiodicarb, but decreased the slope for methyl parathion. No significant changes in slope were observed for cypermethrin, profenofos, endosulfan, and cypermethrin plus thiodicarb. Thus, no consistent pattern in slope change was observed with the combination of chlordimeform with insecticides for resistant neonate TBW larvae.

Slopes of response lines to each insecticide or insecticide combination alone and combined with chlordimeform were also compared for the susceptible strain (Table 2). Chlordimeform combination with insecticides in most cases did not change the slopes of response lines for susceptible neonate larvae compared with the slopes for the insecticides only. However, chlordimeform significantly decreased the slopes for cypermethrin, acephate, and cypermethrin plus thiodicarb.

Resistance spectra were approximately the same for all the resistant strains.

However, resistance levels to insecticides of different classes did not follow a consistent pattern. Chlordimeform synergism against different resistant strains was also variable for different insecticides. In summary, chlordimeform synergized all insecticides tested. The organophosphates, alone or combined with chlordimeform, the carbamate thiodicarb plus chlordimeform, and the combination cypermethrin plus thiodicarb plus chlordimeform are possible alternate toxicants for pyrethroid-resistant TBW control.

Bioassays with the Stoneville and ICI strains of the TBW

Stoneville and ICI neonate larvae were bioassayed extensively with additional insecticides and insecticide combinations. Most tests with resistant larvae were conducted with a second sample of the ICI strain with a resistance level at the LC_{50} to cypermethrin of about 23-fold from generations 1 to 3. Some tests were performed with the fourth generation of those insects . At this time, the resistance of neonate larvae to cypermethrin dropped to about 11-fold. Thus, the resistance level was not constant and declined rapidly in the laboratory.

Results of toxicity tests for pyrethroid insecticides, alone and combined with chlordimeform, with susceptible and ICI neonate larvae are shown in Table 5. Results are also presented for cypermethrin combined with piperonyl butoxide and with chlordimeform and piperonyl butoxide. The toxicity, in LC_{50}, of the pyrethroids to the susceptible strain ranged from 0.012 µg per vial for essfenvalerate to 0.84 µg per vial for permethrin. For the ICI strain the LC_{50}'s varied from 0.41 µg per vial for cyfluthrin to 11.50 µg per vial for fenvalerate. The most toxic pyrethroids to the susceptible strain were also the most toxic to the ICI strain.

Resistance ratios at the LC_{50} and LC_{90} levels for the ICI neonate larvae to the pyrethroids, alone and combined with chlordimeform, are reported in Fig. 1. Resistance extended to all pyrethroids studied. The highest level resistance at the LC_{50} level was

Table 5. Toxicity of pyrethroid insecticides ± chlordimeform (CDF) and/or piperonyl butoxide (PB) to susceptible (S) and resistant (R) neonate TBW larvae

Insecticide	Strain	n[a]	LC_{50} (95% CL)[b]	LC_{90} (95% CL)[b]	Slope (± SD)
Cypermethrin					
	Stoneville(S)	100	0.22 (0.15-0.29)	0.84 (0.57-1.80)	2.19 (± 0.43)
	ICI(R)[c]	170	2.41 (1.21-3.74)	42.79 (16.91-659.62)	1.03 (± 0.27)
Cyperm. + CDF					
	Stoneville(S)	100	0.023 (0.015-0.032)	0.11 (0.066-0.28)	1.94 (± 0.41)
	ICI(R)[c]	110	0.44 (0.25-0.73)	4.86 (2.12-42.75)	1.23 (± 0.30)
Cyperm. + PB					
	Stoneville(S)	225	0.035 (0.025-0.046)	0.24 (0.15-0.57)	1.53 (± 0.26)
	ICI(R)[c]	140	0.47 (0.28-0.68)	4.06 (2.07-22.45)	1.37 (± 0.32)
Cyp.+ CDF + PB					
	Stoneville(S)	225	0.018 (0.013-0.024)	0.12 (0.079-0.24)	1.57 (± 0.22)
	ICI(R)[c]	130	0.071 (0.050-0.096)	0.34 (0.22-0.74)	1.88 (± 0.31)[d]
Permethrin					
	Stoneville(S)	168	0.84 (0.61-1.14)	4.54 (2.93-8.94)	1.75 (± 0.23)[d]
	ICI(R)	154	5.28 (2.65-9.63)	179.73 (56.25-3,526)	0.83 (± 0.20)[d]
Perm.+ CDF					
	Stoneville(S)	238	0.068 (0.045-0.11)	1.35 (0.65-4.22)	0.90 (± 0.12)
	ICI(R)	78	1.23 (0.74-1.65)	3.63 (2.61-7.35)	2.73 (± 0.64)
Essfenvalerate					
	Stoneville(S)	128	0.012 (0.081-0.016)	0.060 (0.039-0.14)	1.84 (± 0.34)[d]
	ICI(R)[e]	275	0.74 (0.54-1.01)	8.17 (4.61-21.28)	1.23 (± 0.17)
Essfenv. + CDF					
	Stoneville(S)	169	0.0046 (0.00015-0.012)	0.55 (0.16-85.66)	0.62 (± 0.20)
	ICI(R)[e]	223	0.25 (0.17-0.35)	3.10 (1.60-10.82)	1.17 (± 0.20)

Table 5. (continued)

Insecticide	Strain	n[a]	LC_{50} (95% CL)[b]	LC_{90} (95% CL)[b]	Slope (± SD)
Deltamethrin					
	Stoneville(S)	322	0.013 (0.0098-0.017)	0.12 (0.074-0.26)	1.35 (± 0.19)
	ICI(R)[e]	175	1.77 (1.06-2.61)	22.61 (10.71-128.39)	1.16 (± 0.25)
Deltam. + CDF					
	Stoneville(S)	191	0.0042 (0.0031-0.0057)	0.025 (0.016-0.048)	1.66 (± 0.20)
	ICI(R)[e]	210	0.40 (0.28-0.61)	4.90 (2.37-17.87)	1.18 (± 0.18)
Tralomethrin					
	Stoneville(S)	221	0.035 (0.019-0.064)	2.42 (0.80-18.22)	0.70 (± 0.11)[d]
	ICI(R)[e]	280	1.79 (1.27-2.60)	29.32 (13.76-116.84)	1.06 (± 0.17)
Tralom. + CDF					
	Stoneville(S)	184	0.015 (0.0094-0.022)	0.19 (0.10-0.56)	1.14 (± 0.17)
	ICI(R)[e]	155	0.33 (0.23-0.44)	1.93 (1.19-4.71)	1.66 (± 0.28)
Biphenthrin					
	Stoneville(S)	187	0.015 (0.0082-0.022)	0.21 (0.11-0.58)	1.12 (± 0.18)[d]
	ICI(R)[e]	220	0.83 (0.45-1.33)	26.54 (10.07-230.30)	0.85 (± 0.18)
Biphent. + CDF					
	Stoneville(S)	189	0.0095 (0.0069-0.013)	0.054 (0.035-0.10)	1.70 (± 0.21)
	ICI(R)[e]	207	0.22 (0.064-2.20)	2.42 ($0.65\text{-}4\text{x}10^{8}$)	1.24 (± 0.34)
Cyhalothrin					
	Stoneville(S)	212	0.040 (0.027-0.057)	0.44 (0.24-1.18)	1.22 (± 0.17)
	ICI(R)[e]	215	2.16 (1.63-2.80)	13.00 (8.46-26.40)	1.64 (± 0.23)
Cyhalot. + CDF					
	Stoneville(S)	254	0.0038 (0.0026-0.0053)	0.045 (0.027-0.097)	1.20 (± 0.14)
	ICI(R)[e]	215	0.46 (0.34-0.61)	3.21 (2.01-6.88)	1.52 (± 0.21)

Table 5. (continued)

Insecticide	Strain	n[a]	LC_{50} (95% CL)[b]	LC_{90} (95% CL)[b]	Slope (± SD)
Cyfluthrin					
	Stoneville(S)	241	0.022 (0.015-0.032)	0.27 (0.16-0.61)	1.18 (± 0.15)
	ICI(R)[e]	100	0.41 (0.17-0.65)	3.31 (1.84-12.73)	1.41 (± 0.33)[d]
Cyflut. + CDF					
	Stoneville(S)	208	0.019 (0.013-0.027)	0.19 (0.11-0.44)	1.28 (± 0.16)
	ICI(R)[e]	60	0.17 (0.12-0.22)	0.40 (0.29-0.76)	3.43 (± 0.76)
Fluvalinate					
	Stoneville(S)	225	0.66 (0.43-0.95)	6.87 (4.28-13.77)	1.26 (± 0.16)
	ICI(R)[e]	80	8.34 (4.02-12.63)	47.70 (27.32-204.62)	1.69 (± 0.44)
Fluval. + CDF					
	Stoneville(S)	164	0.038 (0.022-0.062)	0.69 (0.31-2.93)	1.01 (± 0.17)
	ICI(R)[e]	100	0.67 (0.40-1.01)	4.51 (2.52-15.53)	1.55 (± 0.33)

[a] Number of larvae tested excluding controls.
[b] Concentrations are expressed in micrograms of insecticide per vial.
[c] 2nd Sample of the ICI strain after 4 generations in the laboratory without selection pressure with insecticides.
[d] Slopes for insecticide and insecticide + synergist(s) significantly different for the same strain ($P<0.05$; t-test).
[e] 2nd Sample of the ICI strain after 1-3 generations in the laboratory without selection pressure with insecticides.

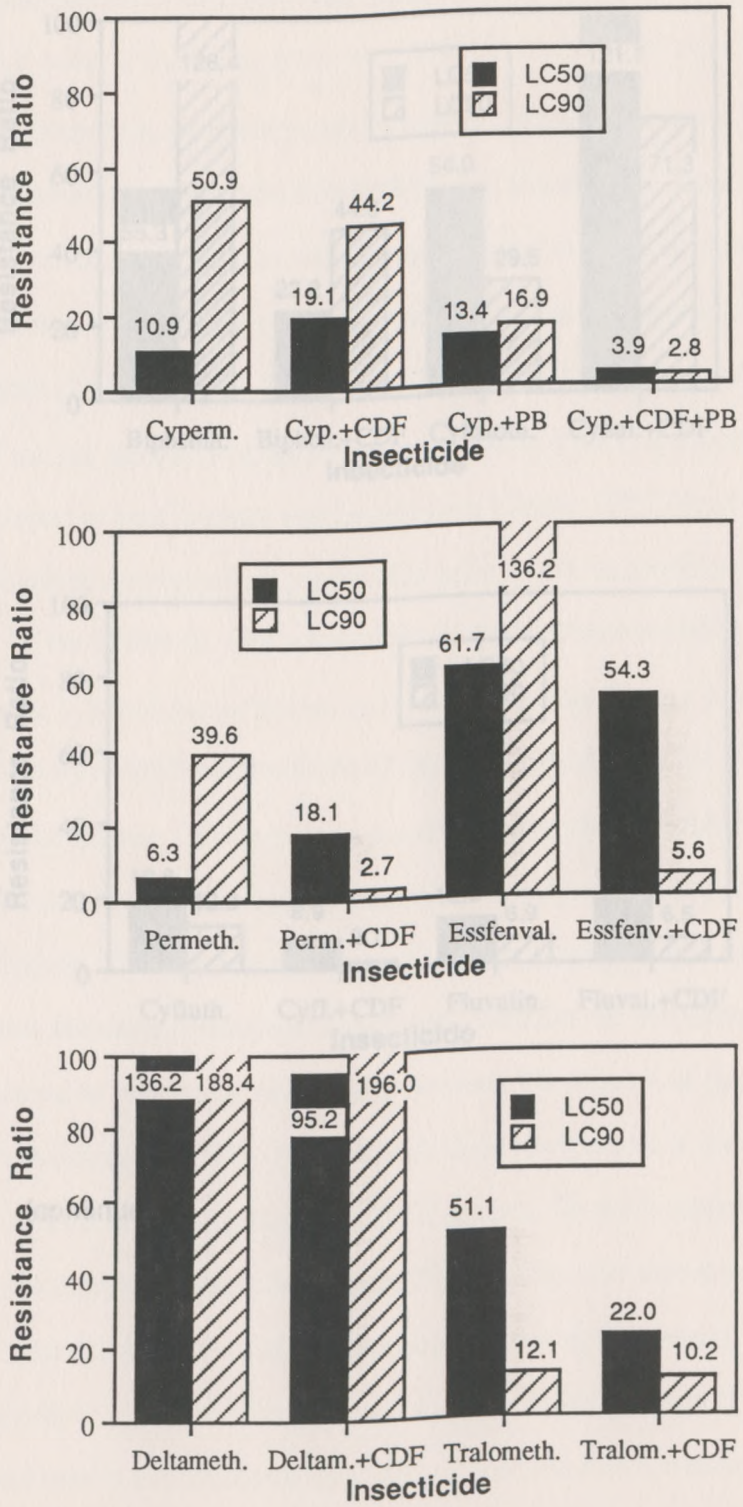

Fig. 1. Resistance ratios for pyrethroid insecticides ± chlordimeform (CDF) and/or piperonyl butoxide (PB) at the LC_{50} and LC_{90} levels for ICI neonate TBW larvae.

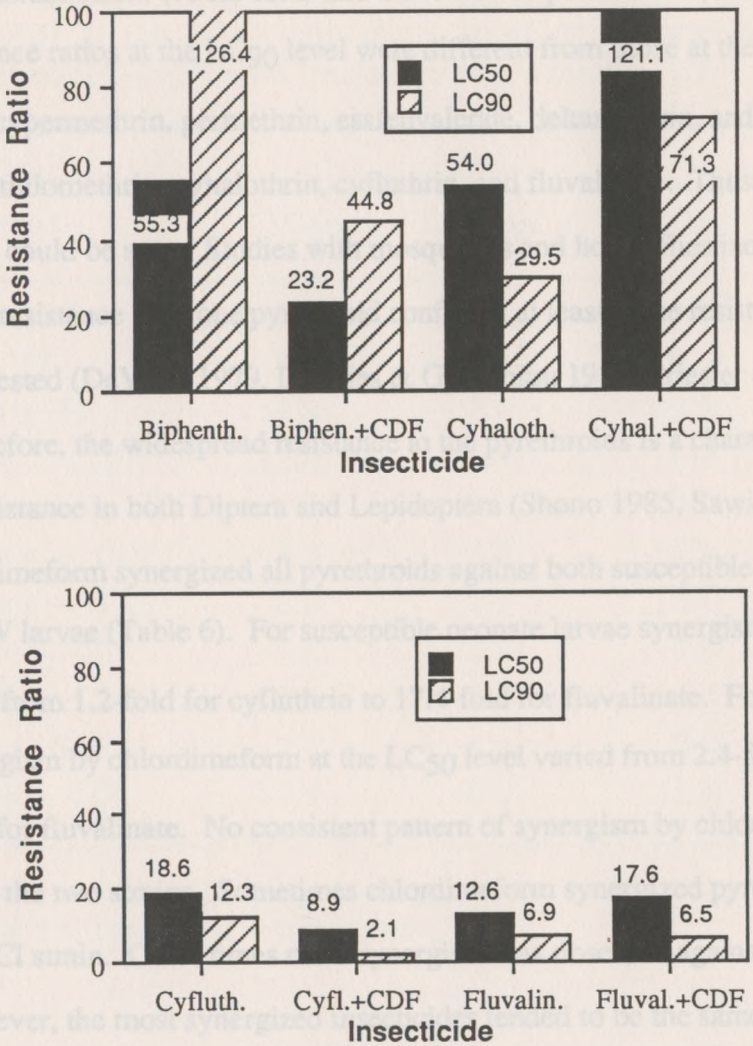

Fig.1. (continued).

observed to deltamethrin (136.2-fold) and the lowest to permethrin (6.3-fold). In most cases, resistance ratios at the LC_{90} level were different from those at the LC_{50}. They increased to cypermethrin, permethrin, essfenvalerate, deltamethrin, and biphenthrin, and decreased to tralomethrin, cyhalothrin, cyfluthrin, and fluvalinate. Thus, no clear pattern of variability could be seen. Studies with mosquitoes and house flies indicated that selection for resistance with one pyrethroid conferred at least some resistance to all other pyrethroids tested (DeVries 1979, DeVries & Georghiou 1980, Priester & Georghiou 1980). Therefore, the widespread resistance to the pyrethroids is a characteristic of target site (kdr) resistance in both Diptera and Lepidoptera (Shono 1985, Sawicki 1978).

Chlordimeform synergized all pyrethroids against both susceptible and resistant neonate TBW larvae (Table 6). For susceptible neonate larvae synergism at the LC_{50} level ranged from 1.2-fold for cyfluthrin to 17.4-fold for fluvalinate. For the ICI neonate larvae, synergism by chlordimeform at the LC_{50} level varied from 2.4-fold for cyfluthrin to 12.4-fold for fluvalinate. No consistent pattern of synergism by chlordimeform was observed for the two strains. Sometimes chlordimeform synergized pyrethroids more against the ICI strain. Other times more synergism was observed against the susceptible strain. However, the most synergized insecticides tended to be the same for both strains.

There seemed to be a linear relationship between the toxicity of pyrethroids and synergism by chlordimeform (Fig. 2). The least toxic compounds tended to be more synergized by chlordimeform against both TBW strains. Overall, chlordimeform was a good pyrethroid synergist against the resistant TBW strains and also it increased the toxicity of pyrethroids with lower effectiveness to the susceptible strain.

Resistance levels were similar with and without chlordimeform (Fig. 1). Thus, while chlordimeform is an insecticide synergist, it does not block resistance. This may be a typical response of target site insecticide synergists.

No consistent pattern of change in slope of response lines was observed with the

Table 6. Synergism[a] by chlordimeform (CDF) and/or piperonyl butoxide (PB) at the LC_{50} and LC_{90} levels for pyrethroid insecticides against susceptible (S) and resistant (R) neonate TBW larvae

Insecticide(s) + Synergist	Stoneville(S)		ICI(R)	
	LC_{50}	LC_{90}	LC_{50}	LC_{90}
Cyperm. + CDF	9.6	7.6	5.5	8.8
Cyperm. + PB	6.3	3.5	5.1	10.5
Cyp. + CDF + PB	12.2	7.0	33.9	125.9
Permethrin + CDF	12.4	3.4	4.3	49.5
Essfenvalerate + CDF	2.6	0.1	3.0	2.6
Deltamethrin + CDF	3.1	4.8	4.4	4.6
Tralomethrin + CDF	2.3	12.7	5.4	15.2
Biphenthrin + CDF	1.6	3.9	3.8	11.0
Cyhalothrin + CDF	10.5	9.8	4.7	4.1
Cyfluthrin + CDF	1.2	1.4	2.4	8.3
Fluvalinate + CDF	17.4	10.0	12.4	10.6

[a] Calculated by dividing the LC_{50} (or LC_{90}) for the insecticide by the LC_{50} (or LC_{90}) for the insecticide + synergist(s).

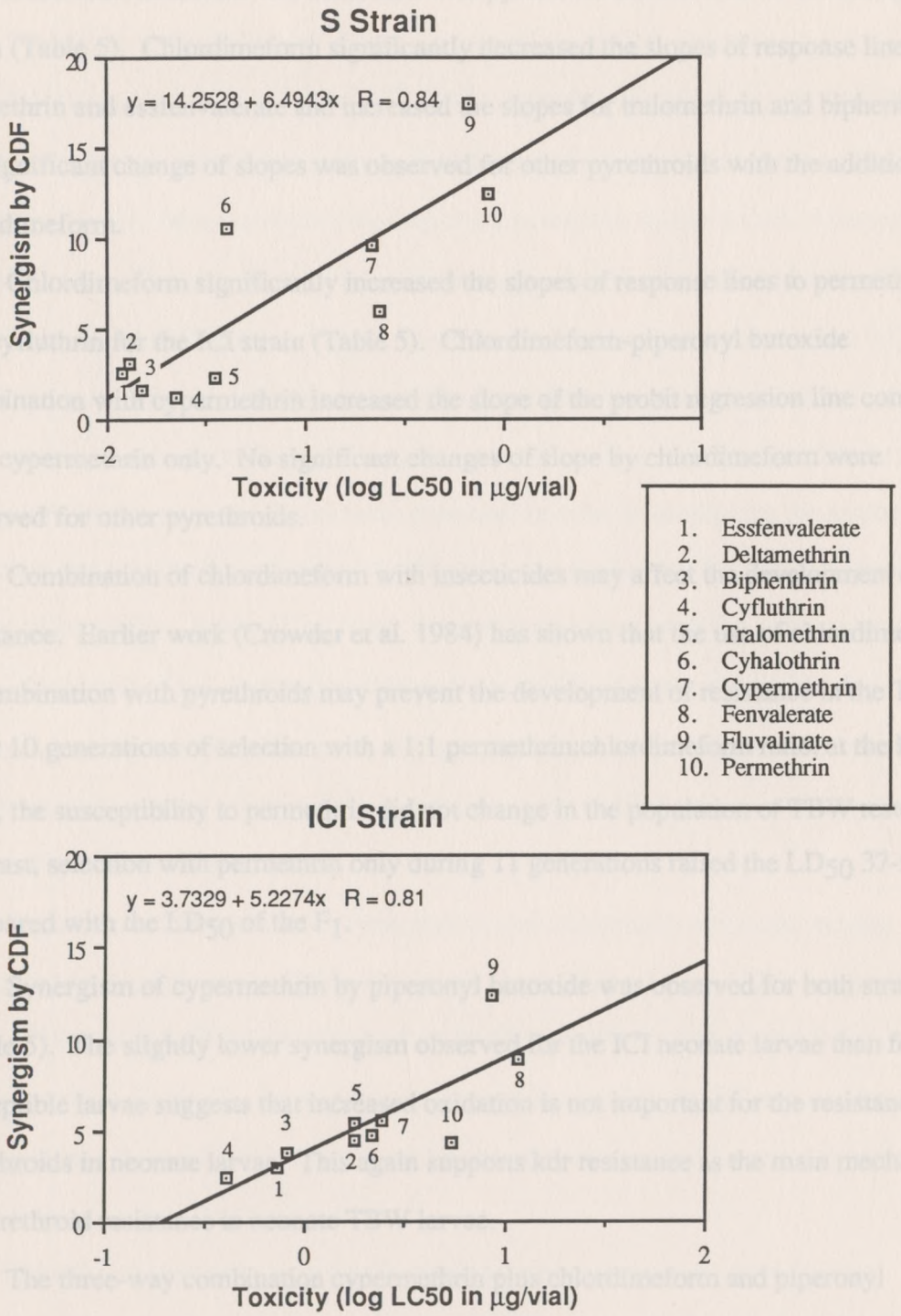

Fig. 2. Relationships between synergism by chlordimeform (CDF) at the LC_{50} level and toxicity of pyrethroid insecticides to susceptible (S) and resistant (R) neonate TBW larvae.

t-test when chlordimeform was combined with pyrethroid insecticides for the susceptible strain (Table 5). Chlordimeform significantly decreased the slopes of response lines for permethrin and essfenvalerate and increased the slopes for tralomethrin and biphenthrin. No significant change of slopes was observed for other pyrethroids with the addition of chlordimeform.

Chlordimeform significantly increased the slopes of response lines to permethrin and cyfluthrin for the ICI strain (Table 5). Chlordimeform-piperonyl butoxide combination with cypermethrin increased the slope of the probit regression line compared with cypermethrin only. No significant changes of slope by chlordimeform were observed for other pyrethroids.

Combination of chlordimeform with insecticides may affect the development of resistance. Earlier work (Crowder et al. 1984) has shown that the use of chlordimeform in combination with pyrethroids may prevent the development of resistance in the TBW. After 10 generations of selection with a 1:1 permethrin:chlordimeform ratio, at the LD_{80} level, the susceptibility to permethrin did not change in the population of TBW tested. In contrast, selection with permethrin only during 11 generations raised the LD_{50} 37-fold compared with the LD_{50} of the F_1.

Synergism of cypermethrin by piperonyl butoxide was observed for both strains (Table 5). The slightly lower synergism observed for the ICI neonate larvae than for the susceptible larvae suggests that increased oxidation is not important for the resistance to pyrethroids in neonate larvae. This again supports kdr resistance as the main mechanism of pyrethroid resistance in neonate TBW larvae.

The three-way combination cypermethrin plus chlordimeform and piperonyl butoxide almost totally blocked resistance in the ICI neonate larvae. At the LC_{90} level, only 2.8-fold resistance to that combination was observed. The LC_{50} and LC_{90} values for the three-way combination for the ICI strain were lower than the respectives values

for cypermethrin only for the susceptible strain.

Toxicity data for additional organophosphate insecticides, alone and combined with chlordimeform, are listed in Table 7. The LC_{50}'s ranged from 0.15 µg per vial for chlorpyrifos for the susceptible strain to 3.35 µg per vial for monocrotophos, also for the susceptible strain. Monocrotophos was much more toxic to resistant than to susceptible neonate larvae. This was the only insecticide tested where I obtained such a result.

Virtually no resistance to organophosphate insecticides was present in the ICI neonate larvae (Fig. 3). For the cases where the resistance ratios were below 1, that is, the insecticides or insecticide combinations were more toxic to the resistant than to the susceptible larvae, negative values were presented in order to emphasize the results. The resistance ratios at the LC_{50} level varied from 0.1 (no resistance) for monocrotophos to 2.0 for chlorpyrifos. At the LC_{90} level, the resistance ratios were the same or lower than at the LC_{50} level. Thus, organophosphates are possible alternate insecticides for pyrethroid-resistant TBW. However, chlorpyrifos and monocrotophos, are likely to disrupt natural enemies and should be restricted for use in IPM programs (Plapp & Vinson 1977, Plapp & Bull 1978).

Organophosphate insecticide synergism by chlordimeform was quite variable against the susceptible strain (Table 8). Levels ranged from 3.2- to 152.3-fold at the LC_{50}. The only significant change in slope by chlordimeform was observed for monocrotophos (Table 7). Synergism was always low against the ICI neonate larvae, varying from 1.8- to 4.8-fold. For this strain chlordimeform significantly decreased the slope of the response line to sulprofos.

Chlordimeform tended to synergize the least toxic compounds more against susceptible than against resistant larvae. However, no clear relationship between toxicity and synergism by chlordimeform could be established for all the organophosphates tested with either strain (Tables 2, 4, 7, 8).

Table 7. Toxicity of organophosphate insecticides ± chlordimeform (CDF) and/or piperonyl butoxide (PB) to susceptible (S) and resistant (R) neonate TBW larvae

Insecticide	Strain	n[a]	LC_{50} (95% CL)[b]	LC_{90} (95% CL)[b]	Slope (± SD)
Chlorpyrifos					
	Stoneville(S)	195	0.15 (0.12-0.18)	0.53 (0.37-0.95)	2.32 (± 0.33)
	ICI(R)[c]	75	0.30 (0.22-0.37)	0.61 (0.48-0.98)	4.19 (± 0.96)
Chlorp. + CDF					
	Stoneville(S)	100	0.047 (0.034-0.061)	0.15 (0.11-0.29)	2.55 (± 0.49)
	ICI(R)[c]	100	0.082 (0.053-0.11)	0.32 (0.22-0.69)	2.17 (± 0.44)
Monocrotophos					
	Stoneville(S)	410	3.35 (2.17-4.45)	50.04 (27.86-155.81)	1.09 (± 0.19)[d]
	ICI(R)[c]	200	0.31 (0.20-0.43)	2.74 (1.66-6.62)	1.36 (± 0.22)
Monocr. + CDF					
	Stoneville(S)	288	0.022 (0.0041-0.047)	4.49 (1.12-206.98)	0.55 (± 0.14)
	ICI(R)[c]	180	0.067 (0.045-0.089)	0.36 (0.25-0.67)	1.74 (± 0.27)
Sulprofos					
	Stoneville(S)	160	0.33 (0.23-0.45)	1.68 (1.13-3.13)	1.81 (± 0.27)
	ICI(R)[c]	145	0.54 (0.36-0.73)	2.53 (1.75-4.75)	1.91 (± 0.32)[d]
Sulpr. + CDF					
	Stoneville(S)	253	0.071 (0.051-0.090)	0.42 (0.28-0.87)	1.67 (± 0.28)
	ICI(R)[c]	123	0.16 (0.082-0.27)	2.64 (1.05-31.90)	1.06 (± 0.26)

[a] Number of larvae tested excluding controls.

[b] Concentrations are expressed in micrograms of insecticide per vial.

[c] 2nd Sample of the ICI strain after 1-3 generations in the laboratory without selection pressure with insecticides.

[d] Slopes for insecticide and insecticide + CDF significantly different for the same strain ($P<0.05$; t-test).

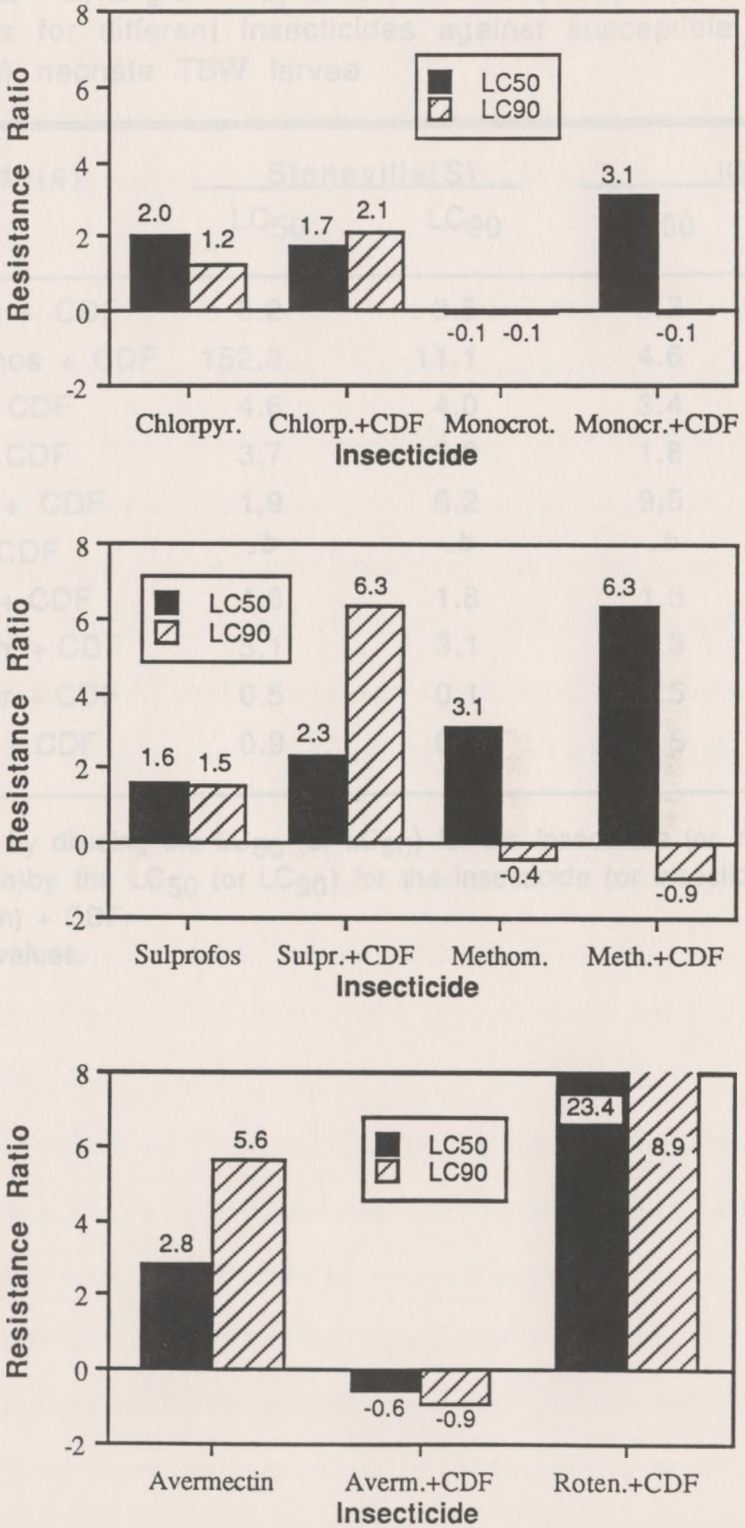

Fig. 3. Resistance ratios for different insecticides ± chlordimeform (CDF) at the LC_{50} and LC_{90} levels for ICI neonate TBW larvae.

Table 8. Synergism[a] by chlordimeform (CDF) at the LC_{50} and LC_{90} levels for different insecticides against susceptible (S) and resistant (R) neonate TBW larvae

Insecticide(s) + CDF	Stoneville(S)		ICI(R)	
	LC_{50}	LC_{90}	LC_{50}	LC_{90}
Chlorpyrifos + CDF	3.2	3.5	3.7	1.9
Monocrotophos + CDF	152.3	11.1	4.6	7.6
Sulprofos + CDF	4.6	4.0	3.4	1.0
Methomyl + CDF	3.7	2.8	1.8	1.3
Avermectin + CDF	1.9	6.2	9.5	38.3
Rotenone + CDF	-[b]	-[b]	-[b]	-[b]
Cyp. + Prof. + CDF	4.6	1.8	1.8	1.3
Cyp. + Aceph. + CDF	5.1	3.1	5.3	16.0
Cyp. + M. Par. + CDF	0.5	0.1	8.5	1.8
Cyp. + Meth. + CDF	0.9	0.7	1.5	2.1

[a] Calculated by dividing the LC_{50} (or LC_{90}) for the insecticide (or insecticide combination)by the LC_{50} (or LC_{90}) for the insecticide (or insecticide combination) + CDF.

[b] Very high values.

Since chlordimeform synergized organophosphate insecticides more against susceptible than against resistant larvae, resistance ratios were higher for the insecticide-chlordimeform combinations than for the insecticides alone (Tables 3 and 8). Only for chlorpyrifos and profenofos was there a slight reduction in the resistance ratio at the LC_{50} level when they were combined with chlordimeform. At the LC_{90} level, a reduction in resistance was observed only for acephate. Nevertheless, chlordimeform increased the toxicity of these compounds to the ICI neonate larvae, making them equally or more toxic to resistant larvae than the insecticide only to the susceptible larvae.

The toxicity of methomyl, with and without chlordimeform, to the susceptible and the ICI neonate larvae is given in Table 9. Methomyl was the most toxic insecticide tested. The LC_{50} for this toxicant was 0.015 µg per vial for the susceptible strain and 0.047 µg per vial for the ICI strain. The former is close to the LC_{50} for deltamethrin for the susceptible strain; the latter is the lowest LC_{50} for any insecticide tested with the ICI strain.

Slight tolerance (3.1-fold at LC_{50}) was observed with methomyl (Table 8). This is similar to what was found for another oxime carbamate, thiodicarb, as reported earlier in this chapter. Chlordimeform was not promising in combination with this toxicant since synergism was less than 2-fold.

Avermectin and rotenone were tested as alternate compounds for control of pyrethroid-resistant TBW and the results, with and without chlordimeform, are shown in Table 9. Avermectin was quite toxic to both susceptible and resistant neonate TBW larvae. A slight level of tolerance seemed to be present in ICI larvae (Fig. 3). However, the tolerance was blocked when the insecticide was combined with chlordimeform. In addition, a high level of avermectin synergism by chlordimeform was observed against the ICI larvae (38.3-fold at the LC_{90} level) (Table 8). In this case chlordimeform significantly increased the slope of the response line to avermectin (Table 9). However,

Table 9. Toxicity of methomyl, avermectin, and rotenone ± chlordimeform (CDF) to susceptible (S) and resistant (R) neonate TBW larvae

Insecticide	Strain	n[a]	LC_{50} (95% CL)[b]	LC_{90} (95% CL)[b]	Slope (± SD)
Methomyl					
	Stoneville(S)	248	0.015 (0.0091-0.023)	0.37 (0.18-1.15)	0.93 (± 0.13)
	ICI(R)[c]	145	0.047 (0.035-0.060)	0.16 (0.12-0.27)	2.40 (± 0.40)
Meth. + CDF					
	Stoneville(S)	243	0.0041 (0.0024-0.0065)	0.13 (0.059-0.46)	0.85 (± 0.12)
	ICI(R)[c]	175	0.026 (0.00032-0.059)	0.12 (0.055-503.60)	1.90 (± 0.53)
Avermectin[d]					
	Stoneville(S)	106	0.29 (0.034-0.52)	3.58 (1.71-92.51)	1.18 (± 0.40)
	ICI(R)[c]	189	0.82 (0.32-1.35)	19.94 (8.57-166.56)	0.93 (± 0.22)[e]
Averm. + CDF[d]					
	Stoneville(S)	100	0.15 (0.088-0.21)	0.58 (0.40-1.19)	2.16 (± 0.45)
	ICI(R)[c]	398	0.086 (0.063-0.12)	0.52 (0.33-1.18)	1.64 (± 0.25)
Rotenone					
	Stoneville(S)	100	>5,000	>>5,000	-
	ICI(R)	104	>5,000	>>5,000	-
Roten. + CDF					
	Stoneville(S)	195	0.10 (0.048-0.15)	1.10 (0.59-6.07)	1.25 (± 0.31)
	ICI(R)	115	2.34 (1.60-3.14)	9.79 (6.64-19.83)	2.07 (± 0.37)

[a] Number of larvae tested excluding controls.

[b] Concentrations are expressed in micrograms of each insecticide per vial.

[c] 2nd Sample of the ICI strain after 1-3 generations in the laboratory without selection pressure with insecticides.

[d] Responses at 48 h.

[e] Slopes for insecticide and insecticide + CDF significantly different for the same strain ($P<0.05$; t-test).

improvements in the formulation of avermectin to increase stability in the field will probably be necessary before this compound can be extensively used.

Rotenone was tested because it was recommended, in combination with piperonyl butoxide, in the northeasthern U. S. for the control of pyrethroid-resistant Colorado potato beetles (J. M. Clark, personal communication). Rotenone alone was nontoxic to both susceptible and resistant larvae (Table 9). Less than 20% mortality was obtained when larvae of both strains were exposed to 5,000 µg rotenone per vial. A very high rotenone synergism by chlordimeform was observed. LC_{50}'s higher than 5,000 µg per vial were brought to 0.10 and 2.34 µg per vial for the susceptible and ICI strains, respectively. Thus, chlordimeform synergized rotenone against both strains and there was 23-fold resistance to the combination.

In the field, the incidence of pyrethroid-resistant TBWs is more frequent late in the season (Plapp 1987). In an attempt to improve the control of resistant TBW at this time, I tested combinations of cypermethrin with insecticides of other classes and the combination of chlorpyrifos plus sulprofos against neonate larvae of both strains.

Toxicity data for insecticide combinations, with and without chlordimeform, are reported in Table 10. The cotoxicity coefficients for cypermethrin combined with profenofos, acephate, methyl parathion, or methomyl for ICI neonate larvae at the LC_{50} level were 9.4, 2.8, 2.4, and 1.6, respectively. For the susceptible strain, the cotoxicity coefficients for those insecticide combinations were 0.8, 2.2, 1.0, and 1.0, respectively. Therefore, synergism was observed with all combinations tested with ICI larvae, but no synergism was observed with cypermethrin combined with profenofos, methyl parathion, or methomyl against susceptible larvae. Also, synergism with insecticide combinations was always higher against the resistant than against the susceptible strain.

The combination of chlorpyrifos plus sulprofos showed a very high synergism (cotoxicity coefficient = 7.7) against resistant neonate larvae. Synergism of this

Table 10. Toxicity of combinations of insecticides ± chlordimeform (CDF) to susceptible (S) and resistant (R) neonate TBW larvae

Insecticide	Strain	n[a]	LC_{50} (95% CL)[b]	LC_{90} (95% CL)[b]	Slope (± SD)
Cyp. + Profen.					
	Stoneville(S)	111	0.069 (0.057-0.086)	0.16 (0.13-0.26)	3.42 (± 0.53)[c]
	ICI(R)	98	0.14 (0.11-0.18)	0.37 (0.27-0.61)	3.06 (± 0.48)
Cyp.+Prof.+ CDF					
	Stoneville(S)	120	0.015 (0.0072-0.022)	0.090 (0.055-0.29)	1.65 (± 0.40)
	ICI(R)	94	0.076 (0.056-0.11)	0.29 (0.18-0.71)	2.22 (± 0.42)
Cyp. + Acephate					
	Stoneville(S)	106	0.097 (0.045-0.14)	0.71 (0.39-3.45)	1.49 (± 0.38)
	ICI(R)	112	0.63 (0.15-1.17)	4.47 (2.65-11.58)	1.51 (± 0.38)[c]
Cyp.+Aceph.+CDF					
	Stoneville(S)	189	0.019 (0.013-0.028)	0.23 (0.12-0.69)	1.19 (± 0.18)
	ICI(R)	75	0.12 (0.087-0.16)	0.28 (0.21-0.47)	3.63 (± 0.79)
Cyp. + M. Parat.					
	Stoneville(S)	100	0.074 (0.058-0.097)	0.21 (0.15-0.39)	2.85 (± 0.48)[c]
	ICI(R)	264	0.11 (0.081-0.13)	0.59 (0.41-1.02)	1.71 (± 0.21)[c]
Cyp.+M.Par.+ CDF					
	Stoneville(S)	100	0.16 (0.084-0.30)	2.04 (0.72-97.14)	1.15 (± 0.35)
	ICI(R)	121	0.013 (0.0046-0.024)	0.33 (0.10-38.33)	0.92 (± 0.29)
Cyp. + Methomyl					
	Stoneville(S)	170	0.014 (0.0096-0.019)	0.083 (0.054-0.17)	1.64 (± 0.24)
	ICI(R)[d]	155	0.029 (0.0069-0.062)	0.11 (0.055-9.72)	2.25 (± 0.56)
Cyp.+Meth.+CDF					
	Stoneville(S)	166	0.016 (0.011-0.023)	0.12 (0.068-0.29)	1.49 (± 0.22)
	ICI(R)[d]	70	0.019 (0.014-0.025)	0.052 (0.036-0.12)	2.91 (± 0.68)

Table 10. (continued)

Insecticide	Strain	n[a]	LC_{50} (95% CL)[b]	LC_{90} (95% CL)[b]	Slope (± SD)
Chlorp. + Sulpr.					
	Stoneville(S)	165	0.029 (0.024-0.033)	0.055 (0.047-0.070)	4.60 (± 0.72)
	ICI(R)[d]	45	0.025 (0.019-0.031)	0.041 (0.032-0.071)	5.91 (± 1.56)

[a] Number of larvae tested excluding controls.
[b] Concentrations are expressed in micrograms of each insecticide per vial.
[c] Slopes for insecticide combinations with and without CDF significantly different for the same strain ($P<0.05$; t-test).
[d] 2nd Sample of the ICI strain after 1-3 generations in the laboratory without selection pressure with insecticides.

combination against the susceptible strain (cotox. coeff. = 3.6) was lower than against the resistant strain .

Fig. 4 lists resistance ratios for the insecticide combinations. Resistance ratios below 1 were changed to negative numbers. The resistance ratios for most insecticide combinations were low, that is, close to or below 2-fold. The highest resistance level (6.5-fold at the LC_{50}) was observed with cypermethrin plus acephate. However, no resistance was found to the combination of chlorpyrifos plus sulprofos. Results indicated that the best possible combinations for pyrethroid-resistant neonate TBW larval control are cypermethrin plus profenofos and chlorpyrifos plus sulprofos. Furthermore, the use of insecticide combinations may be appropriate not only for controlling resistant neonate TBW larvae, but also for preventing resistance development, as has been observed with other insects and mites (Burden et al. 1960, Asquith 1961, Graves et al. 1967, Ozaki et al. 1973, Georghiou 1983, Brindley & Selim 1984).

Chlordimeform substantially synergized only some of the insecticide combinations (Table 8). The highest synergism levels at the LC_{50} were obtained with cypermethrin plus methyl parathion (8.5-fold) and cypermethrin plus acephate (5.3-fold) for ICI larvae. In addition, chlordimeform tended to decrease the resistance ratios for most combinations (Fig. 4). No resistance was observed in ICI larvae with the addition of chlordimeform to cypermethrin plus methyl parathion, cypermethrin plus acephate, and chlorpyrifos plus sulprofos. Therefore, the three-way combinations cypermethrin plus methyl parathion plus chlordimeform and cypermethrin plus acephate plus chlordimeform seem also to be possible alternates to pyrethroids for controlling resistant neonate TBW larvae.

Addition of chlordimeform showed no consistent pattern of change in slopes of response lines to non-pyrethroid insecticides and insecticide combinations (Table 10). For the susceptible strain the only significant changes in slopes by chlordimeform were observed with cypermethrin plus profenofos or methyl parathion. In these cases

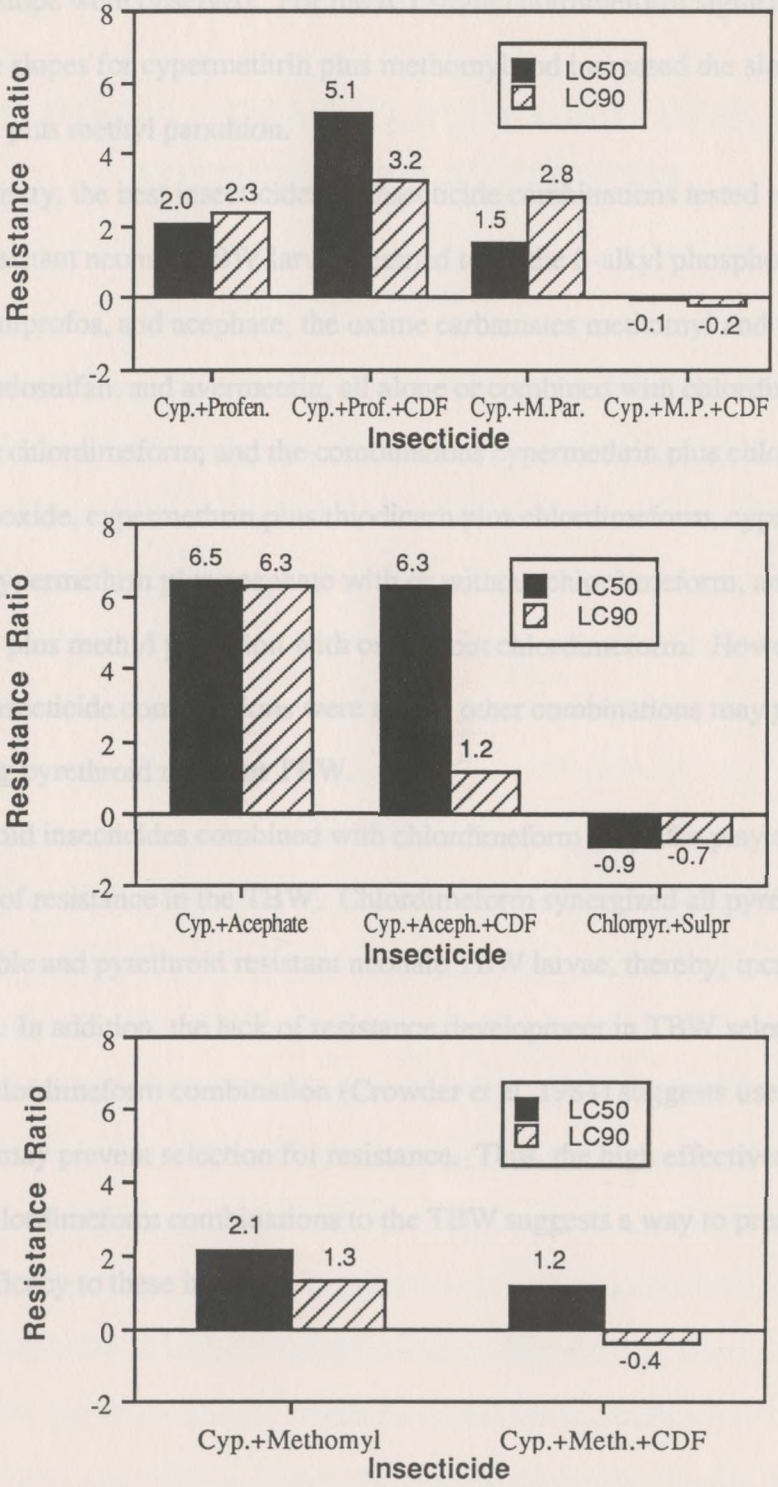

Fig. 4. Resistance ratios for different insecticide combinations ± chlordimeform (CDF) at the LC_{50} and LC_{90} levels for ICI neonate TBW larvae.

decreases in slope were observed. For the ICI strain chordimeform significantly decreased the slopes for cypermethrin plus methomyl and increased the slopes for cypermethrin plus methyl parathion.

In summary, the best insecticides or insecticide combinations tested to control pyrethroid-resistant neonate TBW larvae seemed to be the S-alkyl phosphorothiolates profenofos, sulprofos, and acephate, the oxime carbamates methomyl and thiodicarb, the cyclodiene endosulfan, and avermectin, all alone or combined with chlordimeform; rotenone plus chlordimeform; and the combinations cypermethrin plus chlordimeform and piperonyl butoxide, cypermethrin plus thiodicarb plus chlordimeform, cypermethrin plus profenofos, cypermethrin plus acephate with or without chlordimeform, and cypermethrin plus methyl parathion with or without chlordimeform. However, since not all possible insecticide combinations were tested, other combinations may prove useful for controlling pyrethroid resistant TBW.

Pyrethroid insecticides combined with chlordimeform may also play a role in the management of resistance in the TBW. Chlordimeform synergized all pyrethroids against both susceptible and pyrethroid resistant neonate TBW larvae, thereby, increasing their effectiveness. In addition, the lack of resistance development in TBW selected with pyrethroid-chlordimeform combination (Crowder et al. 1984) suggests use of the combination may prevent selection for resistance. Thus, the high effectiveness of pyrethroid-chlordimeform combinations to the TBW suggests a way to preserve pyrethroid efficacy to these insects.

CHAPTER IV

THIRD INSTAR TOBACCO BUDWORM: INSECTICIDE TOXICITY AND SYNERGISM

Introduction

The control of large TBW larvae in the field becomes very important as the season progresses and there is an overlap of generations. Large larvae tend to be harder to control compared with earlier stages due to their inherently greater capability to metabolize xenobiotics. Large larvae are more exposed to natural xenobiotics because they eat more than earlier stages, hence they have to detoxify a larger amount of those compounds. This fact leads to the idea that it is probably easier to select for metabolic resistance to insecticides in late than in early larval instars.

Pyrethroid resistance in third instar TBW larvae has been observed in many studies. Progeny of field-collected TBWs in the Imperial Valley, California showed a steady increase in resistance from 1979 to 1981 (Martinez-Carrillo & Reynolds 1983). Resistance levels in third instars to permethrin and fenvalerate increased to 51-fold and 29-fold, respectively, at the end of that period. In Texas, a 21-fold difference in LD_{50}s to fenvalerate was observed between laboratory-susceptible and field-collected strains (Harding et al. 1977). Plapp (1981) tested third and fourth instar TBW larvae collected from cotton fields in Texas and observed about 6- and 2-fold resistance to permethrin and fenvalerate, respectively. In tests with third instars, Staetz (1985) found, in a five-year study, that TBW populations in the southwest states (Texas, Arizona and California) generally appeared somewhat less susceptible to permethrin than those in the southeast (Alabama and Georgia). Tests showed that F_1 third instars of field strains of TBW collected in Louisiana, Texas, Arizona, and Mississippi during 1985 and 1986 exhibited

moderate to high levels of resistance to fenvalerate (2-35 fold), permethrin (1-74 fold), and cypermethrin (2-9 fold) (Leonard et al. 1987). Therefore, tests with third instar TBW larvae have been very important in assessing the level of resistance or tolerance in different populations of that species.

In this study I bioassayed susceptible and pyrethroid-resistant third instar TBW larvae with insecticides of different classes, with and without synergists. The purposes of those tests were to determine alternate insecticides or insecticide combinations for controlling pyrethroid-resistant large TBW larvae, to evaluate the effect of insecticide synergists, and to establish the main resistance mechanisms present in third instar TBW larvae.

Materials and Methods

The same susceptible and ICI resistant strains described previously (Chapter III) were used for bioassays with third instars. The insecticides tested included the pyrethroid cypermethrin; the organophosphates methyl parathion, chlorpyrifos, profenofos, sulprofos, and acephate; the oxime carbamates methomyl and thiodicarb; and the cyclodiene endosulfan. The combinations of cypermethrin with thiodicarb or methyl parathion or sulprofos and chlorpyrifos with sulprofos were also tested. All the insecticides and the insecticide combinations were also evaluated for synergism by chlordimeform or piperonyl butoxide or both.

Test insects were individually reared to third instar in 1.7 cm diameter x 6.3 cm long plastic vials on a standard *Heliothis* spp. diet (Vanderzant et al. 1962) for 6 to 8 days before testing. The vial technique was used to expose larvae to the insecticides. One larva was placed in each vial along with a piece of artificial diet. This was done to avoid cannibalism which commonly occurs when two or more late instar TBWs are caged together. All insecticides were tested with both susceptible and resistant strains, except

for some combinations of insecticides that were tested only with the ICI (R) strain. Insecticide(s) plus chlordimeform or piperonyl butoxide were tested at a 1:10 (wt:wt) ratio. All insecticides were tested with chlordimeform, but only some with piperonyl butoxide. Also, some insecticides were tested with both synergists chlordimeform and piperonyl butoxide at a 1:10:10 (insecticide:chlordimeform: piperonyl butoxide) ratio. Larvae were exposed to three to five concentrations of insecticides. At least 20 larvae were tested per insecticide concentration in at least 4 replicates of 5 larvae each. The readings for mortality were conducted after 72 h exposure. During rearing and bioassays the insects were maintained in an incubator at 25 ± 1°C and a 14:10 (L:D) photoperiod.

Data from all bioassays were corrected for control mortality using Abbott's (Abbott 1925) formula. Thereafter, LC_{50}'s and LC_{90}'s, in µg toxicant per vial, as well as slopes of the response curves were estimated by probit analysis (SAS Institute 1982). Comparisons between slopes for each insecticide and insecticide plus synergist(s) were conducted by the t-test. Resistance levels to the insecticides, synergism of insecticides by chlordimeform or piperonyl butoxide or both, and synergistic effects of the insecticide combinations were calculated as described in Chapter III for neonate larvae.

Results and Discussion

The toxicity of the insecticides studied, with and without synergists, to susceptible third instars is shown in Table 11. The most toxic chemical was methomyl with LC_{50} and LC_{90} values of 0.22 and 0.63 µg per vial, respectively. Following in toxicity were sulprofos, profenofos, and cypermethrin with LC_{50}'s of 1.15, 1.23, and 1.29 µg per vial, respectively. The lowest level of toxicity was observed with acephate (LC_{50} = 17.25 µg per vial). Cotoxicity coefficients at the LC_{50} level for the combinations chlorpyrifos plus sulprofos and cypermethrin plus thiodicarb were 1.5 and 1.9, respectively. Thus, there seemed to be a slight synergism when these combinations were

Table 11. Toxicity of insecticides ± synergists (chlordimeform (CDF) and/or piperonyl butoxide (PB)) to susceptible (S) third instar TBW larvae

Insecticide	n[a]	LC_{50} (95% CL)[b]	LC_{90} (95% CL)[b]	Slope (± SD)
Cypermethrin	145	1.29 (0.92-1.71)	5.58 (3.89-10.12)	2.02 (± 0.32)[d]
Cyperm. + CDF	117	0.25 (0.16-0.41)	2.32 (1.06-13.90)	1.33 (± 0.29)
Cyperm. + PB	128	0.53 (0.41-0.74)	1.86 (1.17-4.64)	2.34 (± 0.42)
Cyp. + CDF + PB	75	0.27 (0.21-0.35)	0.67 (0.48-1.36)	3.21 (± 0.66)
M. Parathion	149	12.57 (8.57-17.15)	72.31 (46.33-157.46)	1.69 (± 0.28)
M. Parat. + CDF	95	3.74 (2.70-5.12)	14.29 (9.09-37.01)	2.20 (± 0.45)
M. Parat. + PB	177	32.90 (17.91-91.10)	1,950.46 (374.70-257.20)	0.72 (± 0.19)
Chlorpyrifos	78	3.57 (2.77-5.59)	9.99 (6.14-39.26)	2.87 (± 0.73)
Chlorpyr. + CDF	141	3.57 (2.77-4.60)	13.65 (9.22-28.13)	2.20 (± 0.37)
Chlorpyr. + PB	67	18.83 (13.10-25.05)	54.03 (37.56-118.75)	2.80 (± 0.63)
Profenofos	73	1.23 (0.97-1.62)	2.99 (2.13-5.93)	3.33 (± 0.66)
Prof. + CDF	70	0.92 (0.71-1.19)	2.16 (1.55-4.47)	3.45 (± 0.75)
Sulprofos	93	1.15 (0.85-1.50)	3.35 (2.42-5.90)	2.76 (± 0.48)
Sulpr. + CDF	229	1.09 (0.92-1.28)	3.19 (2.51-4.54)	2.75 (± 0.32)
Acephate	84	17.25 (10.94-27.09)	99.81 (50.95-663.21)	1.68 (± 0.44)
Aceph. + CDF	75	4.01 (3.13-4.93)	8.70 (6.71-14.51)	3.81 (± 0.77)
Chlorpyr. + Sulpr.	104	0.59[c] (0.49-0.74)	1.35[c] (1.02-2.24)	3.59 (± 0.61)

Table 11. (continued)

Insecticide	n[a]	LC_{50} (95% CL)[b]	LC_{90} (95% CL)[b]	Slope (± SD)
Methomyl	177	0.22 (0.19-0.27)	0.63 (0.48-0.98)	2.85 (± 0.39)[d]
Meth. + CDF	75	0.23 (0.19-0.28)	0.44 (0.35-0.66)	4.57 (± 0.83)
Thiodicarb	95	2.94 (2.05-4.01)	11.69 (7.56-28.48)	2.14 (± 0.44)
Thiodic. + CDF	70	0.61 (0.15-0.94)	3.51 (1.90-66.28)	1.69 (± 0.60)
Endosulfan	78	4.61 (3.51-5.87)	11.95 (8.56-25.29)	3.10 (± 0.69)
Endos. + CDF	74	0.87 (0.65-1.14)	2.35 (1.66-5.03)	2.98 (± 0.65)
Cyp. + Thiodic.	111	0.46[c] (0.34-0.67)	2.08[c] (1.20-6.61)	1.95 (± 0.39)
Cyp. + Thiod. + CDF	123	0.25[c] (0.17-0.35)	1.37[c] (0.82-4.22)	1.75 (± 0.36)

[a] Number of larvae tested excluding controls.

[b] Concentrations are expressed in micrograms of insecticide per vial.

[c] Concentration of each insecticide.

[d] Slopes for each insecticide (or insecticide combination) and insecticide (or insecticide combinations) plus synergist(s) are not significantly different ($P>0.05$; t-test).

tested with susceptible third instar TBW larvae.

The toxicity of different insecticides to ICI third instars is reported in Table 12. When I initiated the bioassays the resistance level to cypermethrin at the LC_{50} was close to 1,000-fold. At the end of testing, after 4 generations without selection with insecticides, the resistance level to cypermethrin was about 100-fold. Thus, in most cases the insecticide-response data represent the averages of tests carried out during 4 different generations. The toxicity was quite variable to ICI third instars. The LC_{50}'s varied from 0.95 µg per vial for methomyl to 1,287 µg per vial for cypermethrin. Profenofos and sulprofos were among the most toxic compounds with LC_{50}'s of 1.16 and 1.76 µg per vial and LC_{90}'s of 3.42 and 5.57 µg per vial, respectively. The low toxicities of cypermethrin and methyl parathion (LC_{50} of 237.79 µg per vial) revealed a high level of resistance in third instar TBW larvae. Furthermore, the high levels of resistance observed for these insecticides, as compared to the lower levels observed in neonate larvae, support metabolic resistance as a possible resistance mechanism in third instar TBW larvae. Increased metabolism was suggested as the cause of resistance to organophosphate insecticides in TBW (Whitten & Bull 1970, 1974, Bull 1980). Also, Sparks (1981) emphasized that the observed low level of cross resistance to pyrethroids in organophosphate-resistant TBW may be due to increased detoxification.

The cotoxicity coefficients at the LC_{50} level for the insecticide combinations for ICI third instars were also variable. They were 41.2, 2.0, 0.7, and 1.4 for cypermethrin plus methyl parathion, cypermethrin plus thiodicarb, cypermethrin plus sulprofos, and chlorpyrifos plus sulprofos, respectively. Hence, only the combinations of cypermethrin with methyl parathion and cypermethrin with thiodicarb showed synergism (cotoxicity coefficient ≥ 2.0) and seem to be promising for use in the control of pyrethroid-resistant third instar TBW larvae. High-level synergism was observed previously when the combination methyl parathion:permethrin (10:1) was tested against TBW larvae from an

Table 12. Toxicity of insecticides ± synergists (chlordimeform (CDF) and/or piperonyl butoxide (PB)) to ICI (R) third instar TBW larvae

Insecticide	n[a]	LC_{50} (95% CL)[b]	LC_{90} (95% CL)[b]	Slope (± SD)
Cypermethrin	125	1,287.46 (594.32-3,325)	55,871.07 (11,362-4x10^7)	0.78 (± 0.25)[d]
Cyperm. + CDF	100	16.74 (8.23-24.82)	104.90 (61.56-385.31)	1.61 (± 0.39)
Cypermethrin. + PB	120	8.10 (5.96-10.84)	32.12 (21.50-64.23)	2.14 (± 0.35)
Cyperm. + CDF + PB	108	4.98 (3.46-7.60)	28.75 (15.42-109.25)	1.68 (± 0.34)
M. Parathion	174	237.79 (171.64-321.63)	1,467.18 (928.00-3,114)	1.62 (± 0.23)
M. Parat. + CDF	106	2.91 (2.25-3.66)	7.84 (5.85-12.77)	2.97 (± 0.48)
M. Parathion + PB	137	48.23 (28.58-78.56)	662.49 (274.39-6,022)	1.13 (± 0.26)
M. Parat. + CDF + PB	75	3.77 (2.38-5.05)	12.60 (8.29-41.69)	2.45 (± 0.66)
Chlorpyrifos	100	10.19 (8.21-12.93)	23.64 (17.59-39.22)	3.51 (± 0.57)
Chlorpyr. + CDF	99	5.31 (4.15-6.95)	16.14 (10.81-40.42)	2.65 (± 0.58)
Profenofos	105	1.16 (0.88-1.47)	3.42 (2.49-6.07)	2.73 (± 0.49)
Prof. + CDF	100	1.38 (1.11-1.71)	2.96 (2.30-4.42)	3.87 (± 0.59)
Prof. + PB	70	2.67 (1.79-4.82)	12.71 (6.23-179.85)	1.89 (± 0.58)
Sulprofos	127	1.76 (1.26-2.24)	5.57 (4.17-9.07)	2.56 (± 0.44)
Sulpr. + CDF	63	1.66 (1.08-2.26)	5.07 (3.39-13.95)	2.64 (± 0.67)
Sulpr. + PB	90	4.46 (3.40-6.14)	13.56 (8.97-30.74)	2.65 (± 0.50)
Chlorpyr. + Sulpr.	59	1.06[c] (0.86-1.33)	1.95[c] (1.51-3.43)	4.87 (± 1.10)

Table 12. (continued)

Insecticide	n[a]	LC_{50} (95% CL)[b]	LC_{90} (95% CL)[b]	Slope (± SD)
Acephate	106	25.60 (18.39-41.42)	126.21 (66.78-522.61)	1.85 (± 0.38)[d]
Aceph. + CDF	83	5.82 (4.96-6.92)	9.89 (8.08-14.12)	5.55 (± 0.96)
Acephate + PB	75	22.08 (15.56-30.40)	74.98 (47.91-225.87)	2.41 (± 0.58)
Aceph. + CDF + PB	77	5.57 (4.15-7.32)	14.51 (10.35-27.85)	3.08 (± 0.61)
Methomyl	109	0.95 (0.63-1.60)	7.26 (3.28-60.96)	1.45 (± 0.35)
Methomyl + CDF	110	0.29 (0.18-0.41)	1.66 (0.99-4.70)	1.69 (± 0.34)
Methomyl + PB	130	0.46 (0.23-0.67)	4.09 (2.04-33.63)	1.35 (± 0.36)
Meth. + CDF + PB	98	0.53 (0.35-0.75)	2.60 (1.52-9.33)	1.85 (± 0.42)
Thiodicarb	148	11.38 (7.44-17.18)	113.7 (55.22-545.27)	1.28 (± 0.51)
Thiodic. + CDF	75	1.95 (1.42-2.52)	5.31 (3.81-10.46)	2.95 (± 0.61)
Thiodicarb + PB	107	7.53 (2.75-18.90)	209.92 (46.91-6x10^6)	0.89 (± 0.34)
Thiodic. + CDF + PB	93	1.84 (1.24-2.84)	10.31 (5.42-50.30)	1.71 (± 0.40)
Endosulfan	100	6.56 (4.75-8.97)	25.99 (16.57-64.39)	2.14 (± 0.42)
Endos. + CDF	125	2.23 (1.64-2.99)	9.31 (6.19-18.42)	2.06 (± 0.32)
Cyperm. + Thiodic.	119	5.58[c] (2.68-8.64)	55.42[c] (28.44-283.25)	1.29 (± 0.30)[d]
Cyp. + Thiodic. + CDF	79	2.07[c] (1.23-3.07)	10.32[c] (5.63-73.28)	1.84 (± 0.53)
Cyperm. + M. Parat.	126	4.88[c] (3.67-7.19)	22.42[c] (12.74-76.40)	1.94 (± 0.39)
Cyp. + M. Parat. + CDF	87	2.99[c] (2.24-3.97)	9.12[c] (6.21-19.54)	2.64 (± 0.51)

Table 12. (continued)

Insecticide	n[a]	LC_{50} (95% CL)[b]	LC_{90} (95% CL)[b]	Slope (± SD)
Cyp. + M. Parat. + PB	98	7.21[c] (5.05-10.97)	34.40[c] (19.57-104.40)	1.89 (± 0.35)
Cyper. + Sulprofos	102	2.45[c] (1.65-3.38)	11.25[c] (7.00-31.17)	1.94 (± 0.41)

[a] Number of larvae tested excluding controls.
[b] Concentrations are expressed in micrograms of insecticide per vial.
[c] Concentration of each insecticide.
[d] Slopes for each insecticide (or insecticide combination) and insecticide (or insecticide combination) plus synergist(s) are not significantly different ($P>0.05$; t-test).

apparently susceptible population (All et al. 1977). A cotoxicity coefficient of 17.5 was calculated for this combination and its good performance against TBW was confirmed by a field test.

Chlordimeform alone was almost nontoxic to the insects tested. The LC_{50}'s were 1,068 and approximately 1,500 µg per vial for susceptible and ICI third instars, respectively. At the LC_{90} level, the concentrations of chlordimeform were 3,642 and approximately 3,800 µg per vial for susceptible and ICI strains, respectively. The low chlordimeform toxicity to TBW larvae agrees with previous studies (Streibert & Dittrich 1977, Plapp 1979). Piperonyl butoxide alone was also nontoxic to third instars. The LC_{50}'s were not determined because of their high values, but were greater than 5,000 µg per vial for both TBW strains.

Chlordimeform tended to synergize insecticides more against the resistant than against the susceptible TBW strain (Table 13). Unlike neonate larvae (Chapter III), no linear relationship could be established for insecticide toxicities and synergism by chlordimeform. For the susceptible strain synergism at the LC_{50} was always close to or below 5-fold. At the LC_{90} level, chlordimeform synergism tended to remain low for the susceptible larvae, ranging from 0.7 (antagonism) for chlorpyrifos to 11.5-fold for acephate. For the ICI larvae, the synergism levels also tended to be low , that is, close to or below 5-fold, except for cypermethrin and methyl parathion where the synergism was about 80-fold. Synergism levels were generally higher at the LC_{90} than at the LC_{50} level and the most synergized insecticides at the LC_{50} also showed the highest synergism by chlordimeform at the LC_{90} level.

The addition of chlordimeform increased the slope of the regression lines for most of the insecticides tested with the ICI strain, although most differences were not significant (Table 12). Thus, the variability of response of ICI third instars to insecticides was diminished. This probably means that chlordimeform synergized insecticides more

Table 13. Synergism[a] by chlordimeform (CDF) and/or piperonyl butoxide (PB) at the LC_{50} and LC_{90} levels for different insecticides against susceptible (S) and resistant (R) third instar TBW larvae

Insecticide + Synergist(s)	Stoneville(S)		ICI(R)	
	LC_{50}	LC_{90}	LC_{50}	LC_{90}
Cyperm. + CDF	5.2	2.4	76.9	532.6
Cyperm. + PB	2.4	3.0	158.9	1,739.4
Cyp. + CDF + PB	4.8	8.3	258.5	1,943.3
M. Parathion + CDF	3.4	5.1	81.7	187.1
M Parathion + PB	0.4	0.04	4.9	2.2
M. Parat. + CDF + PB	-	-	63.1	116.4
Chlorpyrifos + CDF	1.0	0.7	1.9	1.5
Chlorpyrifos + PB	0.2	0.2	-	-
Profenofos + CDF	1.3	1.4	0.8	1.2
Profenofos + PB	-	-	0.4	0.3
Sulprofos + CDF	1.1	1.1	1.1	1.1
Sulprofos + PB	-	-	0.4	0.4
Acephate + CDF	4.3	11.5	4.4	12.8
Acephate + PB	-	-	1.2	1.7
Aceph. +CDF + PB	-	-	4.6	8.7
Methomyl + CDF	1.0	1.4	3.3	4.4
Methomyl + PB	-	-	2.1	1.8
Meth. + CDF + PB	-	-	1.8	2.8
Thiodicarb + CDF	4.8	3.3	5.8	21.4
Thiodicarb + PB	-	-	1.5	0.5
Thiodic. + CDF + PB	-	-	6.2	11.0
Endosulfan + CDF	5.3	5.1	2.9	2.8
Cyp. + Thiodic. + CDF	1.8	1.5	2.7	5.4
Cyp. + M. Par. + CDF	-	-	1.6	2.5
Cyp. + M. Par. + PB	-	-	0.7	0.3

[a] Calculated by dividing the LC_{50} (or LC_{90}) for the insecticide by the LC_{50} (or LC_{90}) for the insecticide + CDF.

against the most resistant individuals in the population. However, no pattern in change of slopes of the regression lines was observed for the susceptible strain.

Insecticide synergism by chlordimeform was variable for different larval instars. For the susceptible strain, synergism at the LC_{50} level was always higher for neonate (Chapter III) than for third instar larvae. For the ICI strain, synergism by chlordimeform of profenofos, sulprofos, chlorpyrifos, and endosulfan was greater against neonate than against third instar larvae. However, synergism of cypermethrin, methyl parathion, acephate, and thiodicarb was higher against third instars .

Synergism by chlordimeform was greater than synergism by piperonyl butoxide with all insecticides studied, except for cypermethrin with ICI larvae (Table 13). At the LC_{50} level, chlordimeform synergism ranged from less than 2-fold for chlorpyrifos, profenofos, and sulprofos against both strains to 76.9- and 81.7-fold for cypermethrin and methyl parathion, respectively, against ICI larvae. Chlordimeform synergized acephate more than the other S-alkyl phosphorothiolates, profenofos and sulprofos, against both TBW strains. Unlike the ICI strain, no synergism with methomyl plus chlordimeform was observed for the susceptible strain.

At the LC_{50} level, piperonyl butoxide produced low synergism or antagonism with insecticides for the susceptible strain (Table 13). The synergism level was 2.4 for cypermethrin, but only 0.4 and 0.2 for methyl parathion and chlorpyrifos, respectively. Thus, antagonism was present for the latter insecticides. Conversely, very high levels of synergism by piperonyl butoxide were observed with cypermethrin for the ICI strain (158.9- and 1,739.4-fold at the LC_{50} and LC_{90}, respectively). This suggests oxidative detoxification, i.e. metabolic resistance, is present in the ICI strain.

Pyrethroid synergism by piperonyl butoxide has been observed with other insect species. Piperonyl butoxide synergized pyrethroids to different degrees in both susceptible and resistant strains of diamondback moth (Liu et al. 1984). Pretreatment of a

resistant strain of diamondback moth larvae with piperonyl butoxide increased the effectiveness of fenvalerate by 15-fold, deltamethrin 13-fold, permethrin 6-fold, and cypermethrin 3-fold. In a study with first and fourth instar *Tribolium castaneum* larvae, piperonyl butoxide synergized the toxicity of *cis*-permethrin, *trans*- and *cis*-cypermethrin, and deltamethrin (Ishaaya et al. 1983). The investigators suggested that oxidases were more important than esterases in pyrethroid detoxification by this species.

Slight synergism by piperonyl butoxide was observed with methyl parathion for the ICI strain. However, almost no synergism was observed with acephate, methomyl, or thiodicarb. Antagonism by piperonyl butoxide was found for profenofos, sulprofos, and the combination of cypermethrin with methyl parathion. Piperonyl butoxide might have inhibited the activation of profenofos and sulprofos in resistant larvae. Since piperonyl butoxide synergized cypermethrin and methyl parathion, the data indicated mixed-function oxidases may be an important factor in the metabolic resistance to pyrethroids in the TBW.

Unlike the ICI strain, piperonyl butoxide was antagonistic to methyl parathion for susceptible third instars. Most likely, piperonyl butoxide inhibited the activation of methyl parathion to methyl paraoxon, the toxic compound. Likewise, an antagonistic effect of piperonyl butoxide was demonstrated with chlorpyrifos, an organophosphate compound belonging to the same chemical group as methyl parathion (phosphorothionates). Some synergism was observed when piperonyl butoxide was combined with cypermethrin for susceptible third instars. This fact supports the idea that there is some inherent capability of insecticide degradation by oxidation (tolerance) in susceptible TBW populations.

The addition of chlordimeform and piperonyl butoxide to cypermethrin was more effective than either synergist alone. An LC_{50} of 1,287 μg per vial for cypermethrin only was brought to 4.98 μg per vial cypermethrin when chlordimeform plus piperonyl

butoxide were combined with that pyrethroid. This level is only about 4-fold higher than that of cypermethrin only against the susceptible strain. Therefore, this three-way combination appears to be promising for controlling pyrethroid-resistant populations in the field. However, the light instability of piperonyl butoxide (Georghiou 1980) may limit its extensive use. Thus, a formulation that improves piperonyl butoxide persistence in the field may be required. For other insecticides such as acephate, methomyl, and thiodicarb the three-way combination (insecticide plus chlordimeform and piperonyl butoxide) was approximately as toxic as chlordimeform only combined with the insecticides . Therefore, there was no improvement in the synergism of those insecticides when piperonyl butoxide was combined with chlordimeform.

Fig. 5 presents the resistance ratios at the LC_{50} and LC_{90} levels for ICI third instars exposed to insecticides, with and without synergists. For the cases where the resistance ratio was below 1, that is, where the insecticides or insecticide combinations were more toxic to resistant than to susceptible larvae, the resistance ratios were made negative to emphasize the results. With the addition of synergists, the resistance level to cypermethrin was considerably reduced (Fig. 5a). A 998-fold resistance to cypermethrin, at the LC_{50} level, was reduced to 67.0-, 15.3-, and 18.4-fold with the addition of chlordimeform, piperonyl butoxide, and chlordimeform plus piperonyl butoxide, respectively. At the LC_{90} level, a 10,012-fold resistance was reduced to 45.2-, 17.3-, and 42.9-fold, respectively. Likewise, the addition of synergists to methyl parathion practically blocked resistance (Fig. 5b). Resistance ratios at the LC_{50} level for methyl parathion with chlordimeform or piperonyl butoxide were 0.8 and 1.5, respectively.

The low level of resistance to chlorpyrifos (2.9-fold at LC_{50}) was decreased with the addition of chlordimeform to 1.5-fold. The resistance ratios were close to 1 for the S-alkyl phosphorothiolates profenofos, sulprofos, and acephate, that is, no resistance was observed to these compounds (Fig. 5c). The addition of chlordimeform hardly affected

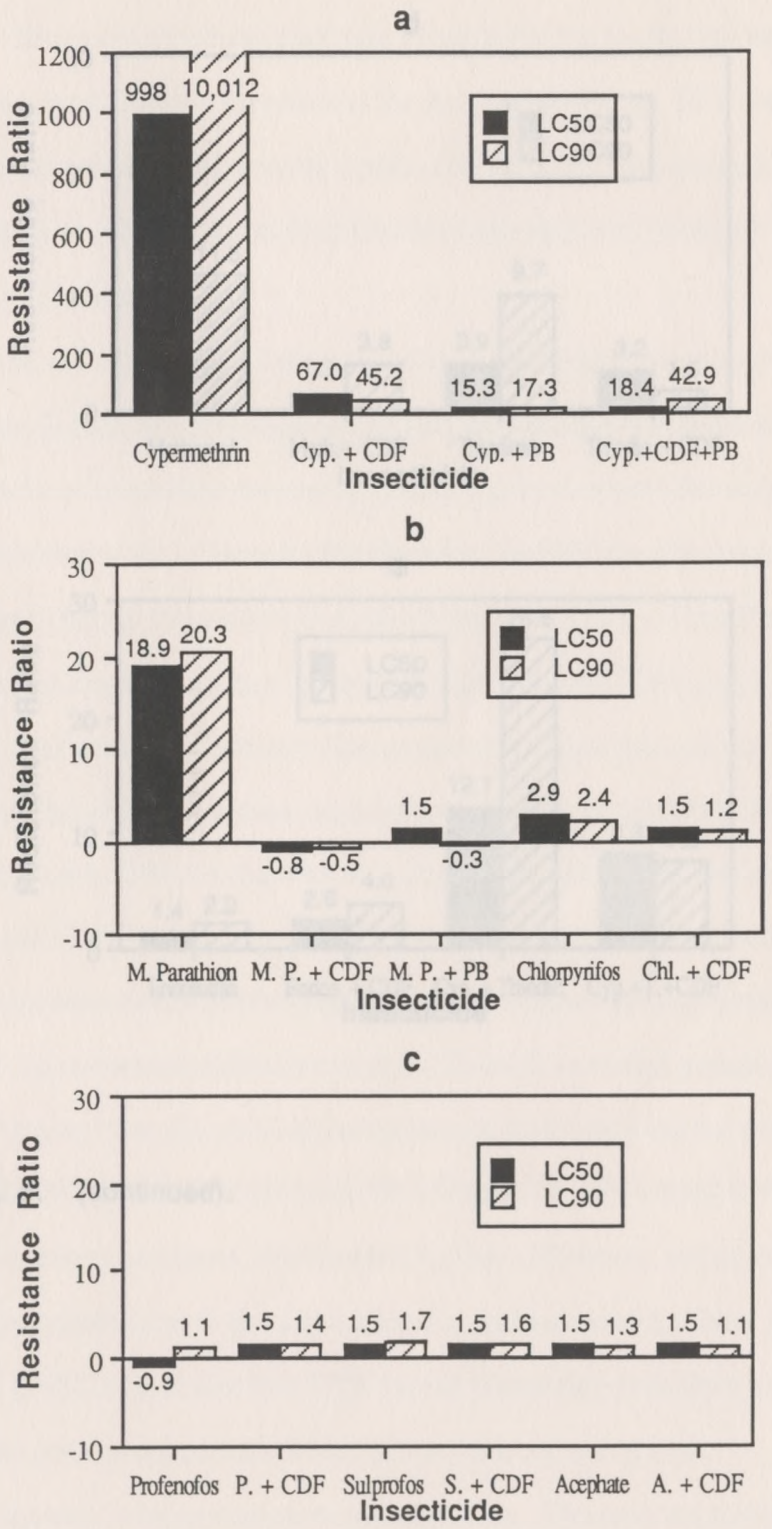

Fig. 5. Resistance ratios for different insecticides ± chlordimeform (CDF) and/or piperonyl butoxide (PB) at the LC_{50} and LC_{90} levels for ICI third instar TBW larvae.

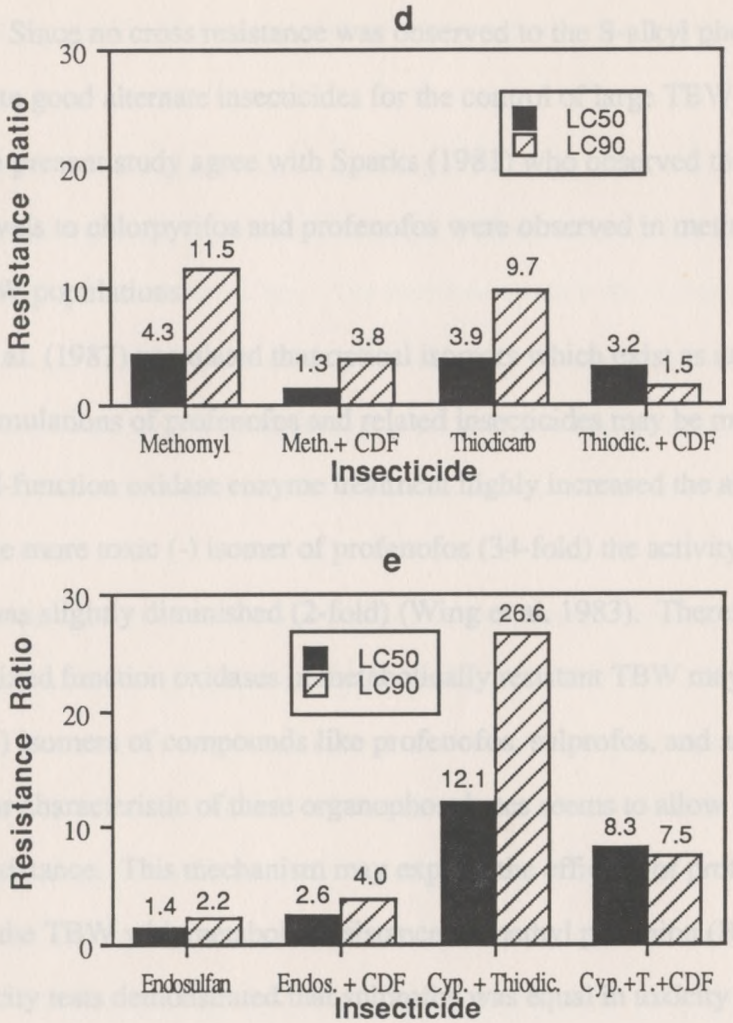

Fig. 5. (continued).

those ratios. Since no cross resistance was observed to the S-alkyl phosphorothiolates, they constitute good alternate insecticides for the control of large TBW larvae. The results of the present study agree with Sparks (1981) who observed that, in general, low resistance levels to chlorpyrifos and profenofos were observed in methyl parathion-resistant TBW populations.

Bull et al. (1987) postulated that optical isomers which exist as racemic mixtures in technical formulations of profenofos and related insecticides may be mutually synergistic. While mixed-function oxidase enzyme treatment highly increased the anticholinesterase activity of the more toxic (-) isomer of profenofos (34-fold) the activity of the less toxic (+) isomer was slightly diminished (2-fold) (Wing et al. 1983). Therefore, the enhanced activity of mixed function oxidases in metabolically resistant TBW may increase the activity of (-) isomers of compounds like profenofos, sulprofos, and acephate. Thus, this particular characteristic of these organophosphates seems to allow them to overcome metabolic resistance. This mechanism may explain the efficacy of profenofos and sulprofos to the TBW with metabolic resistance to methyl parathion (Bull et al. 1987). Topical toxicity tests demonstrated that sulprofos was equal in toxicity to third instar TBW larvae that were susceptible or resistant (25-fold) to methyl parathion (Bull 1980).

Low tolerance was observed to the carbamate methomyl, but the addition of chlordimeform practically overcame it at the LC_{50} level. Also, some tolerance was observed to another carbamate, thiodicarb (Fig. 5d). However, in this case, chlordimeform seemed not to block the tolerance to this toxicant. These results disagreed with Sparks' (1981) conclusion that TBW larvae possessing resistance to methyl parathion also possess appreciable levels of resistance to methomyl.

There seemed to be no resistance to endosulfan. The resistance ratios for this insecticide at the LC_{50} and LC_{90} levels were 1.4 and 2.2, respectively (Fig. 5e). Chlordimeform tended to slightly enhance the resistance level to endosulfan (2.6-fold at

the LC_{50}). Thus, endosulfan seems also to be a good alternate insecticide for controlling pyrethroid-resistant TBWs.

The combination cypermethrin plus thiodicarb did not block resistance (Fig. 5e). With chlordimeform there was a decrease in the resistance level, but still some resistance was observed (7.5-fold at the LC_{50}). The resistance ratios for chlorpyrifos plus sulprofos were 1.8 and 1.4 at the LC_{50} and LC_{90} levels, respectively. Thus, no resistance was present to this combination. I do not have the resistance ratio for the combination cypermethrin plus methyl parathion because I did not test it with susceptible third instars.

In summary, many alternate insecticides or insecticide combinations can be suggested for the control of pyrethroid-resistant large TBW larvae. These include cypermethrin plus chlordimeform and piperonyl butoxide, cypermethrin plus methyl parathion, cypermethrin plus thiodicarb combined or not with chlordimeform, the S-alkyl phosphorothiolates profenofos, sulprofos, and acephate (acephate can be combined with chlordimeform), the cyclodiene endosulfan (with or without chlordimeform), and possibly the oxime carbamates methomyl and thiodicarb, with or without chlordimeform. Usually, chlordimeform was a better synergist than piperonyl butoxide for the insecticides studied. For the cases where the combination of insecticide with chlordimeform did not block resistance, the LC_{50} for the resistant strain became nearly equal or lower than the LC_{50} for the insecticide only for the susceptible strain. Besides target site resistance (Chapter III), metabolic resistance seems also to be present in the TBW. It appears to be mostly due to the enhanced activity of the mixed-function oxidases.

CHAPTER V

ADULT TOBACCO BUDWORM: BIOASSAYS WITH INSECTICIDES

Introduction

Adult insects captured in pheromone traps can be a useful tool for testing for resistance to insecticides. A procedure for detection of resistance to azinphosmethyl or other toxicants in males of the codling moth, *Cydia pomonella* (L.), collected with pheromone traps proved useful in establishing realistic baseline data for insecticide susceptibility, determining discriminating concentration levels for resistance surveys, and mapping the distribution and spread of resistance (Riedl et al. 1985). Likewise, Suckling et al. (1985) carried out bioassays with males attracted to pheromone caps to evaluate the distribution of azinphosmethyl-resistant lightbrown apple moths, *Epiphyas postvittana* (Walker), in an apple orchard district in New Zealand. They found a 5-fold resistance factor whether this technique or the residue exposure of first instars (Suckling et al. 1984) was used. An attracticide method using a modified pink bollworm (*Pectinophora gossypiella* (Saunders)) delta trap was devised (Miller 1987, Haynes et al. 1987). The objective was to study differences in toxicity of carbamate, organophosphate and pyrethroid insecticides to field populations of that insect in cotton-growing areas of Texas, Arizona, California, Mexico, and China . This method was successful in detecting resistance to pyrethroids in adults, the target stage for control with insecticides.

Resistance to insecticides can be manifested in stages of the insect pests other than the one(s) at which chemical control is aimed. The requirement for an efficient and quick monitoring method for pyrethroid resistance in the TBW led Plapp et al. (1987) to develop a system to measure pyrethroid resistance in adult males collected in pheromone

traps. They exposed the moths to different concentrations of cypermethrin using the glass-vial technique. The resistance detected in moths roughly reflected control failures in the field. Thus, the resistance levels in adults were very likely correlated with the resistance levels in larvae. Based on this approach they could detect the occurrence of pyrethroid resistance in most of the U.S. cotton belt. Also, they could follow the fluctuation of pyrethroid resistance over the season, which may improve the implementation of resistance management strategies.

In this chapter I report tests of TBW adults of different strains with different classes of insecticides. I attempted to determine the resistance spectra for adult males and relationships between insecticide toxicities to adults and neonate larvae (generally the target stage for control) of pyrethroid-resistant TBW strains. I also attempted to detect differences in cypermethrin toxicity to adult males and females.

Materials and Methods

The vial technique was used to measure the response of susceptible and resistant adult TBW males to cypermethrin, thiodicarb, methyl parathion, and acephate. TBW females were tested only with cypermethrin. At least 15 adults were tested per insecticide concentration and four or five concentrations were used for each insecticide. For most bioassays I tested two moths per vial, but in a few cases only one moth was tested. In all tests a small piece of cotton wick soaked with 10% sucrose solution in water was supplied as food. Response was determined 24 h after exposure started. Both knocked down (uncontrolled movements and unable to right themselves) and dead moths were considered as responding. During the bioassays the insects were maintained in an incubator at 25 ± 1°C and a 14:10 (L:D) photoperiod. Data for mortality at 24 h were subjected to probit analysis (SAS Institute 1982). Slopes of response lines to insecticides for different strains were compared using the t-test. The resistance level was determined

by dividing the LC_{50} (or LC_{90}) of each toxicant for the resistant strain by the LC_{50} (or LC_{90}) for the susceptible strain.

Results and Discussion

Data on insecticide toxicities to susceptible and resistant adult males are presented in Table 14. Differences in cypermethrin toxicity existed between TBW strains. The LC_{50}'s for cypermethrin varied from 2.95 µg per vial for susceptible males to 50.38 µg per vial for Hearne males.

Thiodicarb was less toxic than cypermethrin. Thiodicarb LC_{50}'s were also variable for different TBW strains, ranging from 127.87 µg per vial for the ICI males to 3,308 µg per vial for the Hearne males. However, thiodicarb was more toxic to ICI males than to susceptible males.

The organophosphates methyl parathion and acephate differed in toxicity to adult TBW males. The LC_{50}'s for methyl parathion for Stoneville and ICI males were 41.1 and 215.29 µg per vial and for acephate were 13.96 and 17.58 µg per vial, respectively. Though methyl parathion and acephate were tested only with the Stoneville and ICI strains, it is clear that there was resistance to methyl parathion, but not to acephate and that acephate was more toxic to TBW males than methyl parathion.

The resistance ratios for insecticides for different TBW strains are shown in Fig. 6. When a resistance ratio was below 1, the insecticide was more toxic to resistant than to susceptible insects. Resistance ratios below 1 were transformed to negative numbers to emphasize the results. Resistance to cypermethrin was observed in all resistant strains. Resistance ratios were 6.3-, 13.8-, and 17.1-fold at the LC_{50} level for ICI, Uvalde, and Hearne strains, respectively. At the LC_{90}, resistance ratios for cypermethrin were 4.8-, 6.9-, and 9.8-fold for ICI, Hearne, and Uvalde males, respectively. Thus, for each strain resistance was higher at the LC_{50} than at the LC_{90} level.

Table 14. Toxicity of insecticides to susceptible (S) and resistant (R) adult TBW males

Insect. Strain	n[a]	LC_{50} (95% CL)[b]	LC_{90} (95% CL)[b]	Slope (± SD)
Cypermethrin				
Stoneville(S)	119	2.95 (2.07-4.43)	17.84 (9.68-61.34)	1.64 (± 0.31)
ICI(R)	251	18.46 (15.11-24.08)	85.13 (53.29-201.58)	1.93 (± 0.31)
Uvalde(R)	144	40.63 (28.31-80.61)	174.47 (85.96-822.46)	2.02 (± 0.40)
Hearne(R)	35	50.38 (35.24-90.66)	122.57 (75.51-1,392)	3.32 (± 1.19)
Thiodicarb				
Stoneville(S)	157	166.22 (128.42-230.16)	652.75 (416.85-1,382)	2.16 (± 0.31)[d]
ICI(R)	78	127.87 (58.17-338.01)	1,706.05 (502.22-...)[c]	1.14 (± 0.43)
Uvalde(R)	45	468.47 (...-...)[c]	5,789.29 (233.20-...)[c]	1.17 (± 0.88)
Hearne(R)	60	3,307.59 (...-...)[c]	134,018.30 (255.80-...)[c]	0.80 (± 1.02)
Methyl Parathion				
Stoneville(S)	127	41.10 (31.19-53.02)	139.44 (98.56-250.66)	2.42 (± 0.38)
ICI(R)	94	215.29 (148.34-347.01)	1,181.09 (606.89-5,949)	1.73 (± 0.39)
Acephate				
Stoneville(S)	79	13.96 (10.51-18.60)	38.73 (26.97-76.53)	2.89 (± 0.54)
ICI(R)	90	17.58 (12.51-25.54)	74.85 (44.50-226.25)	2.04 (± 0.42)

[a] Number of adult males tested excluding controls.
[b] Concentrations are expressed in micrograms of insecticide per vial.
[c] Data too heterogeneous to calculate 95% CL.
[d] Slope for the Stoneville strain significantly different from the mean slope for resistant strains ($P<0.05$; t-test).

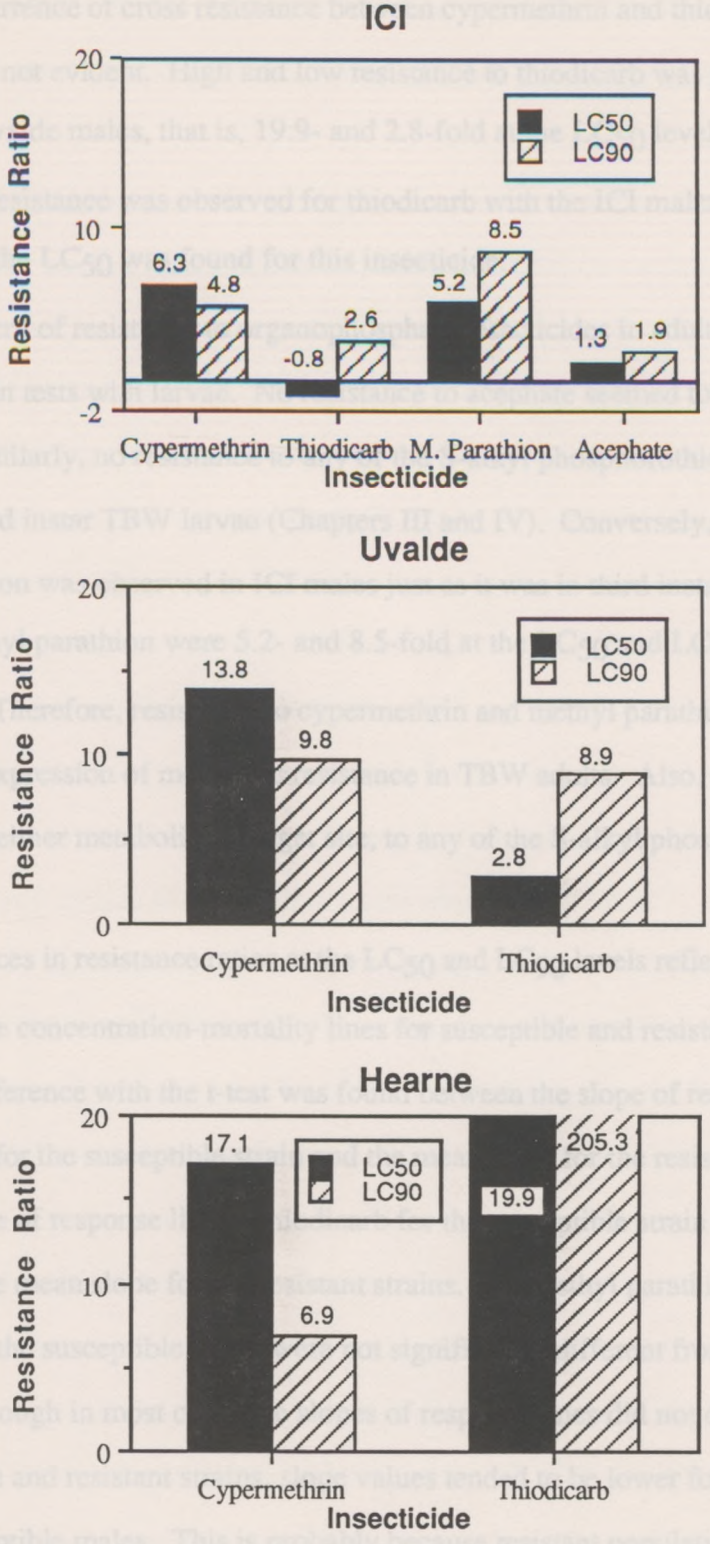

Fig. 6. Resistance ratios for insecticides at the LC_{50} and LC_{90} levels for ICI, Uvalde, and Hearne adult TBW males.

The occurrence of cross resistance between cypermethrin and thiodicarb in adult TBW males is not evident. High and low resistance to thiodicarb was present in the Hearne and Uvalde males, that is, 19.9- and 2.8-fold at the LC_{50} level, respectively. However, no resistance was observed for thiodicarb with the ICI males since a resistance ratio of 0.8 at the LC_{50} was found for this insecticide.

The pattern of resistance to organophosphate insecticides in adults was similar to that observed in tests with larvae. No resistance to acephate seemed to be present in the ICI strain. Similarly, no resistance to any of the S-alkyl phosphorothiolates was found in neonate or third instar TBW larvae (Chapters III and IV). Conversely, resistance to methyl parathion was observed in ICI males just as it was in third instars. The resistance ratios for methyl parathion were 5.2- and 8.5-fold at the LC_{50} and LC_{90} levels, respectively. Therefore, resistance to cypermethrin and methyl parathion in ICI males is evidence for expression of metabolic resistance in TBW adults. Also, there seemed to be no resistance, either metabolic or target site, to any of the S-alkyl phosphorothiolates tested.

Differences in resistance ratios at the LC_{50} and LC_{90} levels reflected the difference in slopes of the concentration-mortality lines for susceptible and resistant strains. No significant difference with the t-test was found between the slope of response lines to cypermethrin for the susceptible strain and the mean slope for the resistant strains (Table 14). The slope of response line to thiodicarb for the susceptible strain was significantly higher than the mean slope for the resistant strains. For methyl parathion and acephate the slopes for the susceptible strain were not significantly different from the slopes for the ICI strain. Though in most cases the slopes of response lines did not differ significantly for susceptible and resistant strains, slope values tended to be lower for resistant males than for susceptible males. This is probably because resistant populations have a mixture of different genotypes and this increases the variability within the population. The

substantial difference between the LC_{50} and LC_{90} values for thiodicarb with the Hearne males may indicate the presence of more than one type of resistance, hence more than one resistance gene.

Insecticide toxicities were always higher to pyrethroid-resistant neonate TBW larvae (Chapter III) than to adult males. However, there seemed to be a relationship between cypermethrin toxicity to adult males and neonate TBW larvae. Uvalde and ICI males were 5- to 6-fold more tolerant to cypermethrin at the LC_{50} than neonate larvae, respectively. The 30-fold tolerance observed in Hearne adult males compared with neonate larvae is not conclusive, since only 35 adults were tested before the strain was lost. Unlike cypermethrin, no clear relationship was present between toxicity of thiodicarb to neonate larvae and adult TBW males. No conclusions could be drawn for methyl parathion and acephate since only Stoneville and ICI males were tested with those chemicals. Differences in response observed with neonate larvae and adults may imply physiological and biochemical differences and, consequently, that resistance mechanisms are not manifested equally in all developmental stages of the TBW.

Table 15 contains the results for cypermethrin toxicity tests for females of susceptible and resistant TBW strains. The LC_{50} for cypermethrin was 1.72 µg per vial for the susceptible strain and varied from 31.81 to 81.69 µg per vial for resistant strains. No significant difference was found with the t-test between the slope of concentration-response line to cypermethrin for the susceptible strain and the mean slope for resistant strains. As with males, females were more tolerant to cypermethrin than neonate larvae. In contrast to males, no clear relationship was present between cypermethrin toxicity to neonate larvae and adult females of resistant strains. The ratios between cypermethrin toxicity (LC_{50}) to females and neonate larvae were 5.2, 10.5, and 50.1 for the Uvalde, ICI, and Hearne strains, respectively. The ratio calculated for the Hearne strain may not be real since only 30 females were tested before the strain was lost.

Table 15. Cypermethrin toxicity to resistant (R) adult TBW females

Strain	n[a]	LC_{50} (95% CL)[b]	LC_{90} (95% CL)[b]	Slope (± SD)
Stoneville(S)[c]	113	1.72 (1.25-2.40)	7.50 (4.55-21.30)	2.00 (± 0.40)[d]
ICI(R)	219	31.81 (21.13-100.44)	248.20 (85.39-7,483)	1.44 (± 0.39)
Uvalde(R)	110	40.87 (28.97-79.01)	144.14 (75.73-627.97)	2.34 (± 0.49)
Hearne(R)	30	81.69 (39.75-...)[e]	555.72 (116.72-...)[e]	1.54 (± 1.10)

[a] Number of adult females tested excluding controls.
[b] Concentrations are expressed in micrograms of insecticide per vial.
[c] Tests were carried out with another sample of the susceptible strain. For Stoneville males the LC_{50} and LC_{90} for cypermethrin were 1.14 (0.59-1.80) and 6.24 (3.25-54.76) µg per vial, respectively.
[d] Slope for the Stoneville strain not significantly different from the mean slope for resistant strains ($P>0.05$; t-test).
[e] Data too heterogeneous to calculate 95% CL.

The resistance ratios for cypermethrin for adult males and females of resistant strains based on tests with another sample of the Stoneville strain (Table 15) are listed in Table 16. Resistance ratios for males were higher than those obtained with a previous sample of the susceptible strain (Fig. 6). No significant differences in resistance seemed to be present between sexes. Results for adults were similar to those obained with neonate larvae, indicating either life stage can be tested to determine the presence of pyrethroid resistance in the TBW.

Females tended to be more tolerant to cypermethrin than males, except for the Uvalde strain, where both sexes showed similar susceptibility to this insecticide. However, the toxicities for both sexes were not significantly different based on the overlap of the 95% confidence limits (CL) for the LC_{50}'s and LC_{90}'s. The present results corroborate those found for the codling moth (Riedl et al. 1985), where the female moths were consistently more tolerant than males and the concentration-response lines were comparable in both sexes. Similarly, studies with house flies indicated that females were more tolerant to pyrethrum (Murray 1938) and DDT (Barber & Schmitt 1948) than males. Also, a study with the *nap*^{ts} strain of *Drosophila melanogaster* showed that females were significantly more resistant than males (Kasbekar & Hall 1988).

Table 16. Resistance ratios[a] for cypermethrin at the LC_{50} and LC_{90} levels for adult males and females of different strains of the TBW

Strain	Males		Females	
	LC_{50}	LC_{90}	LC_{50}	LC_{90}
ICI	16.2	13.6	18.5	33.1
Uvalde	35.6	28.0	23.8	19.2
Hearne	44.2	19.6	47.5	74.1

[a] Calculated by dividing the LC_{50} (or LC_{90}) for the resistant strain by the LC_{50} (or LC_{90}) for the susceptible strain. Tests with the susceptible adults were carried out with another sample of the Stoneville strain.

CHAPTER VI

BIOLOGICAL MEASUREMENTS IN SUSCEPTIBLE AND RESISTANT STRAINS OF THE TOBACCO BUDWORM

Introduction

Insecticide resistance in insects often involves deficiencies in fitness, vigor, behavior, or reproductive potential. Reduced biotic fitness of resistant phenotypes has been reported for several species of arthropods, including the red flour beetle, *Tribolium castaneum* (Bhatia & Pradhan 1968), the armyworm, *Spodoptera littoralis* (Moustafa 1981), the two-spotted spider mite, *Tetranychus urticae* (McEnroe & Naegele 1968), the southern house mosquito, *Culex quinquefasciatus* (Ferrari & Georghiou 1981), and the house fly, *Musca domestica* (Roush & Plapp 1982). However, continued selection may improve fitness through coadaptation of the resistant genome, resulting in more stable resistance (Georghiou & Taylor 1986).

If resistance is associated with biological deficiencies, the resistance gene would decline in frequency when selection pressure is removed. Therefore, by removing selection pressure for one or more generations and using alternate insecticides or other control strategies the frequency of a resistance gene can be decreased to a level where control is once again possible.

There are many cases where the differences in reproductive potential between susceptible and resistant strains are either small or the resistant strain seems to have an advantage (Varzandeh et al. 1954, Thomas & Brazzel 1961, Roush & Hoy 1981). However, resistant genotypes must usually be at a reproductive disadvantage in the absence of insecticides. Otherwise, resistance alleles would be more common prior to selection (Crow 1957).

The assessment of biological characteristics of insecticide-resistant populations can be very important in the management of resistance. Most resistance management tactics involve the reduction of fitness of resistant genotypes relative to susceptible genotypes by either preserving susceptible homozygotes or eliminating heterozygotes and resistant homozygotes (Leeper et al. 1986). This can be achieved by reducing insecticide rates, extending intervals between treatments, using short residual insecticides, or by using alternative insecticides. Susceptible homozygotes can be preserved by creating 'refugia' where part of the population is not treated (Georghiou & Taylor 1977b).

Pyrethroid resistance in the TBW seems to be very unstable. Plapp (1981) observed that a pyrethroid-tolerant population collected in the field and reared in the laboratory in the absence of selection pressure for 8 to 10 generations reached levels of response to pyrethroids very close to those for a susceptible laboratory strain. However, the reason for the resistance decline was not assessed. Also, in the present study pyrethroid-resistant populations of TBW collected in the field showed a marked decline in resistance after being reared for several generations in the laboratory.

Studies on the evaluation of biological differences between susceptible and insecticide-resistant strains of Lepidoptera are scarce. The purpose of the present study was to identify possible causes of pyrethroid resistance reduction in laboratory-reared TBW and of resistance fluctuation in the field, as observed by Plapp (1987). Thus, several biological characteristics such as developmental period for different stages, fecundity, and fertility of a pyrethroid-resistant TBW strain were compared with those of a susceptible strain.

Materials and Methods

Eggs of a susceptible TBW strain were obtained from the Southern Field Crop Insect Management Laboratory, USDA, ARS, Stoneville, MS, where the strain has been

and covered with paper towel for collection of eggs. All jars contained a small plastic bowl with 10% sucrose solution that was replaced as needed. All insects were maintained in an incubator at conditions specified above. Total number of eggs per female was recorded daily. A sample of at least a hundred eggs per female laid up to the 5th day of oviposition was checked for fertility by counting the numbers of neonate larvae. Adult mortality was also checked daily. The intrinsic rate of increase (r) (Andrewartha & Birch 1954) was estimated for each population. Comparisons of mean data between the susceptible and resistant strains were carried out using the t-test.

Results and Discussion

Data referring to the larval stage of susceptible and resistant strains of the TBW are listed in Table 17. The larval developmental period was significantly longer for the resistant strain than for the susceptible strain based on the t-test. Developmental periods for first, third, fourth, and fifth instars were longer for the resistant strain, but no differences were observed between susceptible and resistant second and sixth instars. Mortality at the larval stage was negligible for the TBW strains. Levels of 3 and 2% mortality were observed for the susceptible and resistant strains, respectively.

Only 2 and 1% of the larvae of the susceptible and resistant strains went through the fifth molt (to the sixth instar), respectively. Gunasena (1988) found a higher incidence (11%) of sixth instars in a susceptible TBW strain. Therefore, the occurrence of developmental polymorphism seems not to be constant for different conditions. Factors such as nutrition, temperature, humidity, photoperiod, juvenile hormone, and other growth regulators appear to affect the induction of supernumerary instars (Staal 1975, Schmidt & Lauer 1977).

No significant differences between the susceptible and resistant strains were found for pupal stage length, pupal weight, and pupal mortality (Table 18). However, the

Table 17. Larval developmental period and larval mortality for susceptible (S) and resistant (R) strains of the TBW

Characteristics	S strain	R strain
Initial No. of Neonate Larvae	101	101
Mean Larval Developmental Period (days ± 95% CL)	16.30 ± 0.15	17.84 ± 0.17[a]
First Instars	3.14 ± 0.078	3.47 ± 0.089[a]
Second Instars	2.25 ± 0.12	2.39 ± 0.084
Third Instars	2.05 ± 0.060	2.25 ± 0.071[a]
Fourth Instars	2.32 ± 0.079	2.79 ± 0.079[a]
Fifth Instars	6.54 ± 0.13	6.88 ± 0.11[a]
Sixth Instars	5.00 ± 5.84[b]	7.00 ± ∞[c]
Mortality at Larval Stage (%)	3.0	2.0

[a] Significantly different from the S strain ($P<0.05$; t-test).

[b] Two larvae.

[c] One larva.

Table 18. Sex ratio, mean pupal developmental period, mean pupal weight, pupal mortality, and mean total developmental period for susceptible (S) and resistant (R) strains of the TBW

Characteristics	S strain		R strain	
	Males	Females	Males	Females
No. of Individuals	54	41	52	47
Sex Ratio	1 :	0.76	1 :	0.89
Mean Pupal Developmental Period (days ± 95% CL)	15.04 ± 0.22	13.46 ± 0.23	14.90 ± 0.18	13.28 ± 0.18
Mean Pupal Weight (mg ± 95% CL)	324.07 ± 7.34	318.80 ± 9.05	328.57 ± 5.78	315.36 ± 7.58
Mortality at Pupal Stage (%)	1.8	2.4	1.9	2.1
Mean Developmental Period[a] (days ± 95% CL)	31.30 ± 0.28	29.76 ± 0.35	32.77 ± 0.30[b]	31.02 ± 0.34[b]

[a] Neonate larvae to adults.

[b] Statistically different from the S strain ($P<0.05$; t-test).

developmental period for both sexes tended to be longer for susceptible than for resistant pupae. The mean pupal developmental periods were 15.04 and 14.9 days for susceptible and resistant males, respectively. For females those periods were 13.46 and 13.28 days for the susceptible and resistant strains, respectively. Weights for resistant and susceptible male pupae were 328.57 and 324.07 mg, respectively. Conversely, resistant female pupae were lighter than susceptible female pupae, with mean weights of 315.36 and 318.80 mg, respectively. Pupal mortality was always low (about 2%) for both sexes of the TBW strains.

The developmental period from neonate larvae to adults for each sex was significantly longer for resistant insects (Table 18). This was a result of longer larval period for resistant insects. In addition, females developed significantly faster than males for both TBW strains. It took, on average, 31.3 days for susceptible neonate larvae to develop into adult males, whereas 32.77 days were required for the development of resistant neonate larvae into adults. Those periods averaged 29.76 and 31.02 days for susceptible and resistant females, respectively. The longer developmental time observed for resistant insects may allow for an increase in predation and parasitism on the immature stages and represent an additional factor for fitness reduction in resistant populations under field conditions. In contrast to the present results, a study with organophosphate-resistant southern house mosquito demonstrated that a lower larval survival, a longer pupation period, and a delay in female emergence appeared to be associated with temephos-resistance (El-Khatib & Georghiou 1985).

The sex ratio deviated from unity for both TBW strains (Table 18). The ratios of females per male were 0.76 and 0.89 for the susceptible and resistant strains, respectively. I offer no explanation for this deviation from the expected 1:1 ratio.

The fecundity of susceptible females was significantly higher than that of resistant females (Table 19). The mean numbers of eggs laid by each susceptible and resistant

Table 19. Mean fecundity, mean fertility, mean adult longevity, and intrinsic rate of increase (r)[a] for susceptible (S) and resistant (R) strains of the TBW

Characteristics	S strain		R strain	
	Males	Females	Males	Females
Mean Fecundity (no. eggs laid/female ± 95% CL)	-	2,552.81 ± 232.16 (n = 31)[c]	-	1,270.88 ± 228.68[b] (n = 24)
Mean Fertility (% hatched eggs ± 95% CL)	-	74.77 ± 2.96 (n = 29)	-	71.49 ± 6.59 (n = 15)
Mean Adult Longevity (days ± 95% CL)	21.19 ± 1.45 (n = 54)	17.39 ± 1.44[d] (n = 41)	23.54 ± 2.78 (n = 52)	14.21 ± 1.76[d] (n = 47)
Intrinsic Rate of Increase (r)	-	0.12	-	0.10

[a] $r = (\log_e R_0)/T$, where R_0 is the net replacement rate (no. daughters/female) and T is the mean generation time (Andrewartha & Birch 1954).

[b] Statistically different from the S strain ($P<0.05$; t-test).

[c] n = no. of individuals.

[d] Mean longevity of females significantly different from males of the same strain ($P<0.05$; t-test).

female were 2,552.81 and 1,270.88, respectively. Thus, it was clear that resistant females were at a disadvantage in egg production compared with susceptible females. A difference of this magnitude could obviously be of importance in the loss of resistance in the field. Some studies of this nature have been performed with other insects, but apparently this is the first time a fecundity deficiency has been found for pyrethroid-resistant insects. A laboratory-selected population of *D. melanogaster* showed decreased female fecundity, lower egg-to-adult survival, and slower larval development (Halpern & Morton 1987). In a study with house flies, organophosphate-resistant strains demonstrated reduced fecundities and longer developmental periods (Roush & Plapp 1982).

Curves of egg-lay distributions by day for the susceptible and resistant strains are shown in Fig. 7. Though the egg numbers were different, with more eggs laid by susceptible females, the shape of the curves was approximately the same. Egg laying peaked in both strains 3 days after oviposition began. Then, a marked decline in oviposition was observed for susceptible females between the 3rd and the 10th day, followed by a gradual reduction in oviposition until the death of the females. For resistant females a reduction in oviposition was observed between the 3rd and the 6th day. Then the mean egg numbers per female remained around 100 per day until the 12th day. Afterwards, a slight decline was observed in the egg lay until the death of the females. Thus, the oviposition distribution seemed to be slightly more heterogeneous for resistant females.

Another advantage of the susceptible strain over the resistant strain was that 29 out of 31 of the susceptible females produced offspring while only 15 out of 24 of the resistant females produced offspring.

Fertility did not differ significantly between susceptible and resistant females (Table 19). The mean percentage of hatched eggs was 74.77 and 71.49 for susceptible and

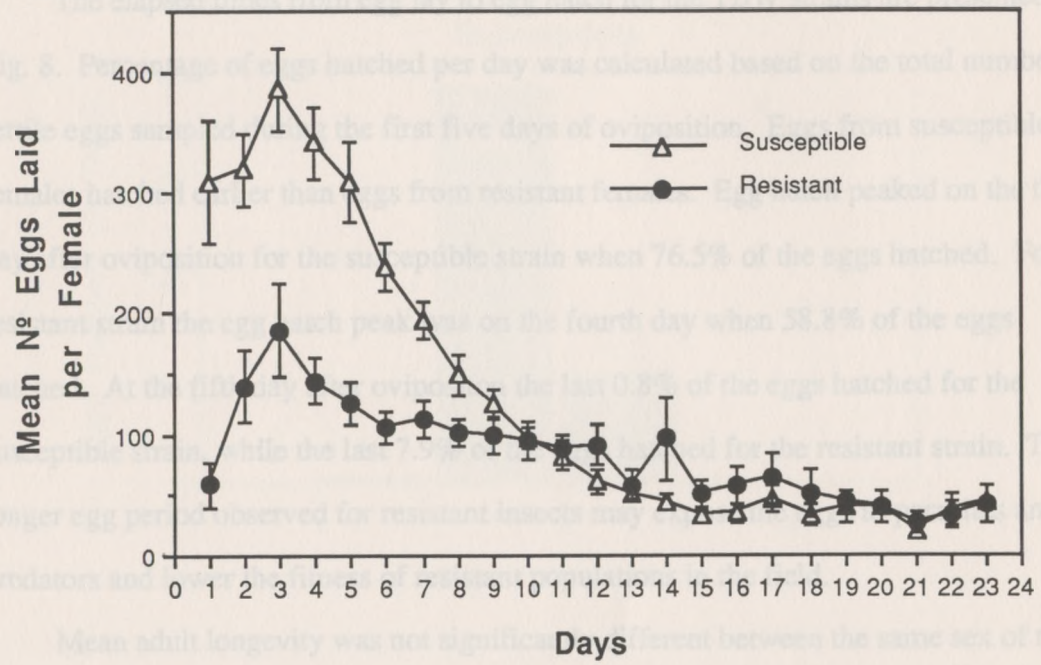

Fig. 7. Egg lay distributions for susceptible and resistant strains of the TBW. Vertical bars represent standard error of the mean.

resistant females, respectively.

The elapsed times from egg lay to egg hatch for the TBW strains are presented in Fig. 8. Percentage of eggs hatched per day was calculated based on the total number of fertile eggs sampled during the first five days of oviposition. Eggs from susceptible females hatched earlier than eggs from resistant females. Egg hatch peaked on the third day after oviposition for the susceptible strain when 76.5% of the eggs hatched. For the resistant strain the egg hatch peak was on the fourth day when 58.8% of the eggs hatched. At the fifth day after ovipositon the last 0.8% of the eggs hatched for the susceptible strain, while the last 7.9% of the eggs hatched for the resistant strain. The longer egg period observed for resistant insects may expose the eggs to parasites and predators and lower the fitness of resistant populations in the field.

Mean adult longevity was not significantly different between the same sex of the TBW strains (Table 19). The mean life spans of susceptible males and females were 21.19 and 17.39 days, respectively. Resistant males and females lived, on average, 23.54 and 14.21 days, respectively.

The intrinsic rate of increase (r) was 20% greater for the susceptible strain (Table 19). The dissimilarity observed for the 'r' values was a consequence of significant differences in both developmental time and fecundity between the strains. According to Roush & Plapp (1982), changes in developmental time have much greater effects on reproductive potential than changes in fecundity. Thus, in the present case, the small discrepancy in the 'r' values occurred because the difference in developmental time between the two strains was smaller than the difference in fecundity. A similar difference (21%) was observed between the 'r' values for a susceptible and a temephos-resistant strain of *C. quinquefasciatus* (El-Khatib & Georghiou 1985).

The biological effects of insecticide resistance may be due either to the pleiotropic effects of the resistance genes or to the effect of closely linked genes which have been

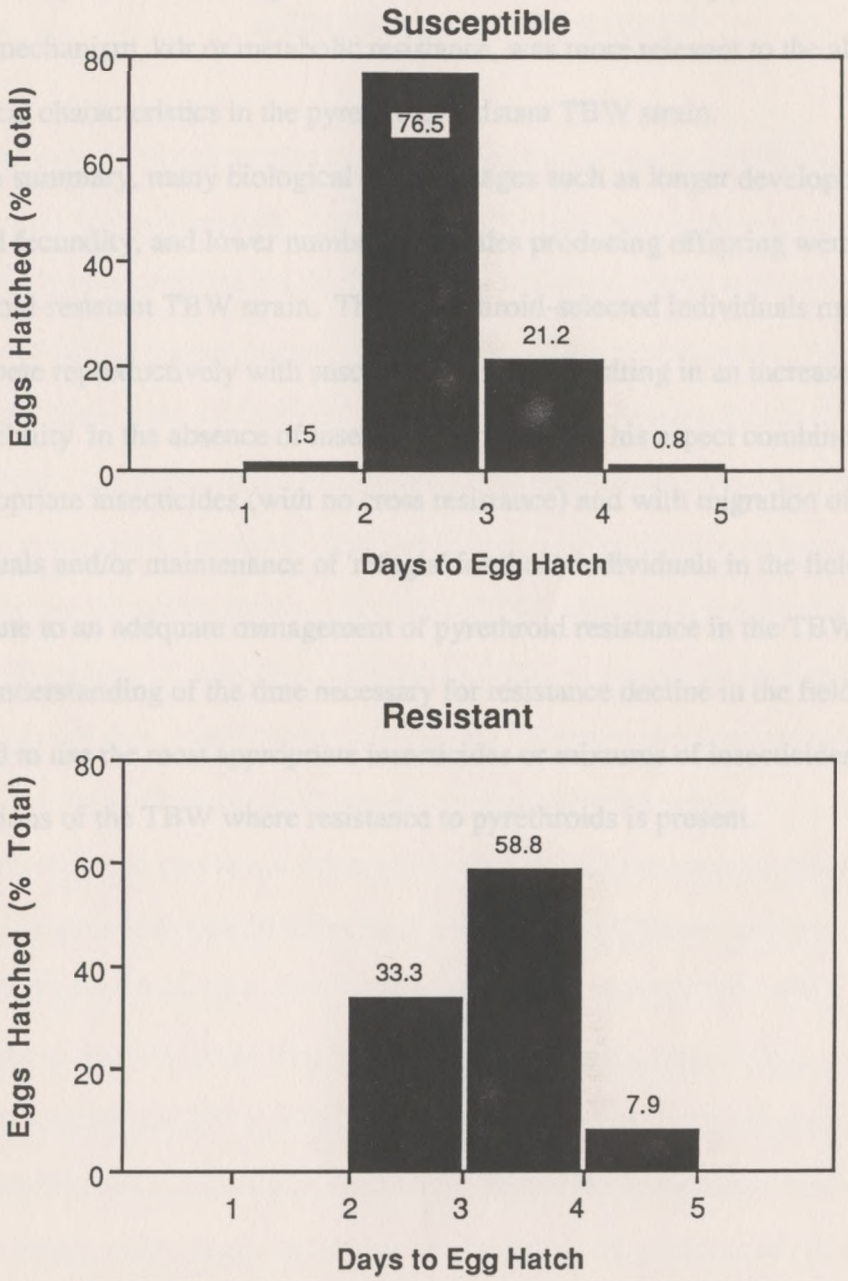

Fig. 8. Egg hatch by day for susceptible and resistant strains of the TBW.

fortuitously selected along with resistance genes (Halpern & Morton 1987). Since in the present study the resistance genes were not isolated, it was not possible to establish which mechanism, kdr or metabolic resistance, was more relevant to the alteration of the biological characteristics in the pyrethroid-resistant TBW strain.

In summary, many biological disadvantages such as longer developmental period, reduced fecundity, and lower number of females producing offspring were present in the pyrethroid-resistant TBW strain. Thus, pyrethroid-selected individuals may not be able to compete reproductively with susceptible insects, resulting in an increased pyrethroid susceptibility in the absence of insecticide exposure. This aspect combined with the use of appropriate insecticides (with no cross resistance) and with migration of susceptible individuals and/or maintenance of 'refugia' for those individuals in the field may contribute to an adequate management of pyrethroid resistance in the TBW. However, a better understanding of the time necessary for resistance decline in the field is still required to use the most appropriate insecticides or mixtures of insecticides to control populations of the TBW where resistance to pyrethroids is present.

CHAPTER VII

GENERAL DISCUSSION

The toxicity of insecticides to both susceptible and resistant TBW larvae decreased as the size of larvae increased. For the Stoneville (susceptible) strain, cypermethrin and methyl parathion toxicities (LC_{50}'s) were 9 and 79 times higher against neonate than against third instar larvae, respectively. For the ICI (resistant) strain, cypermethrin and methyl parathion were 424 and 820 times more toxic to neonate than to third instar larvae, respectively. Therefore, in insecticide-resistance management programs priority should be given to the control of small TBW larvae because they develop lower resistance levels to insecticides than large larvae. Daly et al. (1988) proposed a strategy for the control of pyrethroid-resistant *H. armigera* in Australia based on insecticide applications against a susceptible age class (small larvae). In this way selection pressure would be minimized by avoiding the window of development at which selection occurs.

Differences in toxicity of insecticides were found between adult males and neonate larvae. Cypermethrin was 20 times more toxic to susceptible neonate larvae than to adult males, based on the LC_{50}'s. For the resistant strains, cypermethrin was 5 and 6 times more toxic to neonate larvae than to Uvalde and ICI males, respectively. Methyl parathion toxicity was 257 and 742 times greater to neonate larvae than to adult males for the susceptible and ICI strains, respectively. However, as resistance was expressed in both life stages, either stage can be tested to determine the presence of resistance in the TBW.

Results were similar with adult females. Cypermethrin was about 8 times more toxic to susceptible neonate larvae than to susceptible females. Toxicity ratios of about 5- and 10-fold between females and neonate larvae were calculated for the Uvalde and ICI

strains, respectively. Resistance ratios for cypermethrin were similar for both sexes. Thus, either sex can be tested to monitor for resistance in the TBW.

The comparative toxicity of insecticides to third instars and adult males was also variable for different TBW strains. For the susceptible strain, cypermethrin and methyl parathion were about 2 and 3 times more toxic to third instars than to adult males, respectively. In contrast, for the ICI strain cypermethrin was about 70 times more toxic to adult males than to third instars, while methyl parathion was equally toxic to both stages of the ICI strain. A study with *Spodoptera littoralis* showed that adults and eggs are more susceptible to organophosphate insecticides than larvae, apparently due to higher microsomal cytochrome P_{450} levels in the larvae (Dittrich et al. 1980). This seemed not to be case for the susceptible and resistant TBW strains used in the present study.

The expression of resistance was variable for different developmental stages of the TBW. For the ICI strain, resistance levels to cypermethrin were 10.9-, 998-, and 6.3-fold for neonate larvae, third instars, and adult males, respectively. For methyl parathion, the resistance ratios were 1.8, 18.9, and 5.2 for neonate larvae, third instars and adult males, respectively. The pattern was similar for both insecticides.

Thus, the higher levels of resistance to cypermethrin and methyl parathion observed in third instars than in neonate larvae or adults are evidence for the presence of metabolic resistance in the ICI strain. Also, the presence of resistance to cypermethrin and the absence of resistance to methyl parathion in the ICI neonate larvae are evidence for the expression of target site (kdr) resistance to pyrethroids. Previous studies demonstrated that kdr-resistant houseflies and mosquitoes showed cross resistance to several pyrethroids (Sawicki 1978, DeVries & Georghiou 1980, Priester & Georghiou 1980). Therefore, the presence of resistance to all the pyrethroids tested against the ICI strain is an additional evidence for the presence of target site resistance. Thus, target site resistance appears to be a common mechanism of resistance for all stages of the TBW

while metabolic resistance is manifested mostly in large larvae, which show an inherently higher capability for xenobiotic detoxification.

The patterns of acephate and methyl parathion toxicities against different developmental stages of both TBW strains were similar. However, no resistance to acephate was present in any stage. This seemed also to be the case for the other S-alkyl phosphorothiolates, profenofos and sulprofos.

Thiodicarb demonstrated decreasing toxicities from neonate larvae to adult males for both TBW strains. Susceptible adult males were 85 and 56 times more tolerant to thiodicarb than neonate larvae and third instars, respectively. Resistant adult males were 173 and 11 times more tolerant to thiodicarb than neonate and third instar larvae, respectively. Even though some tolerance to this compound was observed in third instars (3.9-fold), no significant resistance to thiodicarb appears to exist in any stage.

Insecticide resistance in insects has often been found to be the result of one mutant gene (Milani 1960, Brown 1967, Georghiou 1969, Hama & Iwata 1978, Halliday & Georghiou 1985, Plapp 1986, Roush et al. 1986, Yarbrough et al. 1986). A recent study with the TBW showed that permethrin resistance was inherited as a single, major, incompletely recessive, autosomal factor (Payne et al. 1988). Other studies with methyl parathion (Whitten 1978) and methomyl (Roush & Wolfenbarger 1985) also showed that resistance was due to a single, autosomal gene of incomplete dominance. In contrast, our data provide evidence for the presence of two genes for pyrethroid resistance in the ICI TBWs, one for target site and other for metabolic resistance.

Selection for resistance may build up biological disadvantages. Studies concerning the effects of insecticide resistance on the biology of insects performed to date were mostly related to metabolic resistance to organophosphate insecticides (Varzandeh et al. 1954, Singh & Morton 1981, Ferrari & Georghiou 1981, Roush & Plapp 1982, Amin & White 1984, El-Khatib & Georghiou 1985). Those effects can not be generalized since

they are variable for different insect species and for different resistant strains of a same species. Apparently, the affected biological characteristics of a resistant strain are related to the type of resistance present, and ultimately to the gene responsible for it. However, in the present study the contribution of each gene to the biological alterations was not assessed, since the genes for target site and metabolic resistances were concomitantly present in the resistant (ICI) TBW strain.

Chlordimeform synergized most insecticides and insecticide combinations against susceptible and resistant TBW strains. However, synergism was variable for different insecticides against different TBW strains. Levels of pyrethroid synergism by chlordimeform tended to be in the same range for the Stoneville and ICI neonate larvae. The most synergized pyrethroids were cypermethrin and fluvalinate for both strains. For the other insecticides, methyl parathion, monocrotophos, acephate, thiodicarb, and endosulfan were much more synergized against susceptible than against resistant neonate larvae. The remaining insecticides showed low synergism by chlordimeform with similar levels for both TBW strains, except for avermectin that was more synergized against the resistant strain. For third instars, synergism by chlordimeform was much higher with cypermethrin and methyl parathion against the ICI larvae than against the Stoneville larvae. For the other insecticides, synergism levels were equivalent for both TBW strains.

Results of the present study suggested that synergism by chlordimeform was independent of resistance. Even though chlordimeform was a good synergist for the pyrethroids, it did not block resistance to those insecticides in the TBW.

Chang & Plapp (1983b) observed that chlordimeform increased the specific binding of *cis*-permethrin to its target site, i. e., the receptor on nerve membranes. Treacy et al. (1987) and Sparks et al. (1988) concluded that chlordimeform can enhance the efficacy of pyrethroids against *Heliothis* spp. through behavioral mechanisms. The mechanism of

insecticide synergism by chlordimeform was not assessed in the present study; however, a combination of different mechanisms may be involved.

Recommendations for resistance management with insecticides

Based on all the data obtained, a general scheme for resistance management is proposed. The main point is to delay the onset of resistance by one generation early in the season, that is, to displace the build up of resistance from June to July so that the resistance gene frequency would not reach high levels until late in the season, when the yield is already assured (Campanhola & Plapp 1988). The idea is to avoid the use of pyrethroids early in the season, thereby minimizing the selection pressure and increasing the efficacy of those insecticides during the critical mid-season period.

Monocrotophos, profenofos, sulprofos, acephate, methomyl, thiodicarb, or endosulfan, all alone or combined with chlordimeform, and the combinations cypermethrin plus chlordimeform and piperonyl butoxide, cypermethrin plus thiodicarb and chlordimeform, cypermethrin plus profenofos, cypermethrin plus acephate with or without chlordimeform, and cypermethrin plus methyl parathion plus chlordimeform are possible alternate insecticides for the control of pyrethroid-resistant first instars early in the season.

Pyrethroid insecticides can be used for mid-season control of *Heliothis* spp. They are very effective against both bollworms and TBWs, hence should be used at this period to promote good control and assure production of part of the crop.

Chlordimeform synergized all pyrethroids against pyrethroid-resistant neonate larvae. Cypermethrin, the only pyrethroid tested against third instars, was also synergized by chlordimeform against these larvae. Therefore, chlordimeform can be combined with pyrethroids to increase the efficacy of these insecticides during the mid-season period. The use of the three-way combination cypermethrin plus chlordimeform

and piperonyl butoxide also proved efficient in controlling pyrethroid-resistant third instar TBWs. Since chlordimeform will no longer be sold in the United States after 1988, other formamidines may replace it as a synergist. Promising results were obtained with cypermethrin plus amitraz (another formamidine) against pyrethroid-resistant neonate TBW larvae (Bagwell & Plapp 1988). Amitraz has also been an effective synergist for other insecticides such as the S-alkyl phosphorothiolates against pyrethroid-resistant TBWs (R. D. Bagwell, personal communication).

Late-season control of the TBW tends to be more difficult. If control is required, it is desirable to use insecticides other than pyrethroids. At this time larvae of all instars are present in the field due to overlap of generations. Therefore, insecticides or insecticide combinations that provide adequate control of any-size larvae are required. Alternate insecticides such as acephate (with or without chlordimeform), profenofos, sulprofos, endosulfan (alone or combined with chlordimeform), or methomyl and thiodicarb (with or without chlordimeform) can be used to control pyrethroid-resistant TBWs at this period. Some insecticide combinations tested such as cypermethrin plus chlordimeform and piperonyl butoxide, cypermethrin plus methyl parathion, and cypermethrin plus thiodicarb with or without chlordimeform also seemed to be efficient in controlling large pyrethroid-resistant TBW larvae in my tests.

To check the results obtained with insecticide bioassays in the laboratory, a small-scale field test was conducted in the season of 1987 in cooperation with J. R. C. Robinson on the Texas A&M research farm (Appendix A). Even though the variability within plots with the same treatments was high, the results were similar to those obtained in the laboratory.

It is not desirable to use the same class of insecticides or the same insecticide combination season long when resistance is present. Alternation of insecticides with different modes of action can exploit the disadvantage of resistant insects in the absence

of insecticide pressure. However, it is not clear if alternation of insecticides of different classes or combination of two insecticides is better to control resistant populations of insects (Burden et al. 1960, Asquith 1961, Graves et al. 1967, Ozaki et al. 1973, Pimentel & Bellotti 1976, Sasaki & Ozaki 1976, Brown 1977, Knipling 1979, Georghiou 1983, MacDonald et al. 1983, Ozaki 1983, Brindley & Selim 1984, Knipling & Klassen 1984, Mani 1985, Sawicki 1985, Comins 1986). The best approach would probably be the adoption of both strategies during different times of the growing season. In the present case, the use of insecticide plus synergist(s) would substitute for mixtures of insecticides. Thus, the associated use of alternation of insecticides with different modes of action interspaced with use of insecticides plus synergists may promote adequate control of pyrethroid-resistant TBWs while preventing the build up of resistance.

A resistance monitoring system is very useful for evaluating the efficacy of the strategies adopted for resistance management and to detect incipient levels of resistance in the field. In the case of TBW, a monitoring program based on resistance in adult males captured in pheromone traps has been conducted for two seasons and proved very efficient for evaluation of resistance in a region-wide basis (Plapp et al. 1987, 1988). A monitoring system for resistance in TBW based on egg-neonate larval bioassays and larval bioassays is under development and may help in assessing pyrethroid resistance on an individual-field basis (McCutchen & Plapp 1988).

CHAPTER VIII

SUMMARY

Neonate larval biossays with different pyrethroid-resistant strains of the TBW were conducted in the laboratory. Results showed that the resistance spectra were approximately the same for all resistant strains. However, resistance levels to insecticides of different classes did not follow a consistent pattern for different resistant strains. Chlordimeform synergized all insecticides tested, but synergism was variable for different insecticides against susceptible and resistant strains.

Extensive tests with the Stoneville (susceptible) and ICI (resistant) neonate larvae showed that resistance extended to all pyrethroids tested. This is evidence for the presence of target site (kdr) resistance in neonate TBW larvae. The absence of resistance to organophosphate insecticides in this stage showed that metabolic resistance is not significantly expressed in small TBW larvae. Chlordimeform synergized all pyrethroids against both susceptible and resistant TBW larvae. However, no consistent pattern of synergism was observed for the TBW strains. Sometimes chlordimeform synergized pyrethroids more against the ICI strain, other times more synergism was observed against the Stoneville strain.

Adult bioassays with insecticides were performed to support a monitoring program for TBW resistance ongoing in several southern states of the United States based on bioassays with males collected from pheromone traps (Plapp et al. 1987, Plapp et al. 1988). Resistance to cypermethrin was observed in males of all resistant strains. Resistance to methyl parathion was also observed in males, but no resistance to acephate (and probably to other S-alkyl phosphorothiolates) seemed to be present. The occurrence of resistance to thiodicarb in adult males is not clear, since only Hearne males showed

resistance to this insecticide.

Adults were more tolerant to insecticides than neonate larvae for all TBW strains. At the LC_{50} level, Uvalde and ICI males were 5- to 6-fold more tolerant to that insecticide than neonate larvae, respectively. For females, that relationship was more variable, being about 5- and 10-fold for the Uvalde and ICI strains, respectively. Females tended to be more tolerant to cypermethrin than males, but differences in toxicity between sexes were not significant.

The results showed that the best insecticides or insecticide combinations to control pyrethroid-resistant neonate TBW larvae seemed to be profenofos, sulprofos, acephate, methomyl, thiodicarb, endosulfan, or avermectin, all alone or combined with chlordimeform; rotenone plus chlordimeform; and the combinations cypermethrin plus chlordimeform and piperonyl butoxide, cypermethrin plus thiodicarb plus chlordimeform, cypermethrin plus profenofos, cypermethrin plus acephate with or without chlordimeform, and cypermethrin plus methyl parathion plus chlordimeform. However, since not all possible insecticide combinations were tested, other combinations may prove useful for controlling pyrethroid resistant TBWs.

Results of bioassays with Stoneville and ICI third instars showed a tendency for chlordimeform to synergize insecticides more against resistant than against susceptible TBWs. In addition, synergism by chlordimeform was greater than synergism by piperonyl butoxide with all the insecticides studied, except for cypermethrin against the ICI larvae. The addition of chlordimeform and piperonyl butoxide to cypermethrin was more effective than any synergist alone. The high level of resistance to cypermethrin (998-fold at LC_{50}), the high level of cypermethrin synergism by piperonyl butoxide (158.9-fold at LC_{50}), the resistance level observed to methyl parathion (18.9-fold at LC_{50}) in third instars, and the blockage of resistance to methyl parathion by piperonyl butoxide are evidence for the presence of metabolic resistance in the ICI strain.

Therefore, it seems that target site resistance is equally manifested in all the developmental stages of TBW while metabolic resistance is mostly expressed in large TBW larvae.

Many alternate insecticides or insecticide combinations can be suggested for the control of pyrethroid-resistant large TBW larvae. These include profenofos, sulprofos, acephate (acephate can be combined with chlordimeform), endosulfan, possibly methomyl or thiodicarb (with or without chlordimeform), and the insecticide combinations cypermethrin plus chlordimeform and piperonyl butoxide, cypermethrin plus methyl parathion, and cypermethrin plus thiodicarb with or without chlordimeform.

A study was also conducted to detect possible biological differences between the Stoneville and the ICI strains. No significant differences between susceptible and resistant insects were observed for mortality at any stage, pupal weight, sex ratio, fertility, and adult longevity. However, longer developmental period, reduced egg production, and lower number of females producing offspring were observed in the resistant strain relative to the susceptible strain. The intrinsic rates of increase (r) were 0.12 and 0.10 for the susceptible and resistant strains, respectively. These disadvantages of the resistant insects in the absence of insecticide pressure can be exploited in resistance management programs.

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APPENDIX A

FIELD EFFICACY OF INSECTICIDES AND INSECTICIDE COMBINATIONS FOR CONTROL OF THE TOBACCO BUDWORM IN COTTON

Introduction

A field study was conducted in cooperation with J. R. C. Robinson, Research Associate, Dept. of Entomology, Texas A&M University, in order to evaluate the performance of some insecticides and insecticide combinations, with and without synergists, for TBW control. The treatments were based on results obtained with laboratory bioassays.

Materials and Methods

This study was carried out during the 1987 season at the Texas Agricultural Experiment Station Research Farm in Burleson County, near College Station, Texas. An area of approximately 1.6 ha was planted on 9 April with the cotton cultivar Stoneville 825 on rows spaced 1m apart. The cotton was treated with 139.9 g acephate per ha on 29 May against cotton fleahopper, and also with 559.5 g methyl parathion per ha on 17 and 22 June against boll weevil. The field was furrow irrigated on 27 July and received scant rainfall during the experimental period, amounting to 14.2 mm. Nineteen insecticide treatments and an untreated check (Table A1) were compared in a randomized block design with four replications. Plots were 12 rows wide and 13.7 m long. Only the middle six rows were sprayed. Applications were made with a high-clearance, self-propelled sprayer using TX-3 hollow cone nozzles and calibrated to deliver 47.2 l per ha. Insecticide applications started after the first major infestation of *Heliothis* spp. occurred

Table A1. Numbered list of treatments

Number	Treatment	Rate (g/ha)
1	Chlordimeform (CDF)	139.9
2	Cypermethrin (Cyperm.)	44.8
3	Cyperm. + CDF	22.4 + 139.9
4	Cyhalothrin (Cyhal.)	28.0
5	Cyhal. + CDF	14.0 + 139.9
6	Acephate (Aceph.)	1,118.9
7	Aceph. + CDF	559.5 + 139.9
8	Cyperm. + Aceph.	22.4 + 559.5
9	Cyperm. + Aceph. + CDF	22.4 + 559.5 + 139.9
10	Thiodicarb (Thiodic.)	671.4
11	Thiodic. + CDF	447.6 + 139.9
12	Thiodic. + Cyperm.	447.6 + 22.4
13	Thiodic. + Cyperm. + CDF	447.6 + 22.4 + 139.9
14	Cyperm. + Amitraz	22.4 + 139.9
15	Thiodic. + Cyperm. + Amitraz	447.6 + 22.4 + 139.9
16	Thiodic. + Amitraz	447.6 + 139.9
17	Amitraz	139.9
18	Cyperm. + Profenofos[a]	33.6 + 402.8
19	Cyperm. + Profenofos[a]	44.8 + 537.1
20	Untreated Check	-

[a] Cypermethrin only was sprayed four times and the last spray was carried out with profenofos only.

in early July. Treatments were applied on 13, 21, and 31 July, and 11 and 19 August. A post-treatment sample of secondary pests was made 13 August by inspecting 15 randomly selected leaves per plot and counting numbers of aphids and mites with a hand lens. *Heliothis* spp. larval counts and cotton crop damage assessments were made prior to each application of insecticides. Sampling consisted of counting the total number of flower buds (squares) more than 1/3 grown and soft bolls on plants in four separate and randomly selected 1-meter sections of row per plot and recording the number damaged by *Heliothis* spp. larvae. Larvae present on these fruits were also counted. Cotton was hand harvested from four, randomly selected 1-meter sections of treated row per plot. The harvested cotton was extracted, ginned, and weighed. Data were analyzed by analysis of variance, and means were compared using a standard multiple comparison procedure (SAS Institute 1982).

Results and Discussion

The post-treatment seasonal average data for number of *Heliothis* spp. larvae, percent damaged squares, percent damaged bolls, and cotton yield for the insecticides and insecticide combinations tested are listed in Table A2. The post-treatment numbers of secondary pests such as aphids and mites for the different treatments are shown in Table A3.

During the test period the bollworm: TBW ratio varied from 3 to 0.2. However, for most of the experimental period TBW represented more than 50% of the *Heliothis* spp. captured in light traps. Also, the frequency of resistant individuals was highest during this period, according to a monitoring program for pyrethroid resistance conducted in the region (F. W. Plapp, Jr., unpublished data).

We used small plots for testing insecticides (0.017 ha) and observed a very high variability between replicates of treatments for all the variables sampled. Therefore, many

Table A2. Efficacy and yield data for cotton treated with different insecticides and insecticide combinations for control of *Heliothis* spp., Burleson County, Texas. 1987

Treat. Nº	Post-Treatment Seasonal Average Values			
	No. Larvae per 4/10,000 ha	Percent Damaged Squares	Percent Damaged Bolls	Yield (kg lint/ ha)
1	3.1 abc[a]	8.5 abc	5.8 ab	475.3 ed
2	2.2 bc	9.4 abc	6.6 ab	720.2 abcde
3	2.0 c	14.3 a	6.3 ab	764.9 abc
4	4.5 a	7.7 abc	5.4 ab	844.4 ab
5	3.7 abc	7.4 abc	6.1 ab	795.4 abc
6	3.5 abc	7.9 abc	8.9 a	531.1 cde
7	4.2 ab	11.2 abc	8.1 ab	445.7 e
8	3.3 abc	7.2 abc	5.1 ab	714.1 abcde
9	4.3 ab	9.4 abc	7.6 ab	570.5 bcde
10	2.8 abc	7.3 abc	6.7 ab	644.8 abcde
11	2.2 bc	3.3 bc	2.0 b	648.7 abcde
12	2.6 abc	4.0 bc	5.0 ab	699.6 abcde
13	1.7 c	3.0 c	4.5 ab	921.4 a
14	3.4 abc	9.4 abc	5.9 ab	697.4 abcde
15	2.5 abc	5.9 abc	5.1 ab	700.2 abcde
16	2.3 bc	7.5 abc	4.8 ab	581.0 bcde
17	3.2 abc	11.9 ab	7.1 ab	645.4 abcde
18	3.6 abc	11.9 ab	6.2 ab	651.5 abcde
19	3.6 abc	10.4 abc	5.8 ab	752.6 abcd
20	3.8 abc	11.9 ab	7.3 ab	597.6 bcde

[a] Means within a column followed by a common letter are not significantly different ($P > 0.05$; Duncan's multiple range test)

Table A3. Post-treatment sample of secondary pests from cotton treated with different insecticides and insecticide combinations for control of *Heliothis* spp., Burleson Co., Texas. 1987

Treat. Nº	Aphids[a]	Mites[a]
1	113.6 bcde[b]	4.3 ab
2	262.4 bc	4.4 ab
3	441.0 ab	4.4 ab
4	723.6 a	0.5 b
5	445.2 ab	0.1 b
6	27.6 cde	0.4 b
7	1.2 de	0.4 b
8	0.2 e	0.7 ab
9	0.6 de	7.6 ab
10	44.1 cde	0.0 b
11	26.1 cde	1.1 ab
12	147.9 bcd	5.5 ab
13	28.5 cde	4.4 ab
14	73.9 cde	2.2 ab
15	24.1 cde	1.0 ab
16	86.5 cde	0.1 b
17	60.7 cde	2.2 ab
18	83.9 cde	40.6 a
19	104.4 bcde	3.4 ab
20	234.1 bc	0.4 b

[a] Number of insects counted on 15 leaves per plot.

[b] Means within a column followed by a common letter are not significantly different ($P > 0.05$; Duncan's multiple range test following square-root transformation data. Original data used for table presentation).

differences between means of treatments were not detected by the Duncan's multiple comparison procedure (SAS Institute 1982). However, some differences among treatments were identified.

Some measurements performed in the treated plots related well to yield data. Percent damaged squares and percent damaged bolls related well to yield data for the treatments acephate, acephate plus chlordimeform, and cypermethrin plus acephate plus chlordimeform (i.e., high levels of damage with low yields) and the treatment thiodicarb plus cypermethrin plus chlordimeform (i. e., low levels of damage with a high yield). In addition, treatment with cypermethrin plus chlordimeform, cyhalothrin, and cyhalothrin plus chlordimeform resulted in apparently greater yields than many of the other treatments. The data did not reveal any significant differences in yield between chlordimeform mixtures and equivalent amitraz (another formamidine) mixtures, though the latter were associated with slightly lower yields.

Post-treatment differences in secondary pests were observed among treatments. Most treatments containing pyrethroids resulted in large numbers of aphids. In contrast, acephate plus cypermethrin and acephate plus chlordimeform showed control of aphids (statistically lower number of aphids than the untreated check). Numbers of spider mites were negligible throughout the test period.

The field results were similar to results obtained in the laboratory. Very high synergism was found when the combination cypermethrin plus thiodicarb plus chlordimeform was tested in the laboratory against neonate TBW larvae. Also synergism was observed when this combination was tested against third instars.

The combination cypermethrin plus acephate plus chlordimeform seemed not to reduce the *Heliothis* spp. damage and not to increase cotton yield when compared with untreated check. A similar compound, sulprofos, was tested in the laboratory in combination with cypermethrin against third instars and antagonism was observed.

Conversely, synergism by chlordimeform for the 3-way combination cypermethrin plus acephate plus chlordimeform and synergism for the 2-way combination cypermethrin plus acephate was observed against susceptible and resistant neonate larvae.

The satisfactory *Heliothis* spp. control in the field observed with cyhalothrin, alone or combined with chlordimeform, and cypermethrin plus chlordimeform confirmed the laboratory results. Cyhalothrin only tended to be more toxic than cypermethrin only against neonate TBW larvae in laboratory bioassays (Chapter III) and chlordimeform synergized both chemicals, mainly against the resistant strain. Data for cypermethrin plus chlordimeform against resistant third instars also showed high level synergism in the laboratory. Thus, the apparently best treatments observed in the laboratory tended to be confirmed by the results obtained in the field.

VITA

Clayton Campanhola was born on February 11, 1955 in Jundiaí, São Paulo, Brazil. He received the Bachelor of Science in Agricultural Engineering in January, 1977 from the 'Universidade de São Paulo' at Piracicaba. He completed his Master of Science degree in Nuclear Energy in Agriculture (Radioentomology) from the same University in May, 1980. Admitted to the Office of Graduate Studies of Texas A&M University in September, 1985, he qualified as a candidate for the Doctor of Philosophy degree in July, 1988.

During 1979-80 he worked at the 'Centro de Radioisótopos, Instituto Biológico de São Paulo', Brazil. During 1981-82 he taught the courses Applied Entomology, General Agronomy, and Experimental Statistics as a Professor of the Dept. of Agronomy in the 'Fundação Universidade Federal de Mato Grosso', Cuiabá, Brazil. In 1983 he joined the EMBRAPA (Empresa Brasileira de Pesquisa Agropecuária) with which he is presently connected at the 'Centro Nacional de Pesquisa de Defesa da Agricultura', Campinas, São Paulo, Brazil. His permanent address is Rua Natalino Carife, nº 55, Jundiaí 13200, São Paulo, Brazil.