

Chapter 8

TOXICOLOGY OF INSECTICIDES AND ACARICIDES

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INTRODUCTION

The history of insect control on cotton since World War II can be divided into three periods based on the types of insecticides used to control the major cotton insect pests such as the tobacco budworm, *Heliothis virescens* (F.), the bollworm, *Helicoverpa zea* (Boddie) and the boll weevil *Anthonomus grandis grandis* Boheman. The first period was the DDT and organochlorine period, lasting from their introduction just after World War II to the mid- 1960s when widespread resistance and environmental concerns began to outweigh benefits derived from their continued use (Sparks, 1981; also see Chapter 13). The second period was that of the organophosphorus insecticides as exemplified by methyl parathion which came into prominent use as the utility of DDT and other organochlorines declined during the mid-1960s. Although still widely used for control of some cotton insect pests, the organophosphorus insecticide period of predominance declined during the late 1970s when the tobacco budworm developed resistance to many of the organophosphorus insecticides then in use (Sparks, 1981; Sparks *et al.*, 1993a) and the third period, that of the pyrethroid insecticides, began. Currently, pyrethroids are the predominant insecticides used for the control of the primary cotton insect pests such as the bollworm/tobacco budworm. However, because pyrethroid resistance is now present in many parts of the United States (Martinez-Carrillo and Reynolds, 1983; Nicholson and Miller, 1985; Miller, 1987; Campanhola and Plapp, 1987; Leonard *et al.*, 1987, 1988a; Luttrell *et al.*, 1987; Graves *et al.*, 1988; Sparks *et al.*, 1993a; also see this volume), we may be entering a new period of cotton insect control.

Interest in insecticide-related research on cotton insects, as measured by the percentage of publications devoted to the subject in the Journal of Economic Entomology, has fluctuated over the last 40 years (Figure 1). In part, these fluctuations may result from problems with insecticide resistance, environmental concerns and the periodic introduction of new chemistry. For example, the peaks that occur in the mid-1950s correspond with the development of insecticide resistance in the boll weevil, while those in the mid-1970s occur at the time of organophosphorus insecticide resistance appearing in the tobacco budworm and the introduction of pyrethroid insecticides (Sparks,

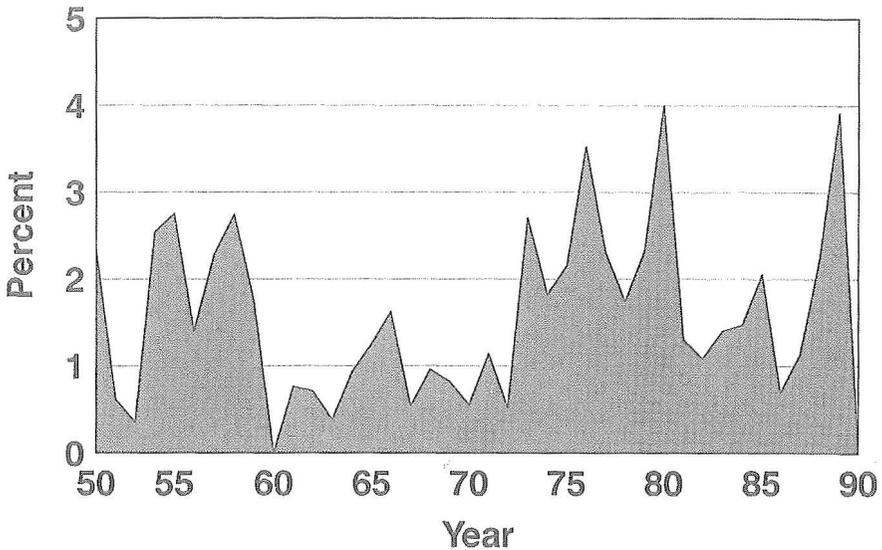


Figure 1. Percentage of articles on the interaction of cotton insects with insecticides (toxicity, metabolism, field efficacy, etc.) published each year in the Journal of Economic Entomology.

1981). The recent peak for 1987-1990 may also reflect the increasing concern over pyrethroid resistance in cotton insects (Sparks *et al.*, 1993a).

Cotton insect control has undergone an evolution from a strictly chemical- based system, to a system of insect pest management, and to what is now being termed resistance management. The appearance of resistance management (National Research Council, 1986) as a concept, reflects the realization that the arsenal of insecticidal compounds for use on cotton or any other crop, is very definitely limited, especially given the increasing concern for the environment, human and animal safety, and the increasing cost of insecticide discovery and development (Georghiou, 1986; Hammock and Soderlund, 1986). Therefore, currently registered and available compounds should be treated as valuable, potentially non-renewable resources, that we can ill afford to lose or waste.

Central to implementing any resistance management program, as well as the successful and safe use of current and future insecticides, is the need to understand the modes of action and mechanisms of detoxification and activation of the insecticide involved. Whole books have been devoted to the subject of insecticide and miticide toxicology (O'Brien, 1967; Brooks, 1974; Eto, 1974; Kuhr and Dorough, 1976; Wilkinson, 1976a; Coats, 1982; Corbett *et al.*, 1984; Matsumura, 1985; Hutson and Roberts, 1985; Kerkut and Gilbert, 1985; Wright and Retnakaran, 1987; Crombie, 1990; Duce, 1992; Duke *et al.*, 1993). This chapter is not intended to provide an exhaustive review of insecticide toxicology, rather the intent is to provide an overview

of insecticide chemistry, mode of action and metabolism within the framework of the cotton pest complex. Given the scope of this book in general and this chapter in particular, many of the lesser insecticide groups will not be considered and the reader is directed to more comprehensive texts for information on these subjects (Corbett *et al.*, 1984; Matsumura, 1985; Kerkut and Gilbert, 1985).

CLASSIFICATION AND MODE OF ACTION

The first critical problem in discussing the toxicology of such a diverse group of compounds is to provide a framework for the reader. Several classification approaches are possible including those based on chemistry, mode of action, origin and method of discovery. The review provided herein will be based on a combination of chemistry and mode of action. A classification based strictly on chemistry can be misleading or allow important connections to be lost. For example, in spite of what appears to be radically different chemistry, DDT and the pyrethroids have the same site of action and the same primary resistance mechanism (knock-down resistance). In fact, in many respects, DDT can be considered the first pyrethroid. Likewise, generally accepted chemical groupings such as the chlorinated hydrocarbon insecticides, which usually consist of DDT and its analogs, the cyclodienes and lindane, are usually treated as a group, and yet they are vastly different in terms of chemistry, mode of action and resistance.

Although not generally viewed as such, almost all modern insecticides can potentially be viewed as having one of two broad modes of action. The first is to mimic or enhance the action of an endogenous (inside the organism) molecule such as a neurotransmitter, while the second is to block or antagonize the action of an endogenous molecule (Table 1). For example, the organophosphorus insecticides can be thought of as functioning by inhibiting acetylcholinesterase which allows increased levels of acetylcholine to stimulate the postsynaptic acetylcholine receptors. Thus, in one sense, the organophosphates can be viewed as having the same effect as mimicking the action of acetylcholine. Similar examples can potentially be made for the carbamates, cyclodienes and pyrethroids (Table 1).

Obviously, this view point has its limitations. Like any classification system, there are difficulties with insecticides that have unclear modes of action, those that act as general metabolic poisons or that act on a variety of systems. This point of view also can become overly simplistic when there is a change in the function or large fluctuations in the titer (chemical balance) of the target compound during the course of the insect's development. While neurotransmitters such as acetylcholine and *gamma*-aminobutyric acid perform the same function throughout the life of the insect, hormones such as juvenile hormone, and perhaps some of the neurohormones, regulate a variety of functions depending on the particular life stage involved. However, keeping this limitation in mind, this approach will hopefully result in a better grasp of the ultimate site of action at the biochemical level.

A majority of the insecticides in use today, including the pyrethroids, the cyclodienes, the organophosphates, carbamates, avermectins, formamidines and nicotinoids, act via the

Table 1. Mode of action of insecticides and acaricides

Insecticide	Target site	Mode of action	Ultimate target			
			Mimic	Antagonist ¹	Endogenous compound	System
DDT & Pyrethroids	Na ⁺ Gate	Open Na ⁺ gate		*	ACh	Nervous
Nicotine	ACh Receptor	Block ACh receptor		*	ACh	Nervous
Nitromethylenes	ACh Receptor	Block ACh receptor	*		ACh	Nervous
Cyclodienes & Phenylpyrazoles	GABA Cl ⁻ channel	Block GABA, Stimulate ACh release	*		ACh	Nervous
Organophosphates	AChE	Inhibit AChE	*		ACh	Nervous
Carbamates	AChE	Inhibit AChE	*		ACh	Nervous
Formamidines	Octopamine receptor	Mimic Octopamine	*		Octopamine	Nervous
Avermectins	GABA Cl ⁻ channel	Increase GABA binding	*		GABA	Nervous
Rotenone	Electron transport	Block malate oxidation		*	ATP	Energy
Dinitrophenols & Pyrroles	Mitochondrial uncoupler binding site	Uncouple ATP production		*	ATP	Energy
Sulfur containing miticides	Mitochondrial ATPase	Inhibition of oxidative phosphorylation		*	ATP	Energy

Table 1. Continued

Benzoylphenyl ureas	Chitin synthesis	Block chitin synthesis	NA ²	NA	Chitin	Structure
Diacylhydrazide	Ecdysone Receptor	Mimic edcysone	*		Ecdysone	Endocrine
Juvenoids	JH receptors	Mimic JH	*		JH	Endocrine
Anti-JHs	JH biosynthesis in CA	Block JH production		*	JH	Endocrine
<i>Bacillus thuringiensis</i>	Gut epithelium	Disruption of gut-hemolcoel barrier	NA	NA	NA	Structure

Abbreviations used: ACh - acetylcholine; AChE - acetylcholinesterase; CA - corpora allata; GABA- *gamma*-aminobutyric acid; JH - juvenile hormone

¹Antagonize or block action of endogenous compound

²Not applicable

insect nervous system (Matsumura, 1985; Table 1). This is because the nervous system of insects, as well as that of mammals, is regulatory in function. Minute changes or disruptions are greatly and rapidly amplified, quickly leading to a breakdown in the system. The nervous system will most likely remain a primary target for new insecticides, as demonstrated by the avermectins. However, other regulatory systems in insects such as the endocrine system may also prove to be good target sites for insecticide action (Sparks, 1990), especially at the neuroendocrine (hormonal system affecting the function of the nervous system) level (O'Shea, 1985, 1986; Holman *et al.*, 1990; Masler *et al.*, 1993).

INSECTICIDE MODE OF ACTION

Although cotton insect control traditionally has accounted for a large proportion of the insecticides used in the United States, cotton insect pests such as the tobacco budworm have not typically been used in studies involving mode of action or structure-activity relationships. In most instances our knowledge concerning insecticide mode of action and structure-activity relationships comes from studies on insects such as the house fly. Likewise, except in selected areas, our knowledge of the basic biochemistry of cotton insect pests is relatively limited. The following overview of insecticide mode of action will be limited to the more important insecticide classes, and where possible, include information derived from studies using cotton insect pests.

DDT AND THE PYRETHROIDS

Although generally viewed as belonging to different insecticide classes, DDT and the pyrethroids share the same mode of action and resistance mechanisms. While DDT and the pyrethroids appear to be quite different chemically (Figure 2), the continual evolution of pyrethroid and DDT chemistry has led to compounds that are DDT-pyrethroid intermediates (Holan *et al.*, 1985). Thus chemically, DDT and the pyrethroids may merely represent opposite ends of a spectrum of compounds that all have the same site of action.

Although DDT, the natural pyrethrins and pyrethroids have been the subject of more than 40 years of research, their exact mode of action and target site requirements still present many unanswered questions. This is in spite of the vital role in agriculture that DDT used to play and that the pyrethroids have largely taken over.

DDT and the pyrethroids act within the central nervous system to disrupt axonal transmission of nerve impulses in insects and mammals (Lund, 1985; Matsumura, 1985; Soderlund and Bloomquist, 1989) and, as an ultimate consequence, disrupt the transmission of information through the axon ultimately disrupting the release of acetylcholine (Table 1). In a nerve axon the passing of a nerve impulse temporarily disrupts the sodium gradient normally present. This change in the sodium gradient results from the rapid opening of the sodium gates leading to a rapid depolarization of the nerve. Although DDT and the pyrethroids are known to affect a variety of systems (Miller and Adams, 1982; Osborne, 1985; Ruigt, 1985), it now appears that the central factor in their action is the disruption of nervous transmission in the central nervous

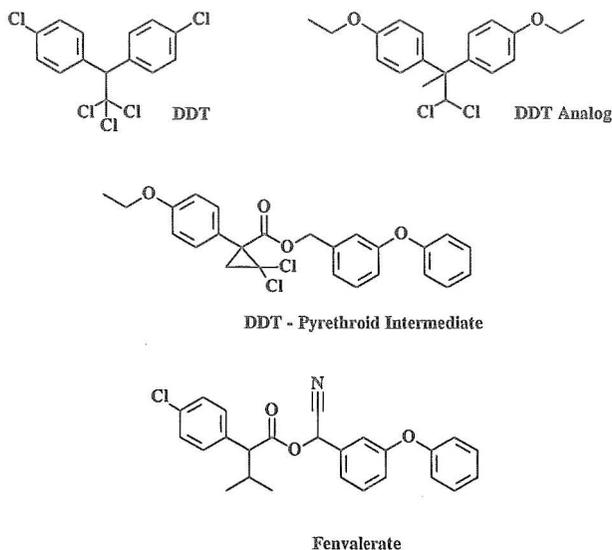


Figure 2. Structures of DDT, a DDT analog (Abu-El-Haj *et al.*, 1979), a DDT-pyrethroid intermediate (Holan *et al.*, 1985) and the pyrethroid fenvalerate.

system (Narahashi, 1987; Soderlund and Bloomquist, 1989). This disruption appears to be the direct consequence of DDT and the pyrethroids binding to voltage gated sodium channels, thereby preventing them from closing properly and leading to a continuous depolarization of the nerve (Matsumura, 1985; Ruigt, 1985; Soderlund and Bloomquist, 1989).

Although possessing the same target site, the actions and symptoms of DDT and the pyrethroids have often been divided into two groups: Type I and Type II. There are several distinctions between these two groups including the generation of repetitive discharges and characteristic whole body tremors by the DDT and the Type I pyrethroids versus a lack of these features by the Type II pyrethroids (Gammon *et al.*, 1981; Matsumura, 1985; Soderlund and Bloomquist, 1989). Type I pyrethroids typically would include the natural pyrethrins, DDT, and the non-*alpha*-cyano pyrethroids, phenothrin and permethrin (Pounce®, Ambush®), while the Type II pyrethroids usually include *alpha*-cyano pyrethroids such as cypermethrin (Ammo®, Cymbush®), fenvalerate (Pydrin®) and deltamethrin (Decis®).

Effects of Temperature — Although generally viewed as increasing in toxicity with decreasing temperature (negative temperature coefficient), recent studies suggest a much more complex relationship. Relative to the tobacco budworm, DDT and the Type I pyrethroids, permethrin and phenothrin, all possessed large negative temperature coefficients, while the Type II pyrethroids, fenvalerate, cypermethrin, deltamethrin

and tralomethrin (Scout®), all possess slightly negative or positive temperature coefficients (Sparks *et al.*, 1983). Based on the results of several studies (Sparks *et al.*, 1982, 1983; Schmidt and Robertson, 1986; Toth and Sparks, 1988, 1990) the response of pyrethroid toxicity to temperature is affected, in part, by the insect species being tested, the pyrethroid being evaluated, the method of application and the temperatures used in the evaluation. Thus, caution should be exercised in relating the effects of temperature on pyrethroid toxicity in the laboratory directly to a field situation.

DDT — DDT was a major component in the control of bollworm/tobacco budworm and the boll weevil during the 1950s and into the early 1960s. Compared to many carbamate and organophosphorus insecticides, DDT possesses good activity against cotton insect pests such as the tobacco budworm (Table 2). Studies of DDT and its structural requirements for activity suggest that the DDT molecule must fit onto a receptor site for which there exist strict size requirements (Coats, 1982; Fukuto and Keadtisuke, 1992). While DDT possesses good activity, there exist other structural variations that also display high biological activity (Coats, 1982; Fukuto and Keadtisuke, 1992). For larvae of the tobacco budworm, the toxicity of many of the pyrethroids is orders of magnitude higher than DDT.

Pyrethroids — As with DDT and its analogs, there also appears to be rather strict structural requirements for good biological activity in the pyrethroids (Elliott, 1985, 1990; Yoshioka, 1992). Due to the complex chemical nature of the pyrethroids, the structural requirements for activity are difficult to define. Most commercial pyrethroids are made up of an alcohol and an acid usually joined by an ester linkage (Figure 3). In the acid, a cyclopropane ring possessing gem dimethyl groups and an unsaturated sidechain (typically 2,2-dihalovinyl), are generally necessary for high activity (Buchel, 1983). Newer pyrethroids such as fenvalerate (Pydrin®) and fluvalinate (Mavrik®) maintain a configuration in the acid similar to the gem dimethyls on the cyclopropane ring by substituting an *alpha*-(1-methylethyl)benzeneacetic acid (Figure 3). In the alcohol a planar ring structure such as benzene or furan with an unsaturated sidechain or benzene ring seems to be necessary. Continued research on pyrethroid chemistry has resulted in the development of a number of non-ester linked pyrethroids (Udagawa *et al.*, 1985; Bushell, 1990; Sieburth *et al.*, 1990; Yoshioka, 1992) that may eventually find application to cotton insect control.

Much of the effort that has gone into detailing the requirements for pyrethroid activity have also dealt with improving environmental stability, since early pyrethroids such as allethrin, were broken down far too rapidly in an agricultural setting to be of use. Permethrin (Pounce®, Ambush®) was the first pyrethroid to truly be successful in an agricultural setting and was quickly followed by a host of other compounds (Elliott, 1977, 1985, 1990). Relative to permethrin, the first pyrethroid available for widespread use in cotton, other widely used pyrethroids are from 2 to nearly 30 times more toxic to the tobacco budworm in topical bioassays (Table 2). More importantly, most pyrethroids are much less toxic to mammals than are many of the organophosphorus insecticides, such as methyl parathion, that they replaced (Table 2).

Table 2: Toxicity of selected cotton insecticides and acaricides.

Compound	Toxicity (LD ₅₀ or LC ₅₀)				
	Tobacco budworm (mg/g)	Bollworm (mg/g)	Boll weevil (mg/weevil)	Twospotted spider mite (ppm)	Rat oral (mg/kg)
DDT & PYRETHROIDS					
DDT	31.5-100.0	30-82	—	—	87
Biphenethrin	1.32	0.71	—	46	55
Cypermethrin	0.241-1.61	1.96	0.2	185	247
Cyfluthrin	1.00	—	—	—	590
<i>lambda</i> -Cyhalothrin	0.929	1.11	—	—	56
Deltamethrin	0.044-0.107	0.071	0.033	287	128
Esfenvalerate	0.429	—	—	—	75
Fenpropathrin	0.51	—	0.36	241	49
Fenvalerate	0.396-1.89	2.64	0.71-0.477	142	451
Flucythrinate	0.254	—	—	266	67
Fluvalinate	1.89	—	—	121	>6,299
Permethrin	1.33-2.79	1.00	0.053	319	>4,000
Phenothrin	2.51	0.770	1.53	—	>10,000
Tralomethrin	0.061	—	—	—	1070
ORGANOPHOSPORUS INSECTICIDES					
Acephate	41.0-74.3	—	>5700.0	—	886
Azinphosmethyl	29.33	14.0	0.062	240	5
Chlorpyrifos	79.5	—	—	3352	135
EPN	37.0	5.67	0.20-0.04	—	14
Malathion	2230.0	150.0	0.66-1.24	3542	885
Methamidophos	85.7-150.0	150.0	128.6	—	13
Methyl Parathion	8.33-20.0	5.67-20.0	0.047-0.061	8112	9
Monocrotophos	29.67	6.00	0.42-1.34	—	21
Profenofos	11.0-11.8	0.07	0.53	234	400
Sulprofos	24.0-25.6	11.3	1.27	—	107
CARBAMATES					
Aldicarb	571.0	—	0.22	21	1
Carbaryl	183.3	193.3	27.7-48.9	—	307
Carbofuran	—	—	0.057	—	8
Methomyl	4.33-30.0	7.00	—	300	17
Thiodicarb	200.0	—	—	—	1,600
FORMAMIDINES					
Amitraz	—	—	—	139	600
Chlordimeform	>400.0	—	—	319	170

Table 2: Continued.

Compound	Toxicity (LD ₅₀ or LC ₅₀)				
	Tobacco budworm (mg/g)	Bollworm (mg/g)	Boll weevil (mg/weevil)	Twospotted spider mite (ppm)	Rat oral (mg/kg)
ACARICIDES					
Cyhexatin	—	—	—	94	180
Dicofol	—	—	—	5	575
Propargite	—	—	—	203	1,480
Tetradifon	—	—	—	127	>5,000
CYCLODIENES					
Endosulfan	73.3	156.7	—	—	18
Endrin	46.7	23.3	—	242	3
AVERMECTINS					
Abamectin	1.16	7.49	1.26	0.05-0.14 ¹	10.6-11.3
MK-244	0.12	—	—	—	—
SPINOSYNS					
Spinosyn A	1.28	—	—	—	3783->5000
PYRROLES					
AC 303,630	—	—	—	1.6 ²	662
CHLORONICOTINYLS					
Imidacloprid	350	—	—	—	450
PHENYLPYRAZOLES					
Fipronil	—	—	—	—	100
SYNERGISTS					
Piperonyl butoxide	>400.0	—	—	—	6,150
IGR'S					
Fenoxycarb	>400.0	—	—	—	16,800
FMev ¹	4000 ³	—	—	—	—
DPH ²	276 ³	—	—	—	—

Tobacco budworm—Topical toxicity to third instar larvae: data adapted from Graves *et al.*, 1964; Adkisson and Nemeč, 1967; Wolfenbarger and Guerra, 1972; Whitten and Bull, 1974; Harding *et al.*, 1977; Nosky *et al.*, 1980; Wolfenbarger and Harding, 1980; Polazzo, 1978; Sparks *et al.*, 1983; Rose and Sparks, 1984; Quistad *et al.*, 1985; Anderson *et al.*, 1986; Bull, 1986; Leonard *et al.*, 1988a,b; Lagadic and Bernard, 1993; Sparks *et al.*, 1995; R. Leonard and J. B. Graves Department of

Table 2: Continued.

Entomology, Louisiana State University, Baton Rouge; and D. Wolfenbarger, USDA, ARS, Weslaco, TX (unpublished data).

Bollworm—Topical toxicity to third instar larvae: data adapted from Graves *et al.* 1963, 1964; Adkisson and Nemeč, 1967; Wolfenbarger and Guerra, 1972; Davis *et al.*, 1977; Polazzo, 1978; Bull, 1986; and Leonard *et al.*, 1988a.

Boll weevil—Topical toxicity to adults: data adapted from Hopkins *et al.*, 1975; Davis *et al.*, 1977; Harding *et al.*, 1977; Sparks *et al.*, 1983; Rose and Sparks, 1984; Wolfenbarger *et al.*, 1985.

Twospotted spider mite— Slide dip bioassay: data adapted from Chang and Knowles, 1977; Dennehy and Granett, 1984; Dennehy *et al.*, 1987; Knowles and El-Sayed, 1985; Hoy and Conley, 1987.

Rat oral data for technical material adapted from Buchel, 1983; Larson *et al.*, 1985; Thompson, 1985; Anonymous, 1988; Addor *et al.*, 1992; Lankas and Gordon, 1989.

¹Fluoromevalonolactone

²3,3-dichloro-2-propenyl hexanoate

³ED50

⁴Leaf-dip bioassay.

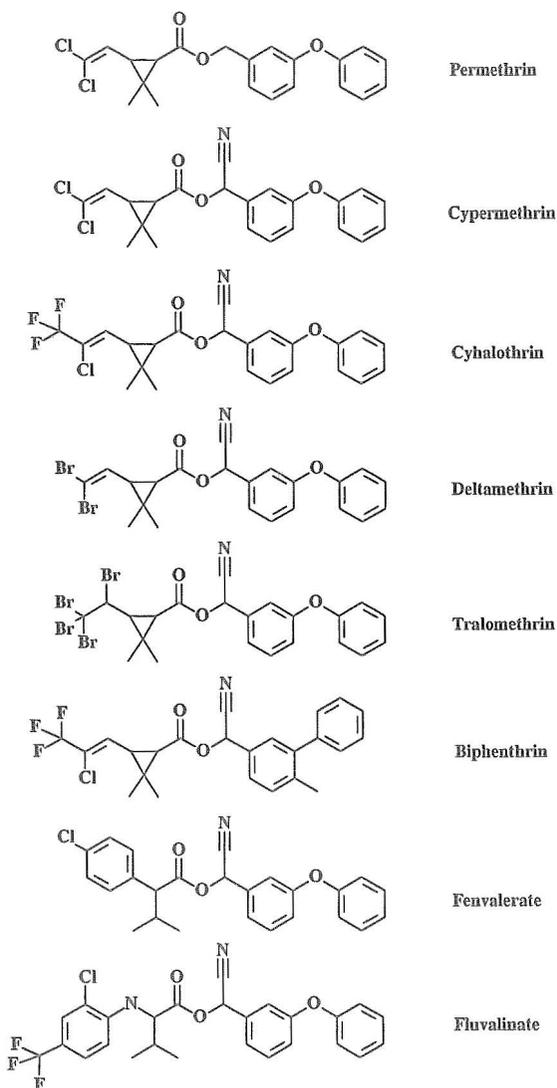


Figure 3. Structures of pyrethroids used for cotton insect control.

ORGANOPHOSPHORUS COMPOUNDS

A critical component of the insect central nervous system is the junction separating two nerve cells, the synapse. At the cholinergic synapse the action potential is translated

to packets of the neurotransmitter acetylcholine (Figure 4) that binds to receptors in the post-synapse causing a depolarization of that nerve cell and a continuance of the nerve impulse. The over stimulation of post-synaptic receptors by acetylcholine is prevented by the presence of an enzyme, acetylcholinesterase, that rapidly breaks down the acetylcholine (Figure 5) before an excess can accumulate at the post-synaptic receptors.

The heart of the active site of acetylcholinesterase, like other serine proteases and carboxylesterases, is a serine hydroxyl group in what is known as the esteratic site (Eto, 1974; Matsumura, 1985). The quaternary nitrogen of the choline group is bound

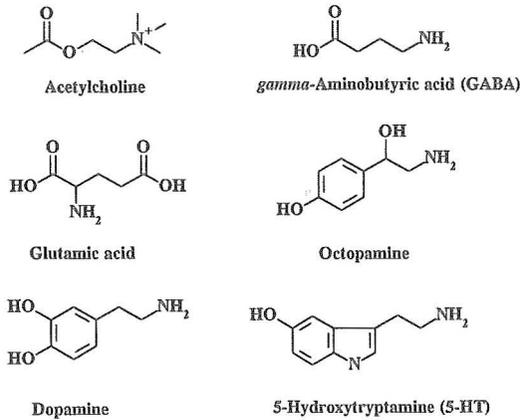


Figure 4. Structures of insect neurotransmitters.

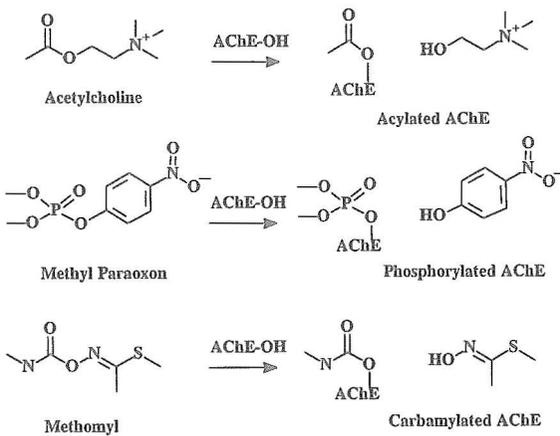


Figure 5. Interaction of acetylcholine, an organophosphorus insecticide (methyl paraoxon) and a carbamate (methomyl) with acetylcholinesterase (AChE).

by an acidic group (probably from aspartate) in what is called the anionic binding site. This binding of the choline to the anionic binding site is probably responsible for the initial complex formation between the acetylcholinesterase and acetylcholine. It is the serine hydroxyl group that attacks the carbonyl carbon of acetylcholine leading to acylation of the acetylcholinesterase and release of choline. The acyl group is then rapidly displaced from the serine hydroxyl group leading to a release of acetic acid and a regeneration of acetylcholinesterase. Inhibition of acetylcholinesterase can, obviously, lead to a build up of acetylcholine at the post-synapse resulting in a total disruption of nerve function, and ultimately cause death.

The organophosphorus insecticides (Figure 6) are a large and diverse group of phosphoric acid esters which can be divided into two broad subclasses: the phosphates which are directly active against acetylcholinesterase and the phosphorothionates that require

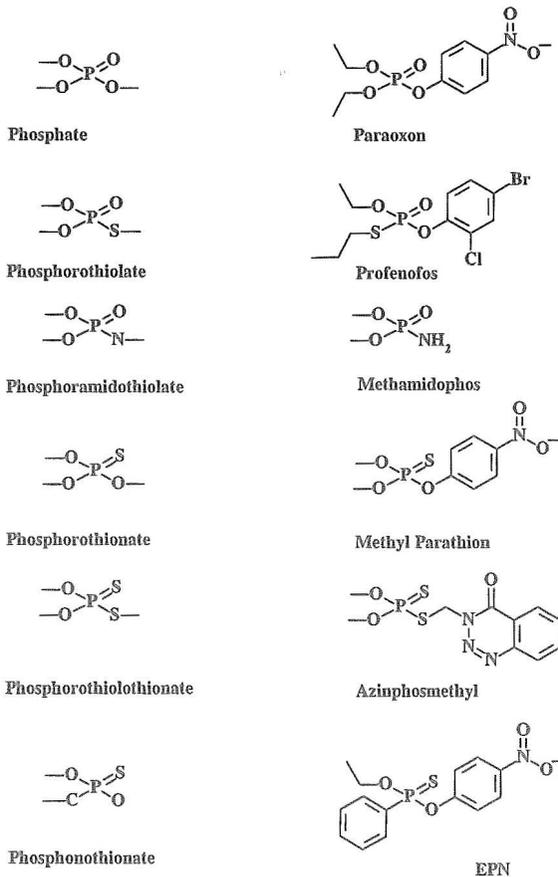


Figure 6. Examples of the different classes of organophosphorus insecticides.

activation in order to inhibit acetylcholinesterase (see section on metabolism). Included in the phosphate subclass are: the phosphates, dicotophos (Bidrin®), monocotophos (Azodrin®), naled (Dibrom®), paraoxon; the phosphorothiolates, profenofos (Curacron®); and the phosphoramidothiolates, acephate (Orthene®), methamidophos (Monitor®) (Figure 6, 14). In the phosphorothionate subclass are: the phosphorothionates, chlorpyrifos (Lorsban®, Dursban®), methyl parathion and parathion; the phosphorothiothionates, azinphosmethyl (Guthion®), dimethoate (Cygon®), malathion, sulprofos (Bolstar®); and the phosphonothionates such as EPN (Figure 6, 14).

Organophosphorus insecticides act by binding with acetylcholinesterase (Matsumura, 1985). Unlike acetylcholine, when organophosphorus insecticides react with the serine hydroxyl group of the acetylcholinesterase active site, the reaction proceeds to the point where the serine hydroxyl is "phosphorylated" (Figure 5) but no further since the final steps in regeneration of the acetylcholinesterase (i.e. the reaction with water) occur only very slowly (Eto, 1974). Thus, phosphorylation of the acetylcholinesterase by organophosphorus insecticides effectively inhibits acetylcholinesterase resulting in an over stimulation of the post-synaptic nerve axon by the excess acetylcholine present.

For the organophosphorus insecticides, the process of phosphorylation is the critical step in determining the activity of a given compound (O'Brien, 1976). A primary factor influencing the efficacy of organophosphorus insecticides is the reactivity of the phosphorus atom to attack by the serine hydroxyl group. In the case of organophosphorus compounds such as paraoxon, and methyl paraoxon, this reactivity is influenced, in part, by the ability of substituents on the phenyl ring (the group that "leaves" when methyl paraoxon reacts with acetylcholinesterase; Figure 5) to make the phosphorus atom more reactive to the serine hydroxyl. Likewise, the size and composition of the alkyl groups also can influence that ability of the organophosphorus insecticide to fit into the esteratic active site. For example, in a series of *O*-alkyl *S*-(4-chlorophenyl) ethylphosphonothiolothionates and *O,O*-dialkyl *O*-(4-nitrophenyl) phosphorothionates, the topical toxicity to tobacco budworm larvae declined as the size of the alkyl group increased from methyl to ethyl to propyl (Wolfenbarger, 1972). There are several excellent reviews of these structure activity relationships (Eto, 1974; Fukuto, 1976, 1979; Magee, P. S., 1982; Fukuto and Keadtisuke, 1992).

CARBAMATES

Carbamates are esters consisting of an alcohol moiety such as naphthol, a substituted phenol, heterocyclic enol or an oxime, and a carbamic acid moiety, most commonly the *N*-methylcarbamic acid. Carbamates used on cotton include: the oxime carbamates, aldicarb (Temik®), methomyl (Lannate®, Nudrin®), thiodicarb (Larvin®), the phenyl carbamates, carbofuran (Furadan®); and the naphthyl carbamates, carbaryl (Sevin®) (Figures 7, 15). Like the organophosphorus insecticides, carbamates act to inhibit the acetylcholinesterase of both insects and mammals. The mechanism of acetylcholinesterase inhibition is very similar to that of the organophosphorus insecticides (Figure 5); however, there are significant differences between the

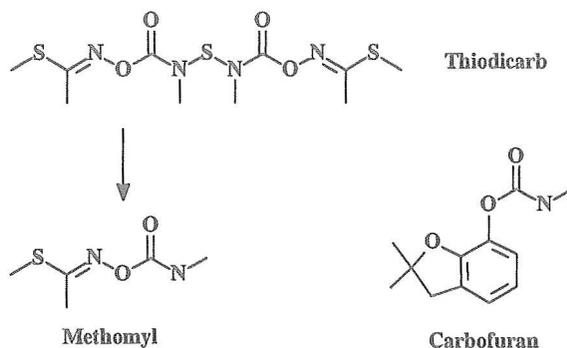


Figure 7. Structures of the carbamates carbofuran, methomyl and thiodicarb.

organophosphorus insecticides and the carbamates, especially relative to regeneration of acetylcholinesterase following inhibition and the structural requirements for activity.

For the organophosphorus insecticides, regeneration of the inhibited acetylcholinesterase is exceedingly slow (several hours to days; Eto, 1974). On the other hand, regeneration of the carbamates is far more rapid (about 15 minutes). This relatively rapid rate of regeneration is virtually universal for all commercial carbamates since most are *N*-methyl carbamates resulting in the identical carbamylated enzyme. The structural requirements for good carbamate activity are also quite different from those necessary for the organophosphorus insecticides. The activity of the oxime carbamates such as aldicarb (Temik®) and methomyl (Lannate®, Nudrin®) appear to be related to their ability to mimic acetylcholine (Magee, T. A., 1982), while activity in the phenyl carbamates such as carbofuran (Furadan®) seems to be closely tied to the electron donating capacity of the substituents and steric requirements that affect ability to bind to one of several proposed binding sites (O'Brien, 1976). The necessity of these structural requirements is supported by kinetic studies of carbamates with acetylcholinesterase, which indicate that the formation of the carbamate-acetylcholinesterase complex is the critical step in the reaction. Very complete evaluations of carbamates structure-activity relationships are given by Fukuto (1976) and Goldblum *et al.* (1981).

Given the high mammalian toxicity of many carbamates such as aldicarb (Temik®), methomyl (Lannate®, Nudrin®), and carbofuran (Furadan®), a great deal of effort has gone into devising analogs, e.g., thiodicarb (Larvin®) (Figure 7) that upon metabolism by insects are converted back to the parent carbamate (e.g., methomyl). When metabolized by mammals, these compounds are converted to non-toxic products (Fukuto, 1984; Drabek and Neumann, 1985).

NITROMETHYLENES AND CHLORONICOTINYLS

As discussed above, the organophosphorus and carbamate insecticides disrupt nervous transmission by preventing the breakdown of the neurotransmitter acetylcholine.

Other insecticides function by binding directly to the acetylcholine receptors to cause an over stimulation of the nervous system. Two classes of these receptors exist in insects and mammals; muscarinic and nicotinic (Matsumura, 1985). In insects the nicotinic receptors appear to predominate while in mammalian systems the predominate acetylcholine receptors appear to be muscarinic (Breer, 1985; Eldefrawi and Eldefrawi, 1990; Eto, 1992) suggesting that it may be a good site for the development of new insecticides (Eto, 1992). The insecticidal activity of nicotine (Figure 8) is well known (Eldefrawi, 1985; Matsumura, 1985) and its mode of action appears to involve binding to the nicotinic acetylcholine receptors, acting as an agonist at low concentrations and as an antagonist at higher concentrations (Eldefrawi, 1985). Although a variety of nicotinoids (synthetic nicotine analogs) have been isolated or synthesized (Eldefrawi and Eldefrawi, 1990) none have led to commercial products. The heterocyclic nitromethylenes (Figure 8) have been identified as acetylcholine agonists at the nicotinic receptor site (Eldefrawi and Eldefrawi, 1990) and some of these compounds have insecticidal activity (Soloway *et al.*, 1978). A hybrid between the nitromethylenes and nicotine is the nitroguanidine or chloronicotinyl insecticide, imidacloprid (Mullins, 1992; Moffat, 1993; Leicht, 1993; Figure 8). Imidacloprid is being developed for the control of sucking insects including aphids, thrips and whiteflies on cotton (Elbert *et al.* 1992; Mullins, 1992). Like nicotine, imidacloprid appears to act on the nicotinic receptor and appears to function as an acetylcholine agonist (Mullins, 1992). Insects resistant to organophosphates and carbamates were not resistant to imidacloprid (Mullins, 1992), an observation consistent with the differences in the respective modes of action.

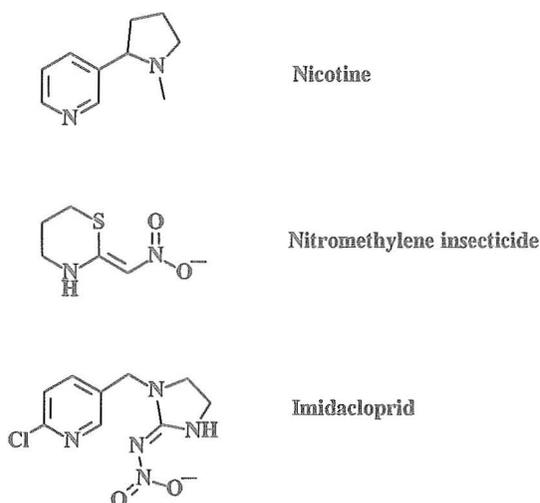


Figure 8. Structures of nicotine, a nitromethylene insecticide and imidacloprid.

AVERMECTINS

An additional target for insecticides in the insect nervous system exists in the form of the peripheral nervous system. Unlike the central nervous system, which is generally agreed upon as using acetylcholine as the synaptic stimulatory neurotransmitter, synaptic transmission in the peripheral nervous system of insects (at least at the neuromuscular junctions) is mediated by a stimulatory neurotransmitter, glutamic acid (Figure 4) and an inhibitory neurotransmitter, *gamma*-aminobutyric acid (Smyth, 1985; Shankland and Frazier, 1985) (Figure 4).

Abamectin (Affirm®, Zephyr®) (mixture of avermectin B_{1a} (Figure 9) and avermectin B_{1b}) is a microbiologically derived insecticide that acts on the insect nervous system (Fisher, 1990; 1993; Lasota and Dybas 1991). Although a number of target sites have been proposed, much of the evidence suggests that the avermectins interact with chloride channels (Turner and Schaeffer, 1989), and in particular *gamma*-aminobutyric acid gated chloride channels. The avermectins appear to open chloride channels acting as *gamma*-aminobutyric acid agonists at binding sites in the chloride channel, enhancing the action of *gamma*-aminobutyric acid at the receptor site or stimulating the presynaptic release of *gamma*-aminobutyric acid (Fisher, 1985; Miller and Chambers, 1987; Turner and Schaeffer, 1989). Although the structural requirements

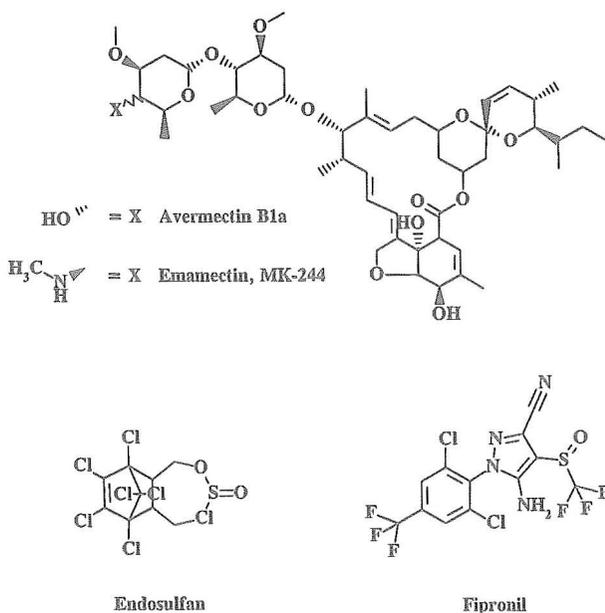


Figure 9. Structures of the avermectins, abamectin and emamectin (MK-244); the cyclodiene, endosulfan; and the phenylpyrazole, fipronil.

for insecticidal activity in the avermectins currently appears to be somewhat restrictive (Fisher, 1985; Fisher and Mrozik, 1989), undoubtedly improvements in avermectin chemistry will occur. These advances will lead to more potent analogs with better field residual and efficacy, as has been demonstrated by the development of the semi-synthetic avermectin analogs MK-243 and MK-244. Abamectin (Affirm®, Zephyr®) is a potent miticide, but is weak on insects such as the lepidoptera (Fisher, 1993). Avermectin derivatives that are more effective against lepidopterans have been a research focus for some time (Fisher, 1990, 1993), and some of the 4''-amino derivatives such as MK-243 (Dybas and Babu, 1988) and the 4''-epi-methylamino-4''-deoxyavermectin (emamectin, MK-244; Figure 9) appear to be much more effective against target lepidopterans than other derivatives of avermectin (Lasota and Dybas, 1991; Fisher, 1993). Topical bioassays of abamectin (Affirm®, Zephyr®) on the tobacco budworm show it to be as active as permethrin (Ambush®, Pounce®) (Table 2). Although there are currently no data available for cotton insect pest resistance to abamectin, information from studies using other insects is available. In some of these studies there was little cross-resistance to abamectin in insects resistant to cyclodiene, organophosphorus and pyrethroid insecticides (Roush and Wright, 1986; Cochran, 1990), while other studies found varying degrees of cross-resistance to abamectin in insects resistant to pyrethroids (Scott, 1989) or multiple insecticides (Abro *et al.*, 1988). Insect resistance to abamectin can result from an altered target site (Konno and Scott, 1991), reduced penetration (Konno and Scott, 1991) or enhanced metabolism (Argentine *et al.*, 1992). Available information suggests that the cross-resistance to abamectin is a function of enhanced metabolism, most likely due to monooxygenases (Abro *et al.*, 1988; Scott, 1989). Thus, while the avermectins are currently only used (in cotton) for mite control, they represent a class of chemistry that may become more important to cotton insect control as problems with resistance to the pyrethroids and other insecticides continue to increase (Campanhola and Plapp, 1987; Leonard *et al.*, 1987; Sparks *et al.*, 1993a).

CYCLODIENES

The cyclodienes are chlorinated insecticides resulting from a Diels-Alder reaction. Like DDT they were discovered during the late 1940s and early 1950s and have long since reached their zenith, falling increasingly into disuse. With the possible exception of endosulfan (Thiodan®) (Figure 9), most of the cyclodienes are highly persistent compounds. This persistence has contributed to the banning by EPA of most of the cyclodienes, and those that remain in the market are relatively little used.

The cyclodienes have for some time been viewed as acting to stimulate the release of acetylcholine from the presynapse (Corbett *et al.*, 1984; Matsumura, 1985). Recent evidence, however, suggests that the cyclodienes may also be acting as *gamma*-aminobutyric acid (Figure 4) antagonists (Matsumura, 1985; Bloomquist *et al.*, 1987; Matsumura *et al.*, 1987), presumably at the picrotoxinin binding site of the chloride ionophore. Since *gamma*-aminobutyric acid may also function as an inhibitory neurotransmitter for chloride channels in the central nervous system of some insects

(Lummis *et al.*, 1987), as well as the neuromuscular junctions (Smyth, 1985), the *gamma*-aminobutyric acid antagonistic activity of the cyclodienes seems consistent with their apparent acetylcholine stimulatory activity.

PHENYLPYRAZOLES

The phenylpyrazoles or fiproles are a new class of promising insecticides that act on the insect nervous system. Currently one member of this chemical family, fipronil (Figure 9), is under development as an insecticide with a wide spectrum of proposed uses including the control of the boll weevil and thrips in cotton (Colliot *et al.* 1992). Some phenylpyrazoles such as fipronil appear to act by blocking the *gamma*-aminobutyric acid gated chloride channel (Colliot *et al.* 1992; Cole *et al.* 1993; Moffat, 1993) in a manner similar to the cyclodienes. Studies indicate that these phenylpyrazoles are not cross-resistant with pyrethroid insecticides (Colliot *et al.* 1992). However, studies also show that cyclodiene (e.g. dieldrin) resistant insects are cross-resistant to at least some of the phenylpyrazoles (Colliot *et al.* 1992; Cole *et al.* 1993), which is consistent with their mode of action.

FORMAMIDINES

Formamidine insecticides, such as chlordimeform (Fundal®, Galecron®) and amitraz (Ovasyn®) (Figure 10), act by affecting the insect nervous system, but not in the

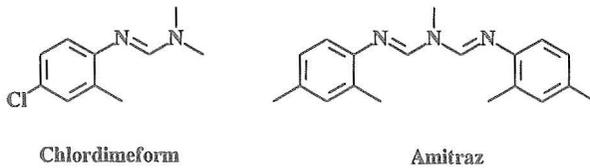


Figure 10. Structures of formamidines chlordimeform and amitraz

manner of the organophosphorus or carbamate insecticides. Available information suggests that the formamidines act as agonists of octopamine (Figure 4) (Hollingworth and Lund, 1982), a biogenic amine that functions as neuromodulator, neurohormone and neurotransmitter. Octopamine is, among other things, involved in the mobilization of carbohydrates and lipids, control of visceral muscles and insect behavior (Evans, 1985; Orchard and Lange, 1987). Extensive structure activity studies (Chang and Knowles, 1977; Knowles, 1982, 1987) support the octopamine agonist concept for insects in that formamidines that are most effective tend to resemble octopamine chemically (Hollingworth and Lund, 1982; Knowles, 1982).

As insecticides and acaricides, the formamidines are somewhat restricted in their spectrum of activity being limited to mites, ticks, lepidopterans and hemipterans (Hollingworth and Lund, 1982). Due, in part, to the rather exacting requirements for activity, commercial development of this class of insecticides has been rather limited.

Only two compounds have found wide commercial use, chlordimeform (Galecron®, Fundal®) and amitraz (Ovasyn®) (Figure 10). Chlordimeform was widely used as an ovicide for the tobacco budworm, but it has been withdrawn from the market. Chlordimeform and amitraz both appear to enhance insecticidal activity when co-applied with pyrethroids or other insecticides in the laboratory and in the field (Plapp, 1987; Campanhola and Plapp, 1987, 1988). This enhanced toxicity may be due, in part, to formamidine induced alterations in insect behavior resulting in increased contact with the pyrethroid or other insecticide (Treacy *et al.*, 1987; Sparks *et al.*, 1988, 1989, 1991), and/or alterations at the target site (Liu and Plapp, 1992).

SPINOSYNS

Spinosad (proposed common name) is a naturally occurring mixture of spinosyn A (A83543A) and D (A83543D) (Figure 11). The spinosyns are a new class of fermentation-derived tetracyclic macrolides (Kirst *et al.*, 1992) that act via the insect nervous system and are especially active against a variety of lepidopterous pests (Sparks *et al.*, 1995). Available information suggests that the mode of action is unique, and is not cross-resistant with the target sites for any other known insect control agents (Anonymous, 1994). Spinosyn A is very effective against the tobacco budworm with activity in topical bioassays in the range of pyrethroids such as permethrin (Table 2). Tests of spinosyn A and spinosad have shown them to be effective on a variety of insecticide resistant field and laboratory (including pyrethroid resistant) strains, with no evidence to date of cross-resistance, and to possess very favorable mammalian toxicity (Table 2) and environmental profiles (Sparks *et al.*, 1995). Given the expanding problems of insecticide resistance in cotton, spinosyns such as spinosad should find a great deal of utility in cotton IPM and resistance management programs.

PYRROLES

A majority of the insecticides in use for cotton insect control act via the nervous system. However, the disruption of metabolic processes can also provide the necessary efficacy to serve as a target for insect control agents. One such metabolic process is mitochondrial respiration. Part of this process involves mitochondrial electron transport whereby NADH is re-oxidized by transferring its electrons through a chain of carriers to oxygen. During the electron transfer process down the electron transport chain, energy is trapped and stored in the high energy bonds of ATP through the process of oxidative phosphorylation. If oxidative phosphorylation becomes disconnected, or uncoupled, from the electron transport process, the production of ATP will be disrupted ultimately leading to death. While the inhibition of the mitochondrial electron transport process (MET) is the basis for the insecticidal activity of rotenone (Fukami, 1985), and apparently several new acaricides (Motoba *et al.* 1992; Hollingworth *et al.*, 1994), the uncoupling of oxidative phosphorylation from MET is the basis for the action of insecticides and acaricides such as the dinitrophenols as well as others (see below).

The insecticidal pyrroles are an outgrowth of the discovery that a natural pyrrolomycin, dioxapyrrolomycin (Figure 11), isolated from a strain of *Streptomyces*

possessed insecticidal activity (Addor *et al.*, 1992; Kuhn *et al.*, 1993). Extensive structure activity studies around the pyrroles led to the discovery of AC 303,630 (Pirate®; Figure 11) (Addor *et al.*, 1992). AC 303,630 is a pro-insecticide that requires biological activation before it can act (Addor *et al.*, 1992; Kuhn *et al.*, 1993). Upon the metabolic removal of the *N*-ethoxymethyl group, the resulting pyrrole (Figure 11) functions as an uncoupler of oxidative phosphorylation (Addor *et al.*, 1992; Kuhn *et al.*, 1993; Moffat 1993). The pro-insecticidal nature of AC 303,630 also imparts a favorable mammalian toxicity profile (Kuhn *et al.*, 1993).

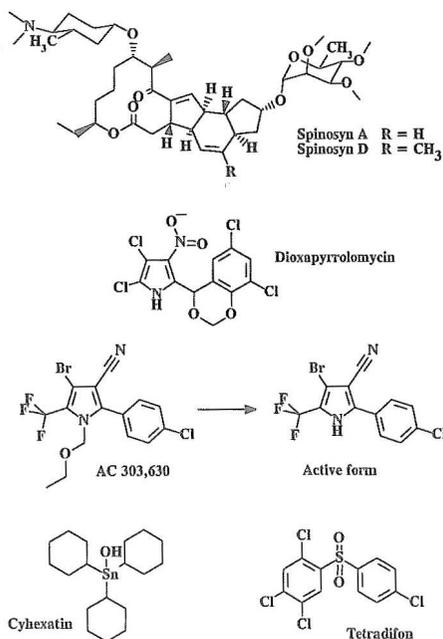


Figure 11. Structures of spinosyns A and D; the natural pyrrolomycin, dioxapyrrolomycin; the pyrrole, AC 303,630 and its bioactivation product; and two miticides, cyhexatin and tetradifon.

ORGANOTIN COMPOUNDS AND SULFUR CONTAINING ACARICIDES

The organotin compounds are exemplified by the miticide cyhexatin (Plictran®) (Figure 11), a tricyclohexylstannate derivative. Sulfur containing compounds such as tetradifon (Gardona®) (Figure 11) in which, typically, two benzene rings are attached to a sulfone, sulfonate or sulfide (Matsumura, 1985) comprise another group of miticides. Both the organotins and the sulfur-containing compounds appear to function as inhibitors of oxidative phosphorylation in mites (Desaiah *et al.*, 1973; Corbett *et al.*, 1984).

INSECT GROWTH REGULATORS

As a group of insecticides, the insect growth regulators (IGRs) encompass a diverse group of chemistries that act in some manner to disrupt insect growth and development (Hammock and Quistad, 1981; Retnakaran *et al.*, 1985; Sparks, 1990). Included in the IGRs are the juvenoids, diacylhydrazides and benzoylphenyl ureas.

Juvenoids — Juvenile hormone is a sesquiterpene epoxide (Figure 12) that is virtually unique to insects (Sparks 1990). Juvenile hormone works in concert with several other insect hormones and neurohormones, including the steroid hormone 20-hydroxyecdysone (Figure 12) and the neuropeptide, prothoracicotropic hormone, to regulate the molting process and, ultimately, insect metamorphosis. High levels of juvenile hormone maintain the larval or immature state while reduced levels of juvenile hormone initiate metamorphosis (Sparks, 1990). Juvenoids are compounds that mimic the action of juvenile hormone thereby disrupting the process of metamorphosis leading to a variety of deleterious effects (Staal, 1975; Hammock and Quistad, 1981; Sparks *et al.*, 1990). A great deal of effort has gone into the synthesis and testing of thousands of juvenoids (Slama *et al.*, 1974; Hemrick 1982; Retnakaran *et al.*, 1985; Miyamoto *et al.*, 1993), some of which [epofenonane and fenoxycarb (Logic®)] (Figure 12) have been evaluated on the bollworm/tobacco budworm (Guerra *et al.*, 1973; Table 2) and the boll weevil (Moore, 1980). Although there currently are no juvenoids in wide use for cotton insect control, new compounds (eg. pyriproxyfen; Figure 12; Miyamoto *et al.*, 1993) and uses (eg. ovicide; Masner *et al.*, 1987) continue to be discovered. Thus, the juvenoids may yet find a role in cotton IPM.

Diacylhydrazides — The diacylhydrazides, a relatively recent and unique class of IGR (Hsu, 1991), are typified by RH 5992 (Figure 12). Although they do not yet have application to cotton insect control, some of these insecticides are effective against a variety of lepidopteran pests (Hsu, 1991; Heller *et al.*, 1992). In insects the molt that occurs at the end of each instar in larval or immature insects is initiated by 20-hydroxyecdysone (Figure 12). The available data suggest that the diacylhydrazides disrupt the molting process by functioning as ecdysone agonists (Wing, 1988; Wing *et al.*, 1988; Wing and Aller, 1990). For insects other than the Lepidoptera, a second non-endocrine mode of action may also be involved in the insecticidal activity observed for these non-steroidal ecdysone agonists. Recent data suggest that the diacylhydrazides can also disrupt the insect nervous system by blocking potassium channels (Salgado, 1992).

Benzoylphenyl Ureas — Unlike the juvenoids and diacylhydrazides, the benzoylphenyl ureas have found a limited use for the control of cotton insect pests such as the boll weevil. The benzoylphenyl ureas are a rather novel class of insecticidal compounds that have their origins in a fortuitous accidental discovery by the scientists at Philips-Duphar in the early 1970s (Verloop and Ferrell, 1977). This discovery very rapidly led to the development of diflubenzuron (Dimilin®) (Figure 12). These insecticidal compounds act only on immature stages and only then during the molting

to a failure in the molting process (Hajjar, 1985; Retnakaran *et al.*, 1985). Since chitin is lacking in plants and mammals, the benzoylphenyl ureas have an inherent selectivity over more conventional broad spectrum insecticides. While it is clear that the benzoylphenyl ureas act by inhibiting chitin synthesis, their exact mode of action is unclear. A number of mechanisms have been proposed for the benzoylphenyl ureas (Hajjar, 1985), including several centering on chitin synthetase, but none yet provide a completely satisfactory answer (Hajjar, 1985; Matsumura, 1985; Cohen, 1987, 1993; Grosscurt and Jongsma, 1987).

In spite of our ignorance concerning the exact mode of action for the benzoylphenyl ureas, a great deal of effort has gone into their development for control of cotton insect pests and other insect pests. A variety of insecticidally-active compounds have been developed (Retnakaran *et al.*, 1985). However, due to limited contact activity, benzoylphenyl ureas other than diflubenzuron (Dimilin®) have yet to find wide use in cotton insect pest control.

BACILLUS THURINGIENSIS Berliner

Bacillus thuringiensis Berliner var. *kurstaki* is a bacterium subspecies that produces a toxin that is toxic to lepidopterous larvae. Other subspecies are active against the Diptera (*Bacillus thuringiensis* var. *israelensis*) and Coleoptera. The toxic principle of *Bacillus thuringiensis* var. *kurstaki* is a crystalline *delta*-endotoxin (Bt toxin) which is activated by the high alkaline pH and proteolytic activity of the gut of lepidopterous larvae. The Bt toxin binds to specific receptors on the brush border membrane of midgut columnar cells (Gill *et al.*, 1992). Multiple receptors may be present, each binding a different group of Bt toxins (Yamamoto and Powell, 1993). The cells of the gut epithelium swell and then separate, disrupting the gut-hemocoel barrier (Luthy *et al.*, 1982; Roe *et al.*, 1985), leading to the death of the insect.

Although *Bacillus thuringiensis* has been available for the control of lepidopterous pests for some time, to varying degrees problems with production, environmental stability and efficacy relative to conventional insecticides have tended to limit their use (Gelernter, 1990; Gill *et al.*, 1992). However, advances in biotechnology have led to the insertion of genes for *Bacillus thuringiensis* toxins into a variety of plants, including cotton (Benedict *et al.*, 1992; Fischhoff, 1992; Periak and Fischhoff, 1993), and consequently this removes some of the problems associated with the use of *Bacillus thuringiensis* and its toxins. This transgenic Bt-cotton has the potential to provide good control of tobacco budworm and cotton bollworm larvae (Benedict *et al.*, 1992; Fischhoff, 1992), but should be used as part of a resistance management program to prevent the rapid selection of resistance to the *Bacillus thuringiensis* toxins (Gould, 1991; Sparks *et al.*, 1993a; Whalon and McGaughey, 1993).

XENOBIOTIC METABOLISM

The following brief discussion of xenobiotic metabolism is meant to illustrate the presence and diversity of the metabolic capabilities present in cotton pest insects.

MONOOXYGENASES

Monooxygenases, also known as microsomal oxidases and mixed function oxidases, are involved in a variety of endogenous reactions including steroid and hormone synthesis, and fatty acid metabolism, all critical to the normal growth and development of insects (Wilkinson, 1985). The monooxygenases also are widely recognized as playing a major role in the metabolism of xenobiotics such as secondary plant compounds allowing the insect herbivores to survive on plants containing potentially toxic allelochemicals (Wilkinson, 1985). The monooxygenases are a family of membrane bound enzymes with broad and often overlapping substrate specificities (Wilkinson, 1983). Since the monooxygenases are particularly adept at dealing with lipophilic molecules and converting them to more polar compounds that can be more easily excreted, it is not surprising to find them playing a critical role in the general activation and catabolism of insecticides and in insect resistance to insecticides. The heart of the monooxygenase system is cytochrome P450 (Nakatsugawa and Morelli, 1976) which plays a critical role in substrate binding and insertion of an activated oxygen molecule into the substrate. The monooxygenases are involved in a number of reactions, all involving the insertion or addition of an oxygen into the substrate including aromatic and aliphatic hydroxylations, *O*, *S*, and *N*-dealkylation, *N*- and thioether oxidation, epoxidation, ester oxidation and desulfuration (Nakatsugawa and Morelli, 1976).

HYDROLASES

A variety of insecticides have ester linkages that are susceptible to hydrolysis by hydrolases that are typically in the extramicrosomal (soluble) fraction. Since both the pyrethroid and organophosphorus insecticides contain a variety of carboxyl, amide and phosphorus ester linkages, the hydrolases can be especially important in the metabolism of these two groups of insecticides. The hydrolases include the phosphotriesterases, carboxylesterases and carboxylamidases, which act on phosphorus triesters, carboxylesters and carboxylamide esters, respectively (Dauterman, 1976, 1985). A fourth group of hydrolases, the epoxide hydrolases, act on epoxide containing insecticides such as dieldrin, epofenonane, or in conjunction with the monooxygenases that epoxidize double bonds, converting the resulting epoxide to diols. Until recently the epoxide hydrolases were thought to be strictly membrane bound enzymes (Dauterman, 1985), however, epoxide hydrolases are now known to occur in the cytosolic fraction as well (Ota and Hammock, 1980).

GLUTATHIONE TRANSFERASES

The glutathione transferases are soluble enzymes that are important in the metabolism of organophosphorus insecticides (Dauterman, 1976, 1985). They require reduced glutathione as a co-factor. *O,O*-dimethyl organophosphorus insecticides are especially susceptible to attack by glutathione transferases leading to the *O*-dealkylation of the organophosphorus insecticide and the formation of an *S*-alkyl glutathione conjugate.

INSECTICIDE METABOLISM BY COTTON INSECTS

There have been numerous studies of insecticide metabolism by cotton insect pests (Table 3). However, for many of the currently used cotton insecticides detailed *in vivo* metabolism studies are lacking. The metabolism of many insecticides used for the control of bollworm/tobacco budworm has been reviewed (Bull *et al.*, 1987). In addition, there have been several extensive reviews of the metabolism of insecticides (Brooks, 1974; Eto, 1974; Kuhr and Dorough, 1976; Hammock and Quistad, 1981; Cool and Jankowski, 1985; Matsumura, 1985).

DDT AND PYRETHROID METABOLISM

As observed for many insects (Matsumura, 1985), DDT is metabolized to DDA by the tobacco budworm (Vinson and Brazzel, 1966) and to DDE via a glutathione-dependent DDT-dehydrochlorinase (Yang, 1976) in the tobacco budworm and bollworm (Gast, 1961; Vinson and Brazzel, 1966; Plapp, 1973).

Although the pyrethroid insecticides have been heavily used for insect control in cotton, information on the metabolism of these insecticides in bollworm/tobacco budworm or the boll weevil has been somewhat limited until recently. Permethrin (Ambush®, Pounce®) metabolism has been studied in the tobacco budworm and bollworm (Table 3) and, as has been observed in other studies (Soderlund *et al.*, 1983; Ruigt, 1985), permethrin is readily metabolized by ester hydrolysis (Figure 13, site 1) and aromatic and aliphatic hydroxylation (Figure 13, sites 2 and 3, respectively) (Bigley and Plapp, 1978; Nicholson and Miller, 1985). Permethrin was metabolized more rapidly by tobacco budworm larvae than bollworm larvae (Bigley and Plapp,

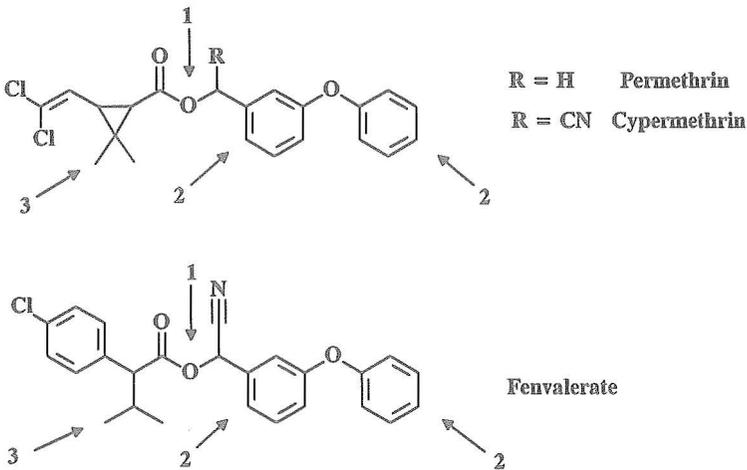


Figure 13. Examples of sites of metabolic attack for pyrethroids. Site 1 - ester hydrolysis. Site 2 - aromatic hydroxylation. Site 3 - aliphatic hydroxylation. All sites result in detoxification of the pyrethroids.

Table 3. Studies of insecticide penetration and metabolism in selected cotton insect pests.

Compound	<i>In vivo</i>	<i>In vitro</i>	Tobacco budworm	Boll-worm	Boll weevil	Twospotted spider mite	References
<u>DDT</u>							
DDT	*			*			Gast, 1961
	*		*				Vinson and Brazzel, 1966
	*	*	*	*			Plapp, 1973
	*	*					Szeicz <i>et al.</i> , 1973
<u>PYRETHROIDS</u>							
Permethrin	*		*	*			Bigley and Plapp, 1978
	*		*				Nicholson and Miller, 1985
	*		*				Payne, 1987
		*	*				Dowd and Sparks, 1987b
	*		*				Dowd <i>et al.</i> , 1987
	*	*	*				Sparks <i>et al.</i> , 1988
Cypermethrin	*		*				Lee <i>et al.</i> , 1989
	*		*				Little <i>et al.</i> , 1989
	*		*				Walker <i>et al.</i> , 1990
<i>lambda</i> -Cyhalothrin	*		*				Sparks <i>et al.</i> , 1988
Tralomethrin		*	*				Dowd and Sparks, 1988
Fenvalerate		*	*				Dowd and Sparks, 1987b
	*	*	*				Grissom <i>et al.</i> , 1989
Fluvalinate		*	*				Dowd and Sparks, 1988
Fenpropathrin	*	*					Dowd and Sparks, 1988
<u>ORGANOPHOSPHORUS INSECTICIDES</u>							
Accephate	*		*		*		Bull, 1979
	*	*	*		*		Rose and Sparks, 1984
Chlorpyrifos	*	*	*				Whitten and Bull, 1974

Table 3. Continued.

Compound	<i>In vivo</i>	<i>In vitro</i>	Tobacco budworm	Boll-worm	Boll weevil	Twospotted spider mite	References
Chlorpyrifos-methyl	*	*	*				Whitten and Bull, 1974
Dicrotophos	*		*	*			Bull and Lindquist, 1964
Dimethoate	*		*	*			Bull <i>et al.</i> , 1963
Fenitrothion	*	*	*	*			Plapp, 1973
Malathion	*		*				Szeicz <i>et al.</i> , 1973
	*	*	*	*			Plapp, 1973
Mephosfolan	*		*				Hollingshaus <i>et al.</i> , 1984
Methyl parathion	*	*	*				Whitten and Bull, 1978
	*		*				Szeicz <i>et al.</i> , 1973
Mono-crotophos	*		*	*	*		Bull and Lindquist, 1966
Phosphamidon	*				*		Bull <i>et al.</i> , 1967b
Sulprofos	*		*		*		Bull, 1980
Trichlorfon	*		*				Bull and Ridgway, 1969
GC-6506-sulfone	*	*	*				Bull and Whitten, 1972
GS-13005	*		*				Bull, 1968
RH-0994	*		*		*		Bull <i>et al.</i> , 1983
<u>CARBAMATES</u>							
Carbaryl	*			*	*		Andrawes and Dorough, 1967
	*	*	*	*			Plapp, 1973
	*		*				Szeicz <i>et al.</i> , 1973
Methomyl	*					*	Gayen and Knowles, 1981
Aldicarb	*		*		*		Bull <i>et al.</i> , 1967a
	*					*	Chang and Knowles, 1978

Table 3. Continued.

Compound	<i>In vivo</i>	<i>In vitro</i>	Tobacco budworm	Boll-worm	Boll weevil	Twospotted spider mite	References
<u>CYCLODIENES</u>							
Aldrin	*	*	*				Bull and Whitten, 1972
	*	*	*	*			Plapp, 1973
	*		*				Whitten and Bull, 1978
Endrin	*		*				Szeicz <i>et al.</i> , 1973
	*		*				Polles and Vinson, 1972
<u>FORMAMIDINES</u>							
Chlordimeform	*					*	Chang and Knowles, 1977
Amitraz	*		*	*			Knowles and Hamed, 1989
	*		*			*	Franklin and Knowles, 1984
	*		*				Sparks <i>et al.</i> , 1989
	*		*				Sparks <i>et al.</i> , 1993b
BTS-27271	*		*	*			Knowles and Hamed, 1989
<u>BENZOYLPHENYL UREAS</u>							
Diflubenzuron	*				*		Still and Leopold, 1978
	*				*		Chang and Stokes, 1979
	*				*		Bull and Ivie, 1980
	*					*	Franklin and Knowles, 1981
Penfluron	*				*		Chang and Woods, 1979
<u>JUVENOIDS</u>							
Fenoxycarb	*		*				Mauchamp <i>et al.</i> , 1989
<u>AVERMECTINS</u>							
Abamectin	*		*	*			Bull, 1986

1978). The rate of ester hydrolysis is influenced by the steric configuration of the acid moiety and whether the alcohol moiety is a primary or secondary alcohol. The *trans* isomer of permethrin is much more readily hydrolyzed than is the more sterically hindered *cis* isomer (Bigley and Plapp, 1978; Dowd and Sparks, 1987a). The addition of a cyano group to the *alpha*-carbon of the 3- phenoxybenzyl alcohol moiety converts the primary alcohol of permethrin (Ambush®, Pounce®) into a secondary alcohol, cypermethrin (Ammo®, Cymbush®) (Figure 13), which is more difficult to hydrolyze (Soderlund *et al.*, 1983; Ruigt, 1985). Although not directly comparable, fenvalerate (Pydrin®), fluvalinate (Mavrik®) and tralomethrin (Scout®) all contain an *alpha*-cyano group in the alcohol and all are hydrolyzed *in vitro* at rates far below that of *trans*-permethrin in the tobacco budworm (Dowd and Sparks, 1988). The activity of the enzymes involved in the hydrolysis of both permethrin isomers and fenvalerate increases during the course of larval development (Dowd and Sparks, 1987b). Recent studies comparing the relative rates of permethrin versus *lambda*-cyhalothrin (Karate®) turnover found the latter to be much more resistant to metabolism (Sparks *et al.*, 1988). *Trans*-cypermethrin penetrated more slowly into pyrethroid-resistant tobacco budworm larvae than into pyrethroid- susceptible tobacco budworm larvae (Little *et al.*, 1988). The pyrethroid- resistant strain appeared to metabolize the *trans*-cypermethrin more rapidly than the susceptible strain. In both strains the 2'/4'-*trans*-cypermethrin and the dichlorovinyl acid from *trans*-cypermethrin were found to be present. This suggests the presence of both oxidative and hydrolytic pathways (Little *et al.*, 1988, 1989). Other studies support the presence of both pathways for cypermethrin (Ammo®, Cymbush®) metabolism (Lee *et al.*, 1989). Although there has been a study of fenvalerate penetration into larvae of the tobacco budworm (Grissom *et al.*, 1989), to date the *in vivo* metabolism of fenvalerate and several other pyrethroids registered for use on cotton including fluvalinate (Mavrik®), tralomethrin (Scout®), biphenethrin (Capture®) and cyfluthrin (Baythroid®) has not been evaluated in either the bollworm/tobacco budworm or the boll weevil. Studies of fenvalerate (Pydrin®) metabolism in the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Soderlund *et al.*, 1987) and horn fly, *Haematobia irritans* (L.) (Bull *et al.*, 1988) indicate that oxidative pathways predominate.

METABOLISM OF ORGANOPHOSPHORUS INSECTICIDES

The organophosphorus insecticides are subject to a variety of metabolic modifications including monooxygenase based reactions: thiophosphate (P=S) to phosphate (P=O) conversion; *O*-dearylation; *O*- and *S*-dealkylation; *S*-alkyl oxidation; and *N*-dealkylation (Figure 14). The hydrolases in the form of phosphotriesterases (Figure 14, site 2), carboxylesterases (Figure 14, site 6) and carboxylamidases (Figure 14, site 7) are important, as are the glutathione *S*-transferases. These latter hydrolases also are important in the *O*- dealkylation of organophosphorus insecticide (Figure 14, site 3), especially where the alkyl groups are *O*-methyl.

The major metabolic pathways for most organophosphorus insecticides include cleavage of *O*- and *S*-aryl and alkyl phosphorus bonds by a combination of phospho-

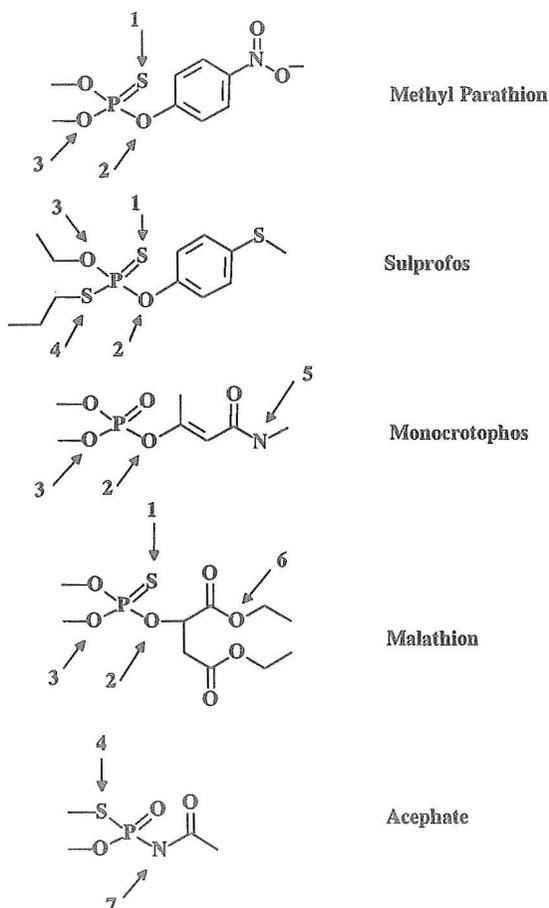


Figure 14. Examples of sites of metabolic attack for organophosphorus insecticides. Site 1 - Oxidative desulfurization ($P=S$ to $P=O$), activation. Site 2 - phosphotriester hydrolysis and/or oxidative dealkylation or dearylation, detoxification. Site 3 - *O*-dealkylation via glutathione transferases or monooxygenases, detoxification. Site 4 - thio oxidation, activation. Site 5 - *N*-dealkylation, activation. Site 6 - carboxylester hydrolysis, detoxification. Site 7 - Carboxylamide hydrolysis, activation (in this particular case).

triesters, glutathione *S*-transferases and monooxygenases (Figure 14, sites 2 and 3). For phosphorothionates (thiophosphates) such as methyl parathion, oxidative desulfuration ($P=S$ to $P=O$) of the phosphorothionate to the oxon is also an important reaction (Figure 14). Typically *O*- and *S*-dealkylation and dearylation are detoxifying reactions, often followed by the rapid conjugation and excretion of the compound (Eto, 1974; Buchel, 1983). Likewise, organophosphorus insecticides such as malathion are also detoxified by carboxylesterases acting on carboxylester linkages (Figure 14, site 6). However, many reactions involving organophosphorus insecticides, including oxidative desulfuration, serve to activate or increase the toxicity of the parent organophosphorus insecticide.

Phosphates such as methamidophos (Monitor®), mevinphos (Phosdrin®), monocrotophos (Azodrin®), naled (Dibrom®) and profenofos (Curacron®) are all active as inhibitors of acetylcholinesterase, whereas phosphorothionates such as azinphosmethyl (Guthion®), chlorpyrifos (Lorsban®, Dursban®), EPN, malathion, methyl parathion, parathion and sulprofos (Bolstar®) all require metabolism (conversion) by monooxygenases to the corresponding phosphates (oxons) before they can effectively inhibit acetylcholinesterase. For example, parathion, malathion (Cythion®) and dimethoate (Cygon®) are 218, 750 and 5357 times less active towards house fly head acetylcholinesterase than their corresponding oxons (Eto, 1974). For many of the thiophosphate insecticides studied in the bollworm/tobacco budworm and the boll weevil, the oxon analogs of chlorpyrifos, chlorpyrifos methyl (Whitten and Bull, 1974), dimethoate (Bull *et al.*, 1963), methyl parathion (Whitten and Bull, 1978) and GS-13005 (Bull, 1968) have been identified as metabolites.

Sulfoxidation by monooxygenases of *S*-alkyl groups of organophosphorus insecticides (Figure 14, site 4) such as profenofos (Curacron®) and potentially sulprofos (Bolstar®) and RH-0994 can also result in increased toxicity (Wing *et al.*, 1982). *S*-alkyl sulfoxidation of methamidophos (Monitor®) has been used to explain the *in vivo* toxicity of an otherwise poor *in vitro* acetylcholinesterase inhibitor (Eto *et al.*, 1977; Magee, P. S., 1982), and the *S*-methyl has been identified as being the leaving group (Thompson and Fukuto, 1982). However, where the *S*-alkyl group is small (i.e. the *S*-methyl of methamidophos) sulfoxidation may not occur (Wing *et al.*, 1982), and may not be necessary to explain the biological activity of this insecticide (Khasawinah *et al.*, 1978; Magee, P. S., 1982; Rose and Sparks, 1984). Studies of sulprofos (Bull, 1980) in boll weevil and tobacco budworm found little in the way of metabolism, but since sulprofos requires biological activation for activity, these reactions were probably not detected due to the low specific activity of the compound used (Bull, 1980). Thioether sulfoxidation can also occur for *S*-alkyl or *S*-aryl substituents on the phenyl rings of organophosphorus insecticides such as sulprofos (Figure 14, site 4) resulting in increased reactivity with acetylcholinesterase (Eto, 1974; Bull, 1980; Bull *et al.*, 1976).

In addition to oxidative desulfuration and *S*-alkyl sulfoxidation, the *N*-dealkylation of organophosphorus insecticides such as monocrotophos (Azodrin®) to the unsubstituted amine also results in increased toxicity (Eto, 1974), but is only a minor pathway in the bollworm and boll weevil (Bull and Lindquist, 1966). The *N*-deacylation of

acephate (Orthene®) to methamidophos (Monitor®) (Figure 14, site 7) by carboxylamidases is also an activation reaction that readily occurs in the tobacco budworm (for which acephate is an effective insecticide) but not in the boll weevil (acephate is non toxic to the boll weevil) (Bull, 1979; Rose and Sparks, 1984).

METABOLISM OF CARBAMATES

Several carbamates have been and continue to be used for the control of cotton insect pests including carbaryl (Sevin®), carbofuran (Furadan®), methomyl (Lannate®, Nudrin®), aldicarb (Temik®), and thiodicarb (Larvin®). Carbamates are primarily metabolized by oxidative reactions and, to varying degrees, by ester hydrolysis (Figure 15) (Kuhr and Dorough, 1976). In the case of carbaryl metabolism by adult boll weevils and bollworm larvae, the hydrolysis product, 1-naphthol, accounted for 5.8 percent and 17.4 percent, respectively, of the applied dose 12 hours posttreatment (Andrawes and Dorough, 1967). However, it is likely that the 1-naphthol originated from the breakdown of an oxidation product, the *N*-hydroxylated carbaryl (Andrawes and Dorough, 1967). The other major metabolite in boll weevils and bollworms was the 5,6-diol of carbaryl, resulting from aryl hydroxylation by monooxygenases. Tobacco budworm larvae metabolize carbaryl faster than do larvae of the bollworm (Plapp, 1973).

As with carbaryl, the principle metabolites of aldicarb (Temik®) are the result of monooxygenase activity and include the *N*-hydroxy-aldicarb, the sulfoxide and the sulfone (Figure 15, site 4). Aldicarb is much more readily absorbed by the boll weevil than by the tobacco budworm (Bull *et al.*, 1967a). As with the organophosphorus insecticides,

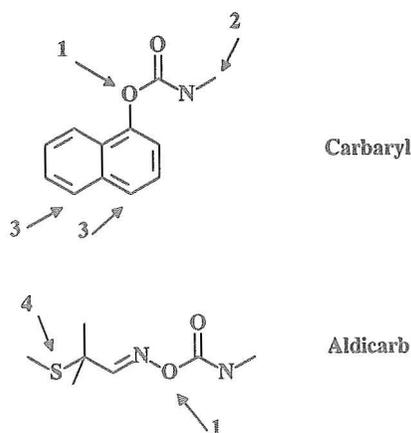


Figure 15. Examples of sites of metabolic attack for carbamate insecticides. Site 1 - carboxylester hydrolysis, detoxification. Site 2 - *N*-alkyl hydroxylation, detoxification. Site 3 - aromatic hydroxylation, detoxification. Site 4 - thioether oxidation, activation.

thioether oxidation to the sulfoxide is an activation reaction for aldicarb and is the predominant reaction for both the boll weevil, tobacco budworm, and twospotted spider mites (Bull *et al.*, 1967a; Chang and Knowles, 1978). The oxidative *N*-demethylation of aldicarb appears to be a very minor pathway for the boll weevil and tobacco budworm. The recovery of oxime sulfoxide and sulfone indicates that hydrolysis of the aldicarb sulfoxide and sulfone occurs to some extent for the boll weevil, tobacco budworm (Bull *et al.*, 1967a) and twospotted spider mite (Chang and Knowles, 1978). In part, the poor toxicity of aldicarb to tobacco budworm larvae versus the boll weevil appears to be due to differences in the sensitivity of their respective acetylcholinesterases (Bull *et al.*, 1967a).

Although methomyl (Lannate®, Nudrin®), carbofuran (Furadan®) and thiodicarb (Larvin®) are also registered for the control of bollworm/tobacco budworm and the boll weevil, there appears to have been no studies of their metabolism in these insects. Based on studies with cabbage loopers (Kuhr, 1973), methomyl does not appear to form the sulfoxides and sulfones observed for aldicarb (Temik®). Rather it seems to decompose to form acetonitrile and carbon dioxide (Kuhr, 1973; Kuhr and Dorough, 1976). When the metabolism of methomyl was studied in the twospotted spider mite (Gayen and Knowles, 1981), methomyl oxime, several unidentified metabolites, and labeled CO₂ were detected. Studies of carbofuran metabolism in insects such as the saltmarsh caterpillar indicate that it is readily metabolized via monooxygenases to form 3-hydroxy carbofuran and its 3-keto analog, as well as the *N*-hydroxymethyl analog (Kuhr and Dorough, 1976).

METABOLISM OF CYCLODIENES

Endosulfan (Thiodan®) remains the only cyclodiene that is recommended for use in the control of bollworm/tobacco budworm on cotton in the United States. Although there are no reports of the metabolism of endosulfan in the bollworm/tobacco budworm or the boll weevil, its metabolism has been studied in other insects (Barnes and Ware, 1965; Brooks, 1974). Compared to other cyclodienes endosulfan is highly biodegradable (Brooks, 1974). The primary metabolite in insects occurs through oxidation of the sulfite moiety to the sulfate (Barnes and Ware, 1965; Brooks, 1974).

The metabolism of endrin has been studied in the tobacco budworm where the primary metabolites were tentatively identified as endrin-aldehyde and endrin-ketone (Polles and Vinson, 1972). Aldrin is more rapidly metabolized in the tobacco budworm than in the bollworm with dieldrin being the primary metabolite for both species (Plapp, 1973).

METABOLISM OF FORMAMIDINES

Metabolism studies of chlordimeform (Fundal®, Galecron®) in the twospotted spider mite indicate that chlordimeform is rapidly taken up and *N*-demethylated to the demethylchlordimeform followed by further *N*-demethylation to didemethylchlordimeform, the 4'-chloro-*o*-formotoluidide and 4'-chloro-*o*-toluidine (Figure 16) (Chang and Knowles, 1977). This pattern of metabolism is consistent with the formation of the more toxic *N*-demethylchlordimeform (Chang and Knowles, 1977) and chlordimeform functioning as an octopamine agonist. Twospotted spider mite metabolism of amitraz (Ovasyn®) produced several metabolites including BTS-27271 (*N'*-(2,4-dimethyl-

phenyl)-*N*-methylformamidine; NOR-AM 49844), 2,4-dimethylformanilide and 2,4-dimethylaniline (Franklin and Knowles, 1984). As observed for chlordimeform, a metabolite (BTS-27271) may be responsible for the biological activity of amitraz (Franklin and Knowles, 1984; Knowles, 1987).

The metabolism of amitraz has also recently been examined in larvae of the tobacco budworm and bollworm (Knowles and Hamed, 1989; Sparks *et al.*, 1989). As observed for the spider mites, amitraz is converted to BTS-27271 (Knowles and Hamed, 1989; Sparks *et al.*, 1989), and other metabolites; 2,4-dimethylformanilide, 2,4-dimethylaniline and polar metabolites (Knowles and Hamed, 1989). Although higher titers of BTS-27271 were found in larvae of the bollworm when compared to larvae of the tobacco budworm (Knowles and Hamed, 1989), there were no differences in the titers of amitraz and BTS-27271 in pyrethroid susceptible and resistant larvae of the tobacco budworm (Sparks *et al.*, 1989). The metabolism of BTS-27271 by larvae of the bollworm and tobacco budworm also proceeded through the 2,4-dimethylformanilide, but not the 2,4-dimethylaniline (Knowles and Hamed, 1989). Eggs of the tobacco budworm also have the capability of converting amitraz to BTS-27271, which may be associated with its ovicidal activity (Sparks *et al.*, 1990, 1993b).

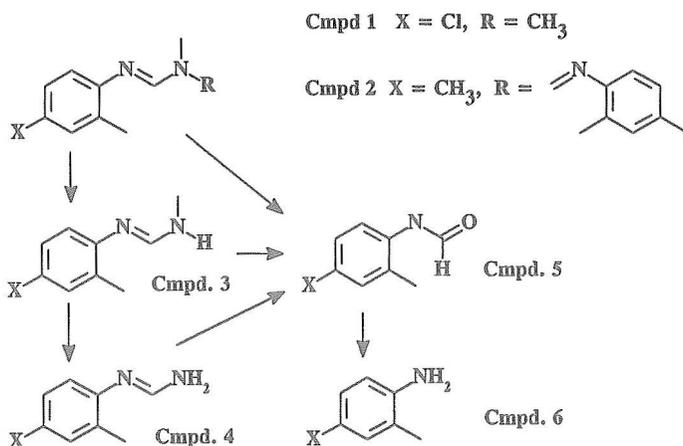


Figure 16. Examples of some of the metabolic pathways for the formamidines chlordimeform (1) and amitraz (2). Compound 3: demethylchlordimeform (X-Cl); BTS-27271 (X-CH₃). Didemethylchlordimeform (4). Compounds 5 and 6 are metabolites for both chlordimeform (X-Cl) and amitraz (X-CH₃). [Information adapted from Chang and Knowles (1977), and Knowles and Hamed (1989).]

METABOLISM OF BENZOYLPHENYL UREAS

The metabolism of the benzoylphenyl ureas has been extensively studied in a variety of insects (Hammock and Quistad, 1981; Sparks and Hammock, 1983; Retnakaran *et al.*,

1985) including the boll weevil. The first studies of diflubenzuron (Dimilin®) metabolism in the boll weevil found only unchanged diflubenzuron internally and in the frass (Still and Leopold, 1978). Subsequent studies (Chang and Stokes, 1979) also found only unchanged diflubenzuron internally, but in the frass observed several conjugates of diflubenzuron hydroxylated in the 2 position of the chloroaniline ring or the 3 position of the difluorobenzamide ring. A further study of diflubenzuron metabolism in the boll weevil (Bull and Ivie, 1980) also found that diflubenzuron accounted for most of the internal radioactivity, but that small amounts of metabolites were produced and evidence suggested both conjugation and hydrolysis reactions. As with diflubenzuron, studies of penfluron (Figure 12) metabolism in the boll weevil found unchanged penfluron to account for virtually all of the internal radioactivity (Chang and Woods, 1979). Likewise metabolism of diflubenzuron by twospotted spider mites also proceeds very slowly, with unchanged diflubenzuron accounting for most of the radioactivity (Franklin and Knowles, 1981). However, major metabolites (5.8 to 7.7 percent of recovered radioactivity) appeared to be the result of hydrolysis while hydroxylation products of the chloroaniline ring were relatively minor (0.8 to 1.4 percent of recovered radioactivity) products.

METABOLISM OF JUVENIDS

Although the metabolism of the insect juvenile hormones and juvenoids have received quite a bit of attention (Hammock and Quistad, 1981; Sparks and Hammock, 1983; Hammock, 1985; Retnakaran *et al.*, 1985), information concerning juvenoid metabolism in pests of cotton is very limited. The metabolism of the juvenoid fenoxycarb (Logic®) has been examined in fourth and fifth instar larvae of the tobacco budworm (Mauchamp *et al.*, 1989). While ester hydrolysis does not appear to be an important metabolic pathway, cleavage of the amide linkage and aromatic hydroxylation did appear to occur (Mauchamp *et al.*, 1989).

METABOLISM OF AVERMECTINS

Although there are numerous studies of ivermectin in mammals (Chiu and Lu, 1989) there is limited information on abamectin (Affirm®, Zephyr®) metabolism in insects. Avermectin B_{1a} was metabolized faster in bollworm larvae than in larvae of the tobacco budworm, and more accumulated in the heads of tobacco budworm larvae than in bollworm larvae (Bull, 1986); however, specific metabolites of avermectin were not identified. Studies with abamectin susceptible and resistant Colorado potato beetles suggest that oxidative metabolism predominates in insects, the major metabolite being the 3''-desmethyl avermectin B_{1a}, followed by the 24-hydroxy avermectin B_{1a} (Argentine *et al.*, 1992; Clark *et al.*, 1992).

SYNERGISM

In the control of cotton insect pests a common practice has been, and continues to be, the mixing of insecticides to either control several different pests with one application, or to increase the activity of a particular insecticide. In the broadest sense, synergism is

the enhancement of biological activity (usually toxicity) over and above that which would normally be expected from the separate components alone. In terms of cotton insect/mite control, synergism can occur when two or more insecticides and/or acaricides are mixed as in the case of toxaphene plus DDT, or when an insecticide and an insecticide synergist, such as piperonyl butoxide, are used together. In many cases, the resulting synergism is due to the detoxification of one component (insecticide) being blocked by another component (another insecticide or an insecticide synergist) (Wilkinson, 1976b). Piperonyl butoxide is commonly used as an insecticide synergist since it is an effective inhibitor of the monooxygenases involved in insecticide detoxification (Wilkinson, 1976b). Likewise, many organophosphorus insecticides are effective inhibitors of the hydrolases involved in the detoxification of pyrethroids and other organophosphorus insecticides (Eto, 1974; Soderlund *et al.*, 1983; Dowd and Sparks, 1987c). For example, the organophosphorus insecticide profenofos (Curacon®) is an effective inhibitor of the esterases responsible for the hydrolysis of pyrethroids (Soderlund *et al.*, 1983; Dowd and Sparks, 1987c). Mixing profenofos with permethrin (Ambush®, Pounce®) increases the topical toxicity of permethrin to larvae of the tobacco budworm by over four-fold (Dowd and Sparks, 1987; Dowd *et al.*, 1987).

Although inhibition of detoxification is one mechanism by which a synergist can function, other possibilities also exist. In recent years chlordimeform (Fundal®, Galecron®) has been found to synergize a variety of insecticides, including the pyrethroids (Plapp, 1976; El-Sayed and Knowles, 1984a,b; Campanhola and Plapp, 1987). It has been suggested that chlordimeform functions by increasing the binding of the pyrethroid at the target site (Chang and Plapp, 1983; Liu and Plapp, 1992). Another potential explanation lies in the octopamine agonist action of chlordimeform (Table 1), resulting in increased motor activity in the insects. Recent studies demonstrate that in contact bioassays, chlordimeform increases the uptake of radiolabeled permethrin or *lambda*-cyhalothrin by tobacco budworm larvae (Sparks *et al.*, 1988, 1989, 1991). Thus, in part, insecticide synergism by chlordimeform may result from increased insecticide contact on the part of chlordimeform-treated insects.

THE FUTURE AND NEEDS

For many cotton growing regions of the United States the pyrethroids currently provide effective control of the tobacco budworm - bollworm complex. However, pyrethroid resistance has become an increasingly important problem for cotton growers in parts of Louisiana, Mississippi and Texas (Sparks *et al.*, 1993a), just as it had for *Helicoverpa armigera* Hübner on cotton in Australia (Daly, 1988; Cox and Forrester, 1992), the horn fly, *Haematobia irritans* (Linnaeus) (Byford and Sparks, 1987), and the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Georghiou, 1986). Although alternatives to the pyrethroids exist in the form of some of the newer organophosphorus and carbamate insecticides, as well as others, these compounds are typically not as active as the pyrethroids and are generally more expensive. In addition, available data now suggest that there may also be resistance to some of these

organophosphorus and carbamate insecticides, possibly endosulfan, as well as the pyrethroids (Martin *et al.*, 1992; Elzen *et al.*, 1993; Kanga *et al.*, 1993; Sparks *et al.*, 1993a). Thus, it has become imperative to expand the search for new insecticides, and at the same time implement programs to preserve available compounds. In many cases, they represent a non-renewable resource (Hammock and Soderlund, 1986). Unfortunately, the cost of discovery for new replacement insecticides is an increasingly expensive process (Georghiou, 1986; Hammock and Soderlund, 1986) with the percentage of compounds making it to market steadily declining. However, there is some new chemistry on the horizon that may be available in the near future to potentially fill in any holes created by the loss of one or more of the currently available insecticides. The pyrrole AC 303,630 (Pirate®) and the avermectin analog emamectin (MK-244) represent two new chemistries that appear to have potential as insecticides for tobacco budworm larvae (Addor *et al.*, 1992; Kuhn *et al.*, 1993; Fisher, 1993; see also above). In addition, insecticides suitable for use against the tobacco budworm and other cotton pests may eventually come out research into the phenylpyrazoles (Colliot *et al.*, 1992), or the diacylhydrazides (Hsu, 1991; Heller *et al.*, 1992).

An important consideration with all of these materials is that a new mode of action does not necessarily mean there will be no cross-resistance from older insecticides to the new insecticide. For example, dimethoate (an organophosphate inhibitor of acetylcholinesterase) resistant house flies were found to be cross-resistant to methoprene, a juvenoid (juvenile hormone mimic) IGR (Hammock *et al.*, 1977). The basis for the dimethoate resistance, and the cross-resistance to methoprene, was an enhanced monooxygenase activity that could effectively metabolize both types of chemistries (Hammock *et al.*, 1977, Sparks and Hammock, 1983). Therefore, new chemistry or new modes of action can be useful tools in resistance management programs only if the resistance mechanisms (which may or may not be based on mode of action) do not overlap for the different insecticides involved. Conversely, compounds with the same mode of action do not necessarily have to be cross-resistant, especially if the resistance mechanism does not involve the insecticide target.

In addition to the chemistries mentioned, other leads for the development of new insecticide/acaricides are needed if we are to insure the future of cotton insect control. While there are a variety of methods available for achieving insecticide/acaricide selectivity and safety (Hollingworth, 1976; Drabek and Neumann, 1985), attacking a target unique to insects is conceptually the most appealing. In this respect the insect endocrine system appears to have some advantages for the development of safer insecticides/acaricides since, in several aspects, it appears to be unique to insects (Sparks, 1990). As already mentioned neither the juvenoids nor the diacylhydrazides have yet to find widespread use in cotton insect/mite control. However, these compounds aptly demonstrate that safe and selective insecticides/acaricides based on the insect endocrine system can be developed. Other approaches to exploiting the insect endocrine system for insect control include the development of anti-juvenile hormones that would affect the early larval development of pest lepidopterans. Given the chemical variety and numerous modes of action for the anti-juvenile hormones that have been identified (Staal, 1986;

Sparks, 1990) antagonism of juvenile hormone biosynthesis or action may yet yield useful insecticides. Indeed, for pest insects such as the tobacco budworm, some of the more recent anti-juvenile hormones (e.g. DPH, Table 2) are as active as some organophosphorus and carbamate insecticides (Quistad *et al.*, 1985).

Available information clearly demonstrates that an appreciation of the basic biochemistry and physiology of insects can be critical in the development of new insecticides. This concept is exemplified in the possibilities now being raised by the isolation, characterization and sequencing of insect neurohormones and neurotransmitters (Sparks, 1990; Masler *et al.*, 1993). These bioactive molecules present a host of new models for the production of synthetic analogs to be used as insecticides. Likewise, the incorporation of the genes for some of these neurohormones into plants or bacterial or viral vectors, presents new opportunities and new approaches for controlling insect pests (Hammock *et al.*, 1993).

However, to take advantage of these new approaches in insect/mite control, more information is needed on the basic insect/mite biochemistry and physiology, as well as on the mode of action of new and existing insecticides. Moreover, insects such as the tobacco budworm, bollworm and pink bollworm should be included as test animals. Some of this information can come from screening programs that have used cotton insect pests such as the tobacco budworm or bollworm in structure optimization studies (Soloway *et al.*, 1979; Henrick *et al.*, 1980; Plummer, 1984; Kuhn *et al.*, 1993), thereby making available very useful information on structure-activity relationships. Unfortunately, such information is typically not made available.

In addition to the search for new chemistry, the many resistance management programs instituted (Anonymous, 1986; Plapp, 1987) throughout the cotton growing areas of the United States hopefully will slow the rate at which pyrethroid resistance is developing (Graves *et al.*, 1988; Sparks *et al.*, 1993a). With programs such as these, the pyrethroids and other insecticides may yet remain useful in cotton insect pest management programs to provide the time needed to develop new and improve upon existing, cotton insect/mite control measures.

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