Chapter 9

RESISTANCE TO PESTICIDES: MECHANISMS, DEVELOPMENT AND MANAGEMENT

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INTRODUCTION

Growers have been aware of resistance to insecticides in cotton pests for many years. They have come to expect that, given a chronic or endemic pest population, a new insecticide or acaricide has a certain life in the field before pest tolerance increases to unacceptable levels (Ivy and Scales, 1954; Brazzel, 1963; Bottrell and Adkisson, 1977; Sparks, 1981; Wood, 1981; Mani, 1990). It is only recently that resistance has become recognized by the industry as being manageable (LaFarge, 1985).

Resistance to insecticides and the related phenomena of pest resurgence and secondary pest outbreaks are said to be predictable from elementary theories of evolution and population dynamics (Berryman, 1991). It is impossible to spray any crop with a full rate dose of any modern residual neurotoxic insecticide without that dose eventually becoming a selecting dose. This is because, through time, the foliar applied dose degrades, usually slowly over a matter of days, from a killing dose to a selecting dose.

Agrochemical realities are driven by a need to produce only broad spectrum insecticides with a long residual activity for major markets like the cotton pest complex (Voss and Neumann, 1992). These characteristics also are ideal for the development of resistance, particularly the residual property (Denholm *et al.*, 1983; ffrench-Constant *et al.*, 1988a,b). Most other candidate insecticides, particularly selective insecticides that would fit ideally into insect pest management programs, are not developed because the return would not pay for the investment.

It is the selecting dose that leads to resistance problems. The natural play of a wide diversity of fitness and other genetic factors combined with high rates of reproduction, drive the response of pest populations toward ever greater tolerance so long as insecticides are being used frequently on a wide scale. These events rarely occur quickly. Instead, resistance gradually "creeps" into the agroeconomic mileu as resistance builds and declines in repeated cycles. This "ratchet up" effect of the alternate build up and decline of resistance to pyrethroid insecticides was seen each season in *Helicoverpa armigera* (Hübner) in Australia (Roush and Daly, 1990) and the same effect was

noticed in resistance monitoring data from tobacco budworm, *Heliothis virscens* (F.) in the United States Cotton Belt (Mullins *et al.*, 1991).

The "classic" signs of resistance are said to be increasing dose rates and decreasing treatment intervals. The onset of resistance is usually measured in years rather than months. In most cases resistance problems are caused in species that tend to be more endemic, because they would be under more constant selective pressure; however, the presence of resistance in pest insects, while almost always suspected, is proven only with a substantial amount of work.

The following quote from Professor Thomas F. Leigh (Personal communication) of the University of California, Davis is pertinent: "We presume there is resistance to several insecticides in our populations of aphid, bollworm [*Helicoverpa zea* (Boddie)], beet armyworm [*Spodoptera exigua* (Hübner)], cabbage looper [*Trichoplusia ni* (Hübner)], saltmarsh caterpillar [*Estigmene acrea* (Drury)] and a number of other pests. However, we have not conducted confirming tests that would verify resistance. While control failures that have occurred frequently could be related to application or weather, we are confident that many failures today relate to the selective pressure of the insecticides that used to be effective."

Dr. Leigh was referring to the pest-cotton complex in the Central Valley of California, but the same remarks with a slightly different range of pests could be true of any part of the Cotton Belt. Representatives from agrochemical industry spend a certain amount of their time verifying rumors of possible resistance (Davies, 1984). Gossip can have a profound influence on the widespread confidence that growers have in a particular pest control product.

REGIONAL PESTS AND RESISTANCE POTENTIAL

Although it is not the purpose of this chapter to document the presence of pest insects, the potential of insect pests to develop resistance is tied closely to species that visit the cotton fields each season. With a few outstanding exceptions like the boll weevil, *Anthonomus grandis grandis* (Boheman), the species that are more endemic and are treated more routinely with insecticides would be expected to develop resistance more readily.

Unfortunately for everyone, the mix of pest species changes every year, sometimes drastically, making predictions about pest pressure virtually impossible. The same impossibility of prediction holds true for gauging the chances of developing resistance, especially over a cotton growing area that extends from California to Virginia. Nevertheless, the insect pressure reported for 1991 (Head, 1992) is instructive and useful as a starting point (Table 1).

Comparing the percent yield reduction due to insect pests from 1989 to 1992 (Table 2), and ranking the pests in terms of causing the greatest damage, boll weevils were the worst pests in 1989, then the bollworm, and tobacco budworm complex caused the greatest losses in 1990 and 1992, while aphids caused the worst losses in 1991.

By comparing the five worst pests in terms of yield reduction across the Cotton Belt, aphids were fifth in 1989 with 0.55 percent yield reduction, 0.64 percent in 1990 and

Table 1. Cotton insect losses in the United States reported for 1991 growing season. (From Head, 1992.)[1]

Pests	Acreage infested	Number of insecticide applications[2]	Yield reduction	Bales lost
	(1,000 acres)		(%)	(1,000 bales)
Boll weevil	6,122	0.7	0.81	146.00
Bollworm/tobacco budworm	11,340	1.6	1.68	300.00
Fleahoppers	4,534	0.2	0.13	23.60
Lygus bugs	5,109	0.4	0.47	85.00
Leaf perforator	315	0.0	0.00	0.39
Pink bollworm	512	0.1	0.08	15.00
Spider mites	1,816	0.1	0.08	14.40
Thrips	7,035	0.3	0.13	22.50
Beet armyworm	2,305	0.1	0.02	3.66
Fall armyworm	1,805	0.0	0.03	5.03
Minor pests	3,018	0.1	0.08	13.80
Aphids	10,067	0.9	2.01	360.00
New pests	1,584	0.1	0.11	19.40
Western flower thrips	2,339	0.0	0.00	0.11

[1]Total acres harvested was 13,022,000; average yield was 1.38 bales/acre.
[2]Per acre for infested acreage.

they were the worst pest in 1991 with a yield reduction of 2.01 percent, and in 1992 dropped off the five worst list to be replaced by the sweetpotato whitefly, *Bemisia tabaci* (Gennadius). Over the same four years, lygus bugs as pests went from second and 2.05 percent yield reduction to 0.47 percent and fourth worst pest.

Table 2. Annual yield reduction of total United States cotton production ranked by top insect pests for the period 1989-1992. (From Head, 1990, 1991, 1992, 1993.)

Rank	1989		1990		1991		1992	
	Pest	Yield reduction	Pest	Yield reduction	Pest	Yield reduction	Pest	Yield reduction
		(%)		(%)		(%		(%)
1.	Boll Weevil	2.75	Bollworm	1.73	Aphid	2.01	Bollworm	2.2
2.	Lygus	2.05	Mites	1.24	Bollworm	1.68	Boll weevil	2.1
3.	Bollworm	1.87	Lygus	0.91	Boll weevil	0.81	Lygus	0.8
4.	Mites	1.11	Aphid	0.64	Lygus	0.47	Whitefly	0.5
5.	Aphid	0.55	Boll weevil	0.60	Leafhopper	0.13	Thrips	0.3

One would expect from this type of exercise that the insects listed in these tables are the prime candidates for the development of resistance. Indeed, research funding aimed at studies of insecticide resistance tends to be driven more by the magnitude and immediacy of current pest problems fueled by grower concerns, than by any rational, stable, longer-term approach.

For example, spider mites caused 1.11 percent yield reduction in 1989 and 1.24 percent yield reduction in 1990; however, in 1991 mites were credited with causing a 0.08 percent yield reduction and in 1992 a 0.2 percent reduction. Not only did they fall off of the worst five list, interest and support for resistance management waned. Thus, like cotton pests in general, insecticide resistance problems are a dynamic target.

An even more revealing statistic on these tables is the number of insecticide applications (Table 1). Costs are related to the number of applications. They also reflect the insecticide sales market. Older compounds like methyl parathion and malathion, long out of patent, would tend to be lower in cost. Insecticides like the pyrethroids would tend to be less expensive because several pyrethroid products are now competing for a cotton pest control market, that reportedly is worth US$ 300 million a year in the United States (Anonymous, 1990b).

If one is allowed to make risky conclusions based on these figures alone, one might be tempted to suspect that those cotton pest insects that are more endemic (show up on the five worst insect list every year) and show higher per application costs would be those insects that pose the greatest insecticide resistance problems. This is only partially correct because resistance can occur in smaller populations of regional pests that are not at the top of these lists (see, for example, the entries in Table 1 for "minor pests" and "new pests").

In 1991, aphids became significant cotton pests and were said to be responsible for the greatest losses attributed to one pest. If one follows the gradual increase in the severity of cotton pest control problems with aphids, a steadily increasing problem is documented starting in 1989; and, being a member of the top five cotton pest problems, aphids represent a significant insecticide resistance problem.

Unseen in these figures is the gradual increase in whitefly problems that occurred in parallel with the aphid population increases. Both of these species are believed to have increased in numbers recently because of insecticide-induced killing of beneficial insects (Newsom and Smith, 1949; Kerns and Gaylor, 1991). Whiteflies and aphids have thus acquired resistance to certain insecticides before becoming significant pests which must now be taken into account in designing treatment strategies (Byrne *et al.*, 1992).

In the San Joaquin Valley of California, the predominant pests, and potential resistance problems, are spider mites (*Tetranychus* sp.), and occasionally the western lygus bug, *Lygus hesperus* Knight. The beet armyworm and cabbage looper can be found occasionally in cotton, but never consistently. Only rarely do tobacco budworm or bollworm present problems, even though both are present on other hosts in the area (Anonymous, 1984; Tom Leigh, 1993, personal communication, University California [Davis], Shafter, CA).

The western lygus bug has gradually developed resistance to insecticides over the years. By 1953, resistance was reported to DDT (Andres *et al.*, 1955). During the 1960s resistance to other organochlorines and to several widely used organophosphates was established by Leigh and Jackson (1968). The list of resistant compounds was extended by Leigh *et al.* in 1977. Presently, acephate (Orthene®), methamidophos (Monitor®) and dicrotophos (Bidrin®) are effective as foliar sprays against the western lygus bug while methidathion (Supracide®) is not always highly effective, suggesting the development of resistance.

In desert growing areas of California and Arizona, the pink bollworm, *Pectinophora gossypiella* (Saunders), is the key pest of cotton (Anonymous, 1984). Since about 1966 the pink bollworm has been distributed from Texas across New Mexico and Arizona to southern California and in adjacent Mexican cotton fields. Although the pink bollworm distribution covers almost the entire western part of the Cotton Belt, its seriousness as a pest problem, and therefore as a resistance threat, varies drastically (Noble, 1969).

In West Texas and from El Paso east along the Rio Grande Rider, the pink bollworm has been held in check largely by cultural control practices including adoption of a short growing season strategy (Bottrell and Adkisson, 1977). Further up the Rio Grande at Las Cruces, New Mexico, the pink bollworm is a late-season pest, probably because it does not overwinter locally, but rather reinvades each year from warmer climates downriver. The same is true at the higher elevations of central and eastern Arizona, where only a few spray treatments may be necessary to control pink bollworm yearly.

Attempts to control the pink bollworm have caused some resistance problems in the past, but given the amount of insecticides used to control pink bollworm yearly in the chronically infested areas, and given the large endemic populations, it is remarkable that resistance problems have not been more severe (Haynes, *et al.*, 1986, 1987; Bariola, 1985; Bariola and Lingren, 1984).

Attempts at chemical control of the pink bollworm have often led to secondary outbreaks of tobacco budworm, cotton leafperforator, *Bucculatrix thurberiella* Busck, and sweetpotato whitefly. The insecticide-induced secondary pest problems in the Imperial Valley of California have become especially severe since 1981 with the insecticide resistant whitefly (Prabhaker *et al.*, 1988; Youngman *et al.*, 1986) building up in cotton in the fall and fouling the lint with honeydew which allows growth of sooty mold. The whitefly later transmits lettuce yellows virus to lettuce and melon crops into the late fall growing season.

While the bollworm is relatively abundant in the cotton growing areas of Arizona and California, it is not an important or chronic pest of cotton. The tobacco budworm is difficult to find in the southwestern desert cotton growing areas of the United States until usually in mid-August when numbers increase markedly, but it, too, is an inconsistent pest of cotton in the southwestern desert.

The tobacco budworm and bollworm are key pests in the Mid-South and eastern cotton growing areas of the United States. This includes the area in Texas along and north of the lower Rio Grande River; the delta growing area roughly bordering the

Mississippi River at and near the juncture of the states of Louisiana, Arkansas and Mississippi; and to a lesser extent the additional cotton growing areas of the Cotton Belt from Mississippi further east through Alabama and Georgia to South and North Carolina.

The boll weevil has been a traditional cotton pest also in the Southeast and Mid-South sections of the Cotton Belt. For the past several years the USDA has been conducting a boll weevil eradication program that reports to be successful starting in North Carolina (Cousins, 1991). In the West, the boll weevil became a problem in the first half of the 1980s when infestations were noted in many cotton growing areas bordering the Colorado River. Eradication attempts started in 1985 and were successfully completed along the Colorado River including Mexico a few years later (Cousins, 1991).

Therefore, boll weevil is no longer a significant pest in cotton growing areas of the far western United States. The boll weevil is also reduced as a pest in the greater Rio Grande Valley of Texas where cultural control in the form of short-season strategy keeps the pest in check (Bottrell and Adkisson, 1977).

In South Carolina, the only recommended treatment for infestations of both the boll weevil and budworm in the same field was the (2:1) mixture of methyl parathion and EPN. This is due to the relative ineffectiveness of pyrethroid insecticides against boll weevil. Although installing a resistance management program aimed at boll weevil has crossed many minds in the past, one has never been developed. The boll weevil eradication program as conducted by the USDA is simplicity itself, extensive monitoring locates the weevil, then localized blanket spraying with malathion follows and this program is repeated at a low population trigger for spraying.

Malathion control of the boll weevil has not shown any tendency to decline in effectiveness. It is reported that, unlike the larval stages, the adult boll weevil lacks any mechanism with which it can develop resistance to organophosphorus insecticides (Brattsten, 1987a,b). Although there are few reports of boll weevil resistance, Teague *et al.* (1983) did report a 3- to 6- fold tolerance to azinphosmethyl (Guthion®) in a field strain obtained from the Lower Rio Grande Valley. The work was done in response to grower reports of problems controlling boll weevil.

In the Southeast, the ecology of cotton pests is quite different from the West, with smaller fields of non-irrigated cotton often surrounded by wooded areas or other crops such as soybean, corn or tobacco. Damage from bollworms and budworms is just as severe in the southeastern states of North and South Carolina and Florida as it is in Texas, Louisiana, Arkansas and Mississippi.

The tobacco budworm is generally more difficult to control in cotton than the bollworm. It is often the predominant species once insecticides have been applied, and it must be considered the more serious threat to the crop, i.e., the more endemic. The tobacco budworm was more resistant against 10 of 13 insecticides when tested on both species (Sparks, 1981). The three compounds against which tobacco budworm was more susceptible were permethrin (Ambush®, Pounce®), fenvalerate (Pydrin®) and carbaryl (Sevin®). However, these data were gathered soon after the introduction of pyrethroid insecticides, and the situation changed within a few years when tobacco budworm resistance to pyrethroid insecticides developed (Plapp *et al.*, 1990).

It is highly instructive that pyrethroid resistance did not develop in the bollworm when budworm resistance was documented. Although both of these major cotton pests are present in cotton, they are also pests of a range of other crops, e.g., bollworm on corn and tobacco budworm on tobacco. There is some speculation that host selection plays a role in this process, as the bollworm would tend to maintain a reservoir population of individuals susceptible to insecticides on untreated corn, its preferred host. The same speculation assumes that the tobacco budworm would remain on cotton and therefore under greater selection pressure.

A vital clue to the response by the tobacco budworm to insecticides comes from population studies. With a dividing line somewhere around New Mexico or West Texas, tobacco budworm populations in the United States are said to split into western and eastern prototypes (Sluss and Graham, 1979). This study was based on about 16 locations and may not have resolved other subpopulations which might be revealed by considering many more locations. This possibility might explain why the tobacco budworms are key pests east of this line and not in the Far West.

Identification of species is at the heart of both resistance management and insect pest management. Insecticide resistance spreads most rapidly in a fully interbreeding population. Substrains of populations might have the effect of delaying resistance by holding a critical mass of susceptible genes away from selective pressure in ordinary cropping cycles. It is possible that the bollworm is doing a similar thing by its host selection behavior.

Defining a possible subpopulation of a pest insect was shown to be vital in the study of another major new cotton pest, the older sweetpotato whitefly. After considerable study, this very old cotton pest was determined to be present in two forms, termed strains A and B. The name silverleaf whitefly was recently suggested for the B strain to show its uniqueness and virtual isolation from the original species (Perring *et al.*, 1993).

It is suspected that these two strains are reproductively isolated one from the other. There is continuing debate and disagreement over whether sweetpotato and silverleaf whiteflies are actually different species or not, but the debate merely underlines the critical importance of understanding the biology of pest populations, and the fact that insect populations are dynamic, ever changing, and unpredictable from year to year.

RESISTANCE MANAGEMENT TACTICS AND STRATEGIES

The genetic bases of most types of resistance have been determined. We know within a few genetic map units where the various factors for resistance map to specific loci on chromosomes (Plapp, 1976; Oppenorth, 1985). Although most of this information comes from house fly, *Musca domestica* L., whose major advantage is a short enough generation time to make inheritance studies practical, it is tacitly assumed that major resistance mechanisms in other insects have similar bases.

Recently, it was documented that repeated copies of a single gene (a process termed gene amplification) exist in resistant green peach aphids, *Myzus persicae* (Sulzer). While the details of how these repeated copies of the same gene might come about and

how they are activated is currently being studied, it is clear that the insect can synthesize large amounts of single resistance factors such as the esterase enzyme in this case (Devonshire and Field, 1991). The pertinent fact concerning this particular esterase resistance is that it confers cross resistance to pyrethroids, carbamates and organophosphorus insecticides.

While genetic knowledge of this kind has been useful in designing resistance management strategies (Denholm and Rowland, 1992), all tactics used in resistance management schemes are, of necessity, based on those parameters that are within the control of practitioners. Characteristics of the biology of pest insects, for example, that are not manipulatable by cultural or other control approaches must, of necessity, be ignored. What remains is often termed operational factors and these include selection of insecticides, timing and dosage of treatments, area treated and application method (Denholm and Rowland, 1992; Plapp, 1993).

One drawback of these tactics in resistance management is their implied emphasis on chemical control. The best way to manage resistance to insecticides, of course, is to reduce their use drastically and develop truly integrated pest management approaches. It is difficult at the best of times to develop an integrated insect pest management approach because IPM is considerably more difficult to achieve than chemical control. The cotton industry as a whole seems reluctant to adopt newer technologies.

THE AUSTRALIAN PYRETHROID STRATEGY

The most pertinent resistance management program to cotton production in the United States, aside from its own, was the one initiated by the Australian cotton growers in 1983 and was designed to prevent the spread of tolerance to pyrethroid insecticides by *Helicoverpa armigera*.

The Australian strategy (outlined in modified form below) was relatively simple. It was designed to restrict the use of pyrethroid insecticides to one generation of *Helicoverpa armigera* per season. Although the strategy was simple, adopting it was not. All growers of summer crops in a large area of Queensland and New South Wales had to be convinced to adopt the strategy. Because *Helicoverpa armigera* is a multihost pest, selective pressure had to be removed from all sources to be successful. In particular, sorghum growers enjoyed excellent success with a single treatment of a low dose of pyrethroid to control sorghum midge, and the pyrethroid strategy meant they would lose this tool in the middle of their season.

Australian Resistance Management Strategy (1983):

Stage I	Stage II	Stage III
(first spray to Jan. 9)	(Jan. 10 to Feb. 20)	(Feb. 21 to last spray)
endosulfan	endosulfan	**no endosulfan**
monocrotphos	BT/chlordimeform	methomyl
profenofos	profenofos	profenofos

no pyrethroids	methomyl	no pyrethroids
methomyl	pyrethroids	parathion
sulprofos	sulprofos	thiodicarb

In addition to the voluntary restriction in time of both pyrethroids and endosulfan, growers were urged to use no more than three pyrethroid sprays in mid-season during the allowed period. They were also urged to use at least three different groups of insecticides distinguished as having unique modes of action as shown below:

Group A: Endosulfan (Thiodan®) (a cyclodiene acting at the GABA synapse).
Group B Organophosphorus compounds (cholinesterase inhibitors) including sulprofos (Bolstar®), profenofos (Curacron®), acephate (Orthene®), parathion, and monocrotophos (Azodrin®).
Group C: Carbamate insecticides (cholinesterase inhibitors) including thiodicarb (Larvin®) and methomyl (Lannate®, Nudrin®).
Group D: Pyrethroids (acting on the sodium channel) including fenvalerate (Pydrin®), cypermethrin (Ammo®, Cymbush®) and deltamethrin (Decis®).
Group E: (miscellaneous) delta endotoxin of *Bacillus thuringiensis* (Berliner) and chlordimeform (Fundal®, Galecron®).

All results reported to date suggest that the Australian resistance management strategy designed to delay the development of resistance to pyrethroid insecticides has worked (Croft, 1990), despite some early skepticism (Davies, 1984). A five-year continuous survey of discriminating doses showed that resistance to pyrethroids built in mid-season when pyrethroid use was allowed, but the resistance then declined by the start of the subsequent growing season, although usually somewhat above the original level (Roush and Daly, 1990). This phenomenon has been referred to above as a "rachet up" effect and can be seen also in the first few years of monitoring data of cypermethrin resistance in tobacco budworm in the Mid-South and Texas (Mullins *et al.*, 1991) where it is termed a "stair step" annual increase (Rogers *et al.*, 1991).

The pattern of resistance build up and decline was first seen in the Australian situation because of a vigorous resistance monitoring program that was supported by the Australian cotton growers. Resistance monitoring has since become more widespread in cotton growing areas of the Mid-South of the United States and the same results seem to hold true (Clower *et al.*, 1992). Indeed, resistance is now suspected of occurring frequently during the cotton growing season (Rogers *et al.*, 1991).

THE ZIMBABWE RESISTANCE MANAGEMENT STRATEGY

The first nationwide resistance management program for cotton pests was developed in Zimbabwe while it was still Rhodesia in 1972-1973 (Duncombe, 1973). The Zimbabwe plan was devised due to dimethoate resistance that developed in carmine spider mites, *Tetranychus cinnabarinus* (Boisduval) and *Tetranychus lombardinii* Baker and Pritchard.

Critical to these events was a reliable resistance testing scheme which had been developed by 1968. When testing revealed mite resistance to monocrotophos (Azodrin®), one of the few remaining acaricidal compounds available, a rotation scheme was devised:

The Zimbabwe Scheme (Sawicki and Denholm, 1987):

(1) Formamidine and carbamate used for two seasons.
(2) Chlorfensulfide and chlorfenthol (Quibrom®, Dimite®) used for the next two seasons,
(3) Monocrotophos (Azodrin®) and triazophos (Hostathion®) used for the next two seasons; and
(4) Return to (1) above, and continue...

In addition to the rotation scheme shown above, endosulfan (Thiodan®) was recommended for bollworm control instead of DDT which was known to induce mite population flare-ups. Formamidines and carbamates were put into the strategy because they were shown to have increased efficacy on organophosphorus resistant mites in a valuable and fortuitous discovery of negatively correlated resistance development (Dittrich, 1969).

The Zimbabwe scheme was voluntary and achieved success over an extended period of time. Considerable care was taken to explain the program and enlist the support of the growers and agrochemical industry. Competition between agrochemical companies resulted in the country being divided into six, then later three regions so that all of the groups of recommended products were actually used in any given year. The regions were separated enough to ensure an interruption in the flow of resistant gene pools.

When chlordimeform (Fundal®, Galecron®), the formamidine used in the beginning of the strategy, came under regulatory scrutiny for adverse health effects, it was replaced by another formamidine, amitraz (Ovasyn®), with a similar mode of action and chemistry.

After the experience with spider mite resistance, Zimbabwe officials anticipated potential problems expected from the introduction of synthetic pyrethroids in 1977-1979. They directed that cotton growers use pyrethroids only during a defined period of not more than nine weeks that coincided with the maximum flowering period when most pest pressure from bollworms occurred (Blair, 1986). Three winter months were designated as pyrethroid free.

When the Australians decided to develop a pyrethroid resistance management strategy in 1983, they borrowed from the Zimbabwe experience. Indeed, one of the principle architects of the Zimbabwe scheme, John Brettell of the Cotton Research Institute at Kadoma, was invited to Australia to assist in the inauguration of the Australian strategy. Although he could not accept because the African growing season coincides with that in Australia, it turned out that the growers in Australia as a whole were far more amenable to the plan than the industry leaders realized and readily adopted it, as confirmed by its continued success (Croft, 1990).

PYRETHROID RESISTANCE IN TOBACCO BUDWORM IN THE UNITED STATES

Resistance to pyrethroids occurred in the United States in the tobacco budworm similar to the event in Australia and only a few years later. Like Australia, many United States growers had been using exclusively pyrethroids for pest control since their introduction to cotton pest control in the mid-1970s. For the first ten years of pyrethroid insecticide use in the United States, there was no attempt by growers to develop resistance management approaches despite very clear warnings about the consequences (Elliott *et al.*, 1978; Sparks, 1981).

Indeed, up until and even after the first reports of resistance to pyrethroids from the Winter Garden area of Texas, 100 miles west of the city of San Antonio, many refused to accept the reports and were openly skeptical (Staetz, 1985; Plapp *et al.*, 1990; Plapp, 1991). Nevertheless resistance was soon accepted by all concerned, and a resistance management scheme was initiated soon after.

The Tri-state Resistance Management Scheme—The elements of the Tri-state strategy (named for the regions represented by the framers in Louisiana, Arkansas and Mississippi) are fairly straight forward (Anonymous, 1986; Certain, 1988; see also Rogers *et al.*, 1991). The three elements are:

(1) Plant and protect early, harvest early;
(2) Use no pyrethroids until June 30; and,
(3) After July 1, use pyrethroids as necessary until August 15, although there are some local variations (Anonymous, 1990a).

This approach was designed to remove selective pressure by pyrethroids from the first generation of the tobacco budworm/bollworm complex. However, the plan also recommends using mixtures of insecticides, a recommendation that does not have universal acceptance (see section below on using insecticide mixtures).

The Tri-state strategy was adopted from Texas to Alabama with some regional modifications to the exact pyrethroid-free period. Also, the strategy was complicated by local boll weevil eradication procedures being conducted in Alabama, for example (Certain, 1988). One of the arguments used in favor of some self-regulation of pyrethroids was financial. Loss of the relatively inexpensive but effective pyrethroids through resistance would necessitate use of more expensive materials (Anonymous, 1990a). Thus, cultural practices that encourage earliness were stressed along with early harvest.

The adoption of resistance management strategies requires cooperation on a scale not ordinarily practiced in farming communities. One natural characteristic of farming communities is a friendly competition or rivalry between growers. Therefore, it is in a very real sense unnatural for growers to cooperate in an endeavor that involves the way each individual farms, in this case how each individual controls insects. Early indications suggest a less than uniform compliance to the Tri-state strategy on the part of the growers (Croft, 1990; Rogers *et al.*, 1991).

THE ENVIRONMENTAL MOVEMENT AND CONSEQUENCES

The key element that caused the Australians to cooperate and overcome natural competitive instincts was the specter of losing effective insecticides. The worldwide outcry against pesticides that began in 1964 and grew into what we refer to as the environmental movement has wrought much change. The most pertinent change was to add constantly changing layers of governmental regulation to the registration of pesticides with all costs passed on to agrochemical industry.

This has had the effect of temporarily reducing the number of new insecticides (Finney, 1991; Voss and Neumann, 1992). Because the costs are so much higher, searching for new materials is even more of a gamble than before (Voss and Neumann, 1992). As a result the agrochemical industry has undergone, and continues to undergo, a dramatic contraction. Shell agrichemicals in the United States was acquired by DuPont some years ago. Dow and Eli Lilly merged into DowElanco; Sandoz purchased Zoecon, and more recently Wellcome Environmental Health was purchased by Roussel Uclaf and FMC was acquired by Monsanto, to name a few mergers.

It has been projected that by the turn of the century there may be only five very large chemical firms left in the business of marketing pesticides. While growers may begin to see fewer familiar and traditional pesticides, the market for insect control agents has not changed that much yet. The cotton industry still accounts for the lion's share of pest control sales in the United States.

Agrochemical companies have been quietly investigating potential new products under the umbrella of "biopesticide." Biopesticides are said to include pheromones, attractants, microbials and some lists even include the neurotoxic pyrethroids (Simmonds *et al.*, 1992; Anonymous, 1990b; Voss and Neumann, 1992). The non-pyrethroid portion of the biopesticide market was recently projected to grow 11 percent through the year 2000, and to reach US$300 million in sales by 1999. A growth of 15 percent per year was also predicted for sales of bacterial-based pesticides in particular (Anonymous, 1990b), but Marrone and Macintosh (1992) put these at one percent of the world market.

Although this prediction appears rosy at first glance for non-neurotoxic insect control agents or chemicals, reality suggests something else. Perceived as replacements for the present range of neurotoxic carbamates, organophosphorus and pyrethroid insecticides (Hutchins and Gehring, 1993), the biologically based materials that act as growth regulators, behavior modifiers, or bacterial or viral toxins are considered (Voss and Neumann, 1992; Wood and Granados, 1991) "...unreliable, uneconomic, and of a very limited practical value."

The projected world sales of insecticide products is reportedly US$ 7 billion by 1995 (Voss and Neumann, 1992). The non-neurotoxic insecticide part of this is projected to be less than 10 percent. Representatives of agrochemical industry have been quietly pointing out these realities for some years, but the message does not seem to be getting through (Hutchins and Gehring, 1993).

THE MEASUREMENT OF INSECTICIDE TOXICITY

PROBIT ANALYSIS

When most parameters or physical traits are measured in a homogeneous population, the results, when plotted, form a bell-shaped curve, or Gaussian distribution. The measurement of toxicity of a given insecticide is no exception. Since toxicity of a given chemical is measured in populations rather than individuals, a special type of statistical procedure termed probit analysis is used.

Resistance determinations are basically comparisons. Some of our colleagues concern themselves with defining resistance (cited from Muggleton, 1984), which is fundamentally important. To have solid and useful information from field pest insects, one must have reference values to begin with, or a stable susceptible reference population. Although seemingly straight forward, a susceptible population can be rare, difficult to obtain or non-existent. This is especially true of pest insects that are not readily cultured, or new strains that become *de novo* (anew) pest insects.

It is generally appreciated (cited from Gould, 1984, 1991; Devonshire and Field, 1991; Ronis and Hodgson, 1989) that insects have been evolving defense mechanisms against plant toxins as long as both have been co-evolving. Most of these involve metabolic factors, but a host also undoubtedly involves feeding behaviors as well. So one may well wonder what susceptibility really is in the first place.

Probit analysis plots the mortality caused by insecticides in a population of insects against the logarithm of doses used. The probit technique changes the bell-shaped nature of the results into straight lines that are more convenient for analysis. Probit programs are now available that run on personal computers (Raymond, 1985).

The probit analysis of a given insecticide against a homogeneous population will yield a straight line. In the example shown here (Figure 1), the toxicity of fenvalerate (Pydrin®) to a susceptible population (S) is plotted alongside the toxicity to a field population of larval *Helicoverpa armigera* (Gunning *et al.*, 1984). If a portion of the population contains one or more resistance traits, the probit or ldp (log dose probit) line shifts to the right (as shown by the arrow in Figure 1). The non-homogeneity of the strain is indicated by the probit line no longer being straight.

If the field strain in Figure 1 is selected by treating several generations with a dose causing 70 percent mortality (the LD_{70}), then the population would become homogeneous for resistance, and the probit line would be straight, but shifted to the right (indicated by the dashed line labelled R in Figure 1). The log dose probit (ldp) lines of the S and R strains shown in Figure 1 are separated horizontally by about 100 dosage units at their mid points, so we consider the R strain to be 100-fold resistant compared to the susceptible S strain. Therefore, while resistance is developing, the probit lines reflect the change and the heterogeneity of the population by bending to the right at the top.

Note also that the LD_{50} (50 percent mortality) value of the field strain does not show the potential resistance fully. In the example shown, the LD_{50} values of the susceptible strain and field strain are less than 10 dose units apart.

Log probit data are most accurate near the 50 percent mortality points and increasingly less accurate at both lower and higher mortality points. This is the main reason why the LD_{50} value has become the standard measurement for toxicity. But this value only has meaning for homogeneous populations. In most cases, field populations are not homogeneous.

If one percent of a field population contains highly resistant individuals, a probit analysis will yield a line that is very similar to the susceptible line shown in Figure 1, with perhaps a few values far off the curve at the upper end depending on how many insects were tested. The important information about the few individuals that are resistant will almost certainly be lost, even if a very large number of insects is tested for toxicity. This is a limitation of the probit method, and reflects the difficulty of determining resistance.

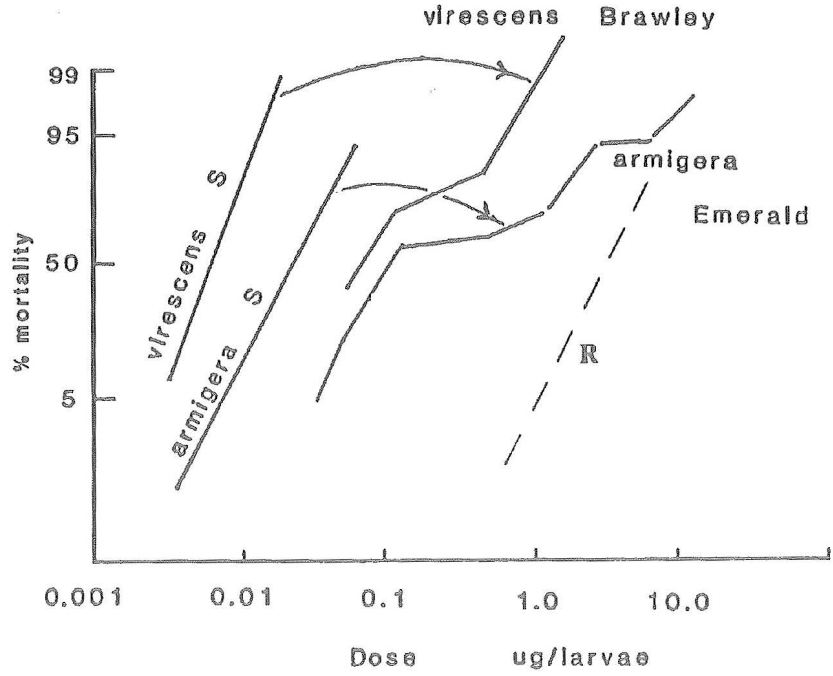

Figure 1. Toxicity of fenvalerate (Pydrin®) to third instar tobacco budworm from Brawley, California and bollworm from Emerald, Queensland, Australia. The log probit plots of susceptible (S) and field collected (lower arrow) *Heliothis armigera* were taken from Gunning et al. (1984). The dashed line (R) shows the result expected if this field population were pressured for several generations by fenvalerate until homogeneous for resistance. Also shown are probit data from susceptible (S) *Heliothis virescens* (tobacco budworm) and from a field strain (upper arrow) collected near Brawley, California in 1984. (Tom Miller, unpublished data.)

To help overcome some of the limitations in log dose probit analysis, a discriminating dose can be used as a diagnostic tool. If the population labelled S in Figure 1 were treated with twice the dose needed to produce 95 percent mortality (two times the LD_{95}), there should be no survivors. If this same discriminating dose were used to treat the field strain, nearly half the population would survive the dose. It can be seen that this is a practical way of rapidly estimating resistance in the field.

To generate the probit line, one needs at least four doses plus a control and at least one replicate. At 20 insects per dose, this amounts to a minimum of 200 insects. One can appreciate that the discriminating dose technique is considerably simpler to perform. However, large samples are still required to document the low percentages of resistant individuals in some populations (Roush and Miller, 1986). Of course, to obtain more detailed information, the probit method must be used, but it is no surprise that the Australians, and later, the Americans, adopted the discriminating dose method in their resistance monitoring programs.

QUASI-SYNERGISM AND PHYSICAL ARTIFACTS

Aside from the drawbacks of probit analysis, there are other complications that are important to keep in mind when trying to determine toxicity. In a classic paper that often escapes notice, Sun and Johnson (1972) documented an artifact in the determination of toxicity of carbaryl (Sevin®) to house flies. When topically applied in acetone, carbaryl gave a toxicity of 900 micrograms per fly. However, when formulated in kerosene and reapplied in exactly the same way, the toxicity was 1.1 micrograms per fly. Sun and Johnson termed this phenomenon "quasi-synergism" because it appeared as though carbaryl toxicity had somehow "improved."

In fact, some insecticides, when applied in acetone, have a physical habit of crystallizing on the cuticle, and thus being unavailable for penetration. This reduces the toxicity of topically applied compounds by an amount that is directly attributable to how much material precipitated on the surface. In some cases this is not significant (Schouest *et al.*, 1983), but in others it is important. Since no insecticides are formulated in acetone, this problem rarely occurs in field applications; instead, it is almost always a possible artifact in the laboratory, where the use of acetone is common.

Probably Sun and Johnson (1972) were experiencing carbaryl precipitation on the surface of the house flies. Whatever the cause, from the time quasi-synergism was discovered, Shell Development Company, where the work was done, switched from routine use of acetone in testing to the use of kerosene. Insecticides such as organophosphorus insecticides, or any other materials that are oily at room temperature, would naturally not have these peculiar physical properties. However, the concept of quasi-synergism is always important to remember when assessing insecticide toxicity.

TYPE OF RESISTANCE

Four major factors responsible for resistance are listed below. They are considered to be the main means by which pest insects develop tolerance to insecticides, and they

can be measured with ease or difficulty. To understand the nature of resistance monitoring, and the strategies behind resistance management, one must be familiar with these factors:

(1) Behavior;
(2) Penetration;
(3) Altered site of action (kdr, AChE); and,
(4) Metabolism (oxidase, esterase, hydrolase, transferase...).

Each of these types of resistance has been demonstrated or measured. For practical purposes, most of these factors of resistance can be considered traits that are genetically inherited. It is popular to consider the development of resistance as an example of Darwinian evolution in action, i.e., survival of the fittest in the face of selection by insecticides.

BEHAVIORAL RESISTANCE

Behavioral resistance means the pests have inherited a behavior pattern that somehow causes them to avoid a toxic dose (possibly by staying on a part of the plant that is protected from exposure to chemical sprays, for example). This type of resistance is the most difficult to measure. Many reports of behavioral resistance are anecdotal observations. Groups of pest insects are seen to be residing on different parts of the plant, such as whiteflies occupying the lower third of the mature cotton plant in late season (Personal communication, N.C. Toscano, University of California-Riverside) or horn fly residing on an untreated part of the steer (Lockwood *et al.*, 1985).

When taken in to the laboratory, it can be appreciated that these insects would test as susceptible by accepted toxicological testing procedures using topical applications of precise amounts. All too often the bioassays are designed for the convenience of the experimenter and miss the more subtle or esoteric forms of resistance. Another view of these groups of insects is that they can represent a pool of susceptibility for diluting the other forms of resistance, assuming they are not already cross-resistant themselves.

Some insects evolve behavioral avoidance of antibiotic crop cultivars which may be an important principle in developing insect resistant varieties (Gould, 1984). Lockwood *et al.* (1984) described behavioral resistance as either stimulus-dependent or stimulus-independent, but they also defined protective avoidance as distinct from behavioral resistance. They gave a number of examples.

Gould (1991) considered that either insecticides with repellent properties or insecticides used with insect repellents can significantly decrease the rate of development of resistance. The entire field of the behavioral response of insect to selective pressure is a much neglected field of study (Lockwood *et al.*, 1984).

PENETRATION RESISTANCE

Penetration usually is not a big factor in resistance and is more readily measured. In some cases insecticides simply do not penetrate inside the resistant insects as rapidly as in a comparison (susceptible) strain. The most convenient way to determine this

information is to measure the rate of uptake of radiolabelled insecticide from topical treatment (Sawicki and Lord, 1970). This can be done by topically applying the labelled insecticide to both susceptible and suspected resistance individuals, then washing the cuticle with solvent after a short wait and comparing amounts of unchanged insecticide (Nicholson and Miller, 1985).

To split hairs, this procedure actually measures what is left on the surface of the insect, not how much actually penetrated and became available as primary toxicant inside. However, determining the latter requires a more extensive toxicological research project. Given the dwindling support for insect toxicology, the effort required may not be justified.

ALTERED SITE OF ACTION RESISTANCE

An altered site of action means the site where the insecticide exerts its primary toxic action is somehow genetically altered so that greater amounts of the insecticide are now needed to produce the same effect as previously.

The term "kdr" means "knock down resistance" to pyrethroid insecticides. Insects with kdr-resistance either do not respond at all to a dose that normally kills the susceptible strain, or the symptoms of poisoning take far longer to appear than in the susceptible strain. In this case the first symptoms of poisoning are termed knockdown. In the case of DDT and pyrethroids, the presence of a kdr-like resistance mechanism normally requires sophisticated electrophysiological equipment for final confirmation. However, kdr-like resistance can be measured by simpler methods as long as complicating factors such as penetration do not interfere with the interpretation.

The kdr-resistance mechanism was originally demonstrated by Busvine (1951) for DDT. Table 3 shows the very simple results reported by Busvine from three house fly, *Musca domestica* L., strains, one susceptible, one with kdr-like genes and a third strain with largely metabolic based resistant genes against DDT. Note that the kdr-like factor was expressed very fast, within minutes of treatment, by a lack of response compared to the susceptible or metabolic resistant strains when all strains were treated by the same dose.

Table 3. Percent of adult female house flies knocked down after exposure to DDT residues (0.1 mg/cm^2) in a 500 ml beaker. (After Busvine, 1951.)

Knockdown time	Strains		
	Rome susceptible	Italian resistant	Sardinian resistant
	(%)	(%)	(%)
Down in 20 minutes	35	0	27
Down in 40 minutes	93	11	80
Down after 24 hours	100	8	11

The hallmark of metabolic resistance is eventual recovery many hours following poisoning. As seen from the information in Table 3, it can be appreciated that the initial responses to poisoning of the susceptible strain and the strain containing metabolic resistance are similar, and major differences express themselves only many hours later.

It was shown that the kdr-like factor is expressed throughout the nervous system. Motor nerve terminals of larval house flies with the kdr-like gene expressed a resistance to pyrethroids (Salgado *et al.*, 1983a,b). The central nervous system also expressed a resistance to pyrethroids in the same strain of house flies with the kdr-like gene (Miller *et al.*, 1979).

Although the symptoms of insecticide poisoning normally express themselves within the first 30 minutes of topical treatment, the ultimate toxicity depends on what happens many hours later. This principle of toxicology is best illustrated by considering unpublished results from Dr. Harry von Keyserlingk of Schering AG in Berlin (Figure 2).

Deltamethrin (Decis®), considered the most toxic of all the pyrethroid insecticides, knocked down adult house flies minutes after topical application (Figure 2). Over a period of seven days, however, the number of insects remaining down began to decrease until about 80 percent fully recovered. If, on the other hand, the deltamethrin dose was delivered along with a nontoxic amount of the synergist, piperonyl butoxide, which blocks oxidative metabolism, the adults never recovered during the following week.

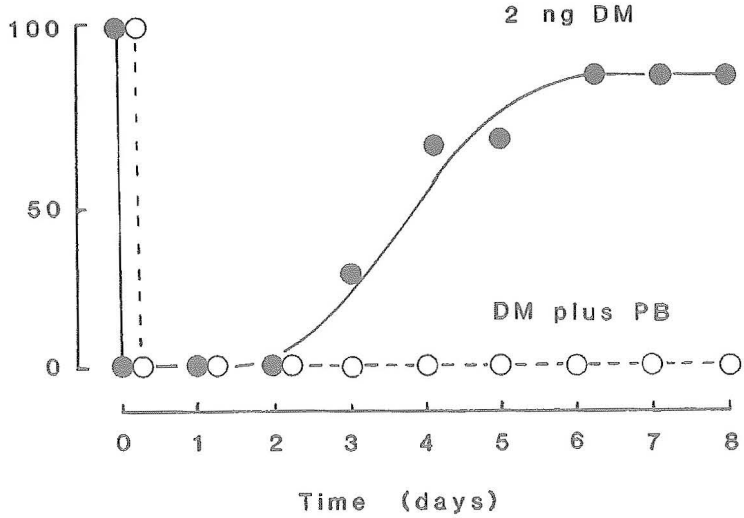

Figure 2. Recovery of adult house flies topically treated with 2 ng DM [deltamethrin (Decis®)] (From Dr. Harry von Keyserlingk, Schering, AG, Berlin). Note recovery took longer than two days to begin even though toxicity is normally determined after 24 hours. In presence of the synergist, piperonyl butoxide (PB), the adults never recovered from the same dose of deltamethrin.

This simple experimental result from Schering shows a powerful concept in a simple example. First, pyrethroids do not "kill" insects. Put another way, insects can survive massive chemical insult to their nervous systems. Secondly, given time, the metabolic machinery of insects can reduce the concentration of active ingredients in the hemolymph (blood-like circulatory fluid in insects) to below toxic levels allowing the insect to recover. Many investigators measure toxicity 24–72 hours after treatment, and ignore longer term recovery.

The important lesson to learn from Figure 2 is that the main metabolic component in insecticide poisoning takes a long time, many hours or even days to fully express itself. It never happens immediately. As shown by Busvine (1951), however, the presence of a kdr-like resistance factor can be tested for in minutes. This fact was the principle upon which the "warm-needle" bioassay for kdr-like resistance was developed. The warm-needle assay was perfected by Jeff Bloomquist (Bloomquist and Miller, 1985, 1986). Figure 3 shows results of this procedure applied to larval house flies.

Following topical treatment, a group of maggots were "probed" at regular intervals. Those failing to respond were scored as paralyzed and the percent paralyzed was recorded over time. Within an hour following topical treatment, larvae with kdr-like genes were easily distinguished from susceptible insects. The amount of kdr-like gene expression was also readily apparent (Figure 3). In the example shown, the resistant

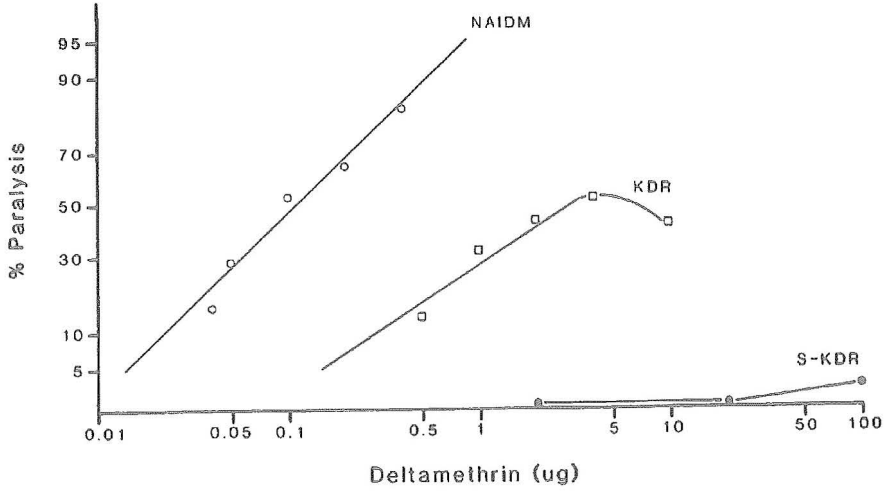

Figure 3. Dose response of deltamethrin (Decis®) topically treated on third instar house fly larvae from three different strains (From Bloomquist and Miller, 1986). Paralysis was determined ten minutes after treatment. The strains are susceptible (NIADM), and 100-fold resistant (S-KDR) and 10-fold resistant (KDR) both of which were selected for single gene kdr-resistance.

strains were 10-fold and 100-fold resistant to deltamethrin (Decis®), respectively, compared to the susceptible strain based on the ratios of the LD_{50} values.

The warm-needle assay was adopted for studies on pink bollworm, and perfected for the horn fly (Crosby *et al.*, 1991). The same principle can be used to demonstrate a kdr-like gene or effect in any insect. Thus, as originally demonstrated by Busvine (1951), the presence of a kdr-like factor can be determined in minutes in any convenient assay. This is essentially what is being done in the vial assay as it was adapted to resistance monitoring of the whitefly (Staetz *et al.*, 1992). In this vial assay, adults are held only a few hours before toxicity is determined. As can be seen from the arguments above, kdr-like resistance would be readily apparent in this assay, but metabolic resistance would not.

Altered site of action resistance to organophosphorus or carbamate insecticides involves an alteration in cholinesterase (ChE), the target enzyme. This normally requires laboratory analysis by biochemical means for final confirmation. In the tobacco budworm, a single gene was shown to be responsible for methyl paraoxon resistant acetylcholinesterase (Brown and Bryson, 1992).

Since the altered cholinesterase factor would be expressed as a general lack of response to cholinesterase inhibitors, it would respond in rapid assay in the first several minutes of treatment in a manner similar to pyrethroid resistant insects with kdr-like mechanisms. While this should be straight-forward to demonstrate with carbamate insecticides, there are technical reasons why it may be more complicated with organophosphorus insecticides (Miller, 1976).

METABOLIC RESISTANCE FACTORS

The final mechanism of resistance, metabolic, is by far the most common and the most complex. Insecticides are basically chemical molecules made up of so-called functional groups. Since most insecticides have a carbon skeleton, nature has a myriad of ways to alter, digest and break apart such structures, usually by enzymatic means. The only exception is the carbon-chlorine or carbon-halide bond which is rare in nature and consequently difficult to reduce. This is the main reason DDT was found to be unacceptable for widespread use; it resisted degradation and eventually accumulated in non- target organisms to unacceptable levels.

It has become popular to point out that insects have been co-evolving for many years with plants. Plants have evolved a spectrum of natural toxins as insect deterrents to which some insects have promptly developed resistance or immunity. The classic case is the tobacco plant with nicotine (an insecticide) and the tobacco budworm. This means that even before insecticides are introduced into crop protection, there are metabolic mechanisms in place that are designed to protect against poisoning.

It is also popular to point out that polyphagous insects (insects that feed on many kinds of food) are more capable of resisting plant toxins presumably because their metabolic machinery is more adaptable. The struggle of coevolution is seen to occur in the larval stages which are largely confined to the locality of their oviposition (egg laying) site. The adult stages not only do not have the same adaptability of metabolic

factors, their diet is considerably simpler. Thus larval stages that are treated, in general, have the greater ability to respond by developing resistance to insecticides (Ronis and Hodgson, 1989; Gould, 1991).

Three main metabolic factors conferring resistance are: (a) mixed function oxidases, (b) glutathione S-transferases, and (c) hydrolases (including esterases) (Devonshire and Field, 1991). All three mechanisms have been demonstrated or are suspected to be present in cotton pest insects (Little *et al.*, 1989; Nicholson and Miller, 1985; Byrne *et al.*, 1992).

Besides insects evolving metabolic and other mechanisms to deal with the natural toxins in plants, the toxins in plants themselves are of great interest. Almost all insecticide products have some remote connection to a natural plant toxin. This even includes the organophosphorus insecticides (Neumann and Peter, 1987). Thus the plants have had to deal with insecticide resistance before through evolution, and perhaps some of their adaptive countertactics might be of some interest in the present context.

SYMBIONT METABOLISM OF INSECTICIDES

A complicating factor in the metabolism of insecticides is the possible activity of symbiotic organisms. Shen and Dowd (1991) reported the presence of esterase enzyme activity in cultures of the yeast-like organism, *Symbiotaphrina kochii* Jurzitza ex. W. Gams and v. Arx., which enjoys a symbiotic relationship with the cigarette beetle, *Lasioderma serricorne* (Fabricius). It was suggested that such symbiotic organisms are able to detoxify a wide range of pesticides, mycotoxins and plant toxins (Shen and Dowd, 1991).

INDUCTION OF METABOLIC ENZYME ACTIVITY

Enzyme induction is a well established phenomenon (Hodgson and Levi, 1987) in which exposure to xenobiotics (foreign chemicals) has the effect of increasing production of certain enzymes to assist in the degradation of the chemical. Diets have been shown to have the same effect. Some of the esterase activity in insects has been shown to be inducible (Yu and Hsu, 1985).

When reared on cotton (Delta Pine 61), corn (Golden Jubilee), chrysanthemum (Florida Marble) or artificial diet (see Shorey and Hale, 1965), tobacco budworm larvae showed little difference in the bands of esteratic activity on gel electrophoresis of hemolymph. Reared on the same host plants, however, analysis of the enzyme activity of the hemolymph of the bollworm showed a greater diet-dependence (Salama *et al.*, 1992).

The bollworm had ten bands of carboxyesterase activity and thirteen bands of cholinesterase activity; whereas, the budworm had eight bands and two bands, respectively, of the same types of enzyme activity. It was concluded that the bollworm larvae with a more diet-dependent esterase activity may have evolved more closely with its preferred host plant while the enzyme complement of the budworm is more stable on different hosts (Salama *et al.*, 1992; Brattsen, 1987a,b).

The toxicity of insecticides on bollworm and tobacco budworm larvae is known to be affected by the diet upon which the larvae are reared. Undoubtedly, enzyme induction in

response to plant chemicals present in specific host plants plays a role in this response to insecticides. Comparing the toxicities of insecticides on the same insect species that originate from different host plants should take these principles into consideration. Bioassay of adult insects alone would certainly miss these subtleties in larval stages.

TYPES OF INSECTICIDE

To understand the development of resistance, the chemistry of insecticide molecules and the exact constituents of insecticides as they are formulated are very important. The Australian resistance management strategy not only restricted the use of pyrethroid insecticides, it restricted the use of endosulfan (Thiodan®) in an effort to preserve this material as well.

The major categories of insecticides are given below to show the common site and mode of action.

DDT and pyrethroid category: DDT, fenvalerate (Pydrin®), permethrin (Ambush®, Pounce®), deltamethrin (Decis®), cypermethrin (Ammo®, Cymbush®), cyhalothrin (Karate®), cyfluthrin (Baythroid®), bifenthrin (Capture®), tefluthrin (Force®), and etofenprox (Trebon®). DDT is sometimes listed as a chlorinated hydrocarbon and incorrectly lumped together with the cyclodienes and lindane (Isotox®). DDT acts at the same site as pyrethroid insecticides. When kdr-resistance was encountered, the original diagnostic test for it was cross-resistance had to be present to all of the other members of this class, i.e., all pyrethroids and DDT.

Cyclodiene category: dieldrin, aldrin, chlordane, heptachlor, endosulfan, lindane, and toxaphene. The cyclodienes include a distinct class of chemicals named after the principle route used in their synthesis, the Diels-Alder reaction. These compounds are now suspected of acting on the chloride ion channel of the GABA synapse. The GABA synapse is named for the neurotransmitter, *gamma* amino butyric acid, that is released at its ending. Endosulfan (Thiodan®) is one of the few compounds of this category remaining in registration for crop protection, and therefore, by virtue of its different mode of action from other major categories of insecticides, is one of the most valuable. The other members of this class were added (lindane, toxaphene, and more recently bicyclophosphates) when their mode of action was discovered.

A nerve cell connects to (or synapses with) other nerve cells (nerve-nerve synapses), muscle cells (neuromuscular synapses), or directly to tissue organs. All nerve cells have neurotransmitter or neuromodulator chemicals that they manufacture and release at their synaptic connections. The release normally occurs when nervous impulses are conducted along the nerve cell axon to its nerve endings or synapses. The nerve cells are normally named by their neurotransmitter chemicals. Thus a nerve that makes and releases *gamma* amino butyric acid (GAB) is a GABA neuron.

When GABA is released at a synapse, it diffuses to the cell downstream (the postsynaptic cell) and excites the postsynaptic membrane, usually after being recognized by a "GABA receptor." Once activated, the GABA receptor in turn causes a brief (milliseconds long) increase in permeability to a specific ion, in this case chloride.

Chloride permeability increases tend to stabilize the postsynaptic cell, or inhibit it from any further activity. Thus GABA neutrons in the central nervous system of insects play a major role in inhibiting other kinds of nervous activity.

Cyclodiene insecticides specifically block the chloride permeability at the postsynaptic membrane of GABA synapses. This leads to an interruption in the inhibitory message and the postsynaptic cells can no longer be inactivated. This is thought to lead to convulsions as motor programs (discrete patterns of nervous activity driving behavior) turn on indiscriminately.

Insects that develop site-insensitive resistance to cyclodiene insecticides are described diagnostically by their cross-resistance to picrotoxinin, a toxic natural product that was used for years to distinguish GABA synaptic transmission. Indeed, all chemical insecticides in this greater cyclodiene category owe their activity to a structural and functional resemblance to picrotoxinin at the site of toxic action in the nervous system. Picrotoxinin acts by selectively and reversibly blocking the chloride ion channel on the postsynaptic membrane of the GABA synapse.

Avermectin category: avermectin. Avermectin is a natural product synthesized by the soil fungus, *Streptomyces avermitilus*. Its structure is so complex that chemical synthesis is impractical. Instead, the product is developed through fermentation tanks and marketed as both a veterinary medicine and an agricultural insecticide product. The outstanding feature of avermectin is that its action on the nervous system seems to be counteracted by picrotoxin. Thus while the cyclodiene insecticides are thought to act by blocking the chloride ion channel at the GABA synapse, avermectin derived products are thought to be active by virtue of increasing the permeability of the chloride channel.

Because avermectin is a natural product that has the unusual property of killing internal parasites in vertebrate animals without harming the host, it is a valuable veterinary product. Being a natural product with a complex structure has hindered development of analogs to avermectin. As a result, the primary manufacturer, Merck Co., Inc., has enjoyed exclusive access to a unique market.

Carbamate category: Carbamate insecticides are considered to be inhibitors of acetylcholinesterase enzyme at cholinergic synapses in the central nervous system of insects. Carbamates were derived from the natural product, physostigmine. The inhibition by carbamates is largely due to a reversible complex formation with the enzyme. Once the enzyme is carbamylated by the insecticide, the carbamate group is hydrolyzed off of the enzyme readily with a half-life of about 25 minutes. This means that poisoning by carbamates is readily reversible, one of the characteristics of carbamate action (Miller, 1976).

Reversibility of carbamylated cholinesterase enzyme, the target of these insecticides, puts carbamates into a different category from organophosphorus (OP) insecticides. The organophosphates act by inhibiting the same cholinesterase enzyme attacked by carbamates, but the half-life of the phosphorylated enzyme is days rather than minutes. Thus the organophosphates insecticides are considered to act longer as insecticides and poisoning symptoms are irreversible, for all practical purposes.

Aryl carbamates category: carbaryl (Sevin®), propoxur (Baygon®), carbosulfan (Advantage®).

Oxime carbamates category: aldicarb (Temik®), methomyl (Lannate®, Nudrin®), oxamyl (Vydate®), thiodicarb (Larvin®).

Members of the oxime carbamate family of insecticides, especially aldicarb, have a unique property in that they are often systemic and are readily taken up and transported in plant tissues where they are effective in controlling plant pests with sucking mouthparts. This sometimes leads to special handling and residue problems and must be treated with caution.

Organophosphorus category: These insecticides are divided into resistance management classes based on the functional groups that are bonded to the phosphorus atom. Once thought to have no equivalent natural toxin in nature, Neumann and Peter (1987) recently reported the isolation and identification of a heterocyclic phosphate from *Streptomyces antibioticus* DSM 1951, that had potent anticholinesterase activity and was equal in insecticidal activity to monocrotophos (Azodrin®).

In general P=S compounds (phosphothionates) require activation to be insecticidal and this occurs rapidly in insects. P=O compounds do not require metabolic activation. Metabolic resistance would be expected to be dependant, in part, on the other groups attached to the phosphorus atom. Because of the potency of their action on cholinesterase, and the difficulty of reactivation of the phosphorylated enzyme, and because of the great amount of structure and activity work done on organophosphorus insecticides, this category is the largest and most diverse group of insecticides (Voss and Neumann, 1992).

Phosphates: monocrotophos (Azodrin®), dicrotophos (Bidrin®). Both of these simple dimethylphosphates have alkyl leaving groups.

Dimethylphosphorothioates: methyl parathion and fenitrothion (Folithion®, Nonathion®) both have aryl leaving groups.

Dimethylphosphorodithioates: azinphosmethyl (Guthion®) and chlorpyrifos (Lorsban®) have an aromatic leaving group and malathion and methidathion (Supracide®) both have alkyl leaving groups.

Diethylphosphorothioates: parathion has an aryl leaving group.

Diethylphosphordithioates: disulfoton (Disyston®) which is a systemic, has a thioalkyl leaving group.

Phosphorodithioate: sulprofos (Bolstar®) with an aryl leaving group has an unusual O-ethyl, S-propyl substitution.

Phosphorothioate: profenofos (Curacron®) is closely related to sulprofos (Bolstar®) with the same O-ethly, S-propyl substitution, but is a P=O compound rather than a P=S.

Phosphonates: EPN is an unusual phenylphosphonothioate with the phenyl group bonded directly to the phosphorus atom, which is unique among the organophosphorus insecticides.

Chlordimeform type: chlordimeform (Galecron®, Fundal®), amitraz (Ovasyn®) and diafenthiuron. These "insecticides" and acaricides have distinct ovicidal activity.

Although chlordimeform registration has been withdrawn, it provided a unique type of control activity in cotton pest control. Known as formamidines in general structure, these compounds have little or no overt topical toxicity. They are widely known to interact with the octopamine receptor in the nervous system, and therefore have a completely unique mode of action, and indeed have a very distinctive structural similarity to octopamine itself. They were suspected of retarding the development of resistance when used with other acutely toxic insecticides, and to have a strongly synergistic effect (Liu and Plapp, 1992).

Diafenthiuron is a new type of octopamine mimic (Kadir and Knowles, 1991). This compound has not been studied fully, nor developed yet, but it is reported to have activities unlike all other insecticides and acaricides except chlordimeform. Since difenthiuron is broken down by oxidation of the thiourea moiety to urea, the parent compound can be thought of as a propesticide. The urea breakdown product appears to have the greater biological activity (Kadir and Knowles, 1991).

Nicotinic type: nicotine, cartap (Caldan®, Sanvex®) and imidacloprid (Confidor®, Gaucho®). The chemical structure of these compounds is based on a natural toxin extracted from marine worms. Cartap is said to interact with the nicotinic acetylcholine receptor at cholinergic synapses in the insect central nervous system. There is a general similarity between the mode of action of cartap and that of nicotine, but little structural similarity between them.

The relatively new compound, imidacloprid (Admire®, Confidor®, Gaucho®) (BAY NTN-33893) is derived from nitromethylene compounds first discovered by Shell Development Company some years ago. Originally, development was delayed due to an instability of the chemicals that appeared to be an inherent property of the chemical structure of the active compounds. The nitromethylenes are also active at the nicotinic cholinergic receptor, and so this category rightly is called a nicotinic type. Nicotinic agents all should inhibit the binding of the specific and highly potent cholinergic ligand, *alpha*-bungarotoxin (Sattelle *et al.*, 1989).

Benzoylurea type. These compounds have undergone considerable development in the past few years and continue to be of interest. They are not neurotoxins. Rather they are considered to be growth regulators with the ability to interrupt development. As a result they are relatively slow acting. Despite this, their efficacy has improved so much in the past few years through structure and activity studies, that they rival the most potent neurotoxic insecticides in field efficacy.

Miscellaneous: B.t., *Bacillus thuringiensis* (Berliner). This bacterium produces an endotoxin protein that when ingested selectively disrupts the midgut of certain chewing insects, especially lepidopterous larvae. Although B.t. has been used in crop protection for many years, more recent advances in B.t. technology have improved the strains and pest control products. B.t. is an ideal component in an IPM scheme because, being selective on chewing insects, it is considered completely safe to beneficial insects.

The B.t. endotoxin gene has been bioengineered into cotton plants and insect resistant transgenic cotton are now undergoing field development (Fox, 1992; Ferro, 1993;

Benedict *et al.*, 1992; Jenkins *et al.*, 1993). Commercial availability on a limited basis is expected for the 1996 growing season.

Resistance to the B.t. endotoxin has already been demonstrated in the diamondback moth, *Plutella xylostella* (Linnaeus) (Tabashnik *et al.*, 1990, 1991). The question of the development of resistance in leaf chewing cotton pests to the transgenic plants in commercial development is now being debated (Fischhoff, 1992; Fox, 1992; Ferro, 1993; Marrone and MacIntosh, 1992). On one hand, the expression of the toxin throughout the plant suggests one hundred percent selection pressure, or close to ideal for resistance development which Ferro (1993) predicts will take as few as four generations. The National Audubon Society considers B.t. a valuable resource and is highly concerned that it might be squandered if vigorous attention is not given now to resistance management (Fox, 1992).

The levels and expressions in the transgenic plants are amenable to manipulation and possibly more than one factor may be engineered into the plant to retard the development of resistance more or less in analogy to the use of rotation or mixtures of ordinary insecticides (Fischhoff, 1992). Although some of the transgenic cotton cultivars are spectacular in their protection against chewing insects in the field, at least one recent report (Benedict *et al.*, 1992) concluded that a low expression of the endotoxin gene conferred little or no protection compared to control plants.

RESISTANCE MONITORING

Here at last is one area that appears to evoke uniform agreement in the field of insecticide resistance. Everyone agrees that monitoring of resistance is needed (Roush and Miller, 1986; Sawicki, 1987; Riley, 1989). Although there may be some minor disagreements on the details, no one can argue with the spectacular data generated by resistance monitoring of pink bollworm in California, tobacco budworm in Texas and the Mid-South, bollworm in Australia, and green peach aphid in England. For the very first time it has been possible to get good information about the resistance of populations in single fields or in localities.

Preliminary results suggest that resistances to both pink bollworm and tobacco budworm in the United States might even be highly localized. This information is particularly important because the original expectation was that one grower generating a resistant population in one field would cause general problems in a local area.

LESSONS FROM BIOASSAY COMPARISONS

Given that resistance monitoring is widely accepted, one of the first lessons to learn about the subject of insecticide resistance is the folly of relying entirely on one resistance monitoring method. One example of this is in the outstanding work conducted by Tim Dennehy on spider mite resistance in the San Joaquin Valley of California. This work epitomizes what can happen with incomplete testing, and shows the difficulty of distinguishing resistance fact from artifact concerning any particular product.

Reports of failures of dicofol (Kelthane®) to control spider mites of the *Tetranychus* genus, prompted Dennehy, Granett and Leigh (1983) to investigate. They first repeated the standard laboratory test for acaricide efficacy, the well known slide-dip test. The slide-dip test is essentially a topical toxicity assay since mites are dipped in dicofol directly. They obtained a resistance ratio for dicofol of 5.7 comparing field strains of twospotted spider mite, *Tetranychus urticae* Koch, with laboratory susceptible strains.

They then employed a less accepted residue test whereby leaf discs are dipped in dicofol and mites are confined to the treated surface. This residue test gave results that were completely different and showed a 544-fold toxicity difference between susceptible spider mites and field collected mites (Figure 4).

Thus, the Dennehy *et al.* work (Figure 4) shows that reliance on one method, even though widely accepted, may yield misleading results. The other lesson to learn from this classic study is that on close examination, all resistance and field control problems were with the twospotted spider mite. The strawberry spider mite, *Tetranychus turkestani* Ugarov & Nikolski that occupied the same cotton niche was controlled with dicofol (Kelthane®) and showed no resistance.

Schreiber and Knowles (1991) also compared topical toxicity with vial bioassay on the bollworm. They found that the adult vial assay gave results that were similar to adult topical tests, but larval vial assay results were significantly different from larval topical toxicity.

Misleading results with topical assays using standard toxicological testing protocols are not new. Arthur and Zettler (1991) found that topical methods did not accurately reflect malathion resistance frequencies in the red flour beetle, *Tribolium castaneum* (Herbst). Roush and Luttrell (1989) reported that topical bioassays did not accurately detect resistance in the tobacco budworm.

The dichotomy between results of topical bioassay versus residue treatments have also been reflected in improved control by space sprays for house flies compared to residual treatments using a variety of insecticides (Taylor, 1982). These and other examples show the inappropriateness of extrapolating laboratory test results to field situations.

Reliance on a single biochemical test for insecticide resistance is cautioned as being myopic (lacking in foresight) since continuous use of one insecticide may result in the selection of additional mechanisms (Sawicki, 1987). Biochemical tests are sophisticated in that they can often give precise quantitative information on specific metabolic enzymes that play a role in insecticide detoxification such as esterase, or carboxylesterase tests (Devonshire *et al.*, 1986; Hemingway *et al.*, 1986) or cholinesterase tests (Voss, 1980).

Biochemical tests, by their nature, normally are restrictive in what they reveal and cannot substitute for topical or other tests of overall toxicity of insecticides to insects.

RESISTANCE MONITORING METHODS

Attracticide Assay Method — A novel resistance monitoring method was created and perfected for pink bollworm. This method, termed the "attracticide resistance monitoring method", employs Delta traps baited with pheromone gossyplure, that are ordinarily used for assessment of populations of male adult pink bollworms.

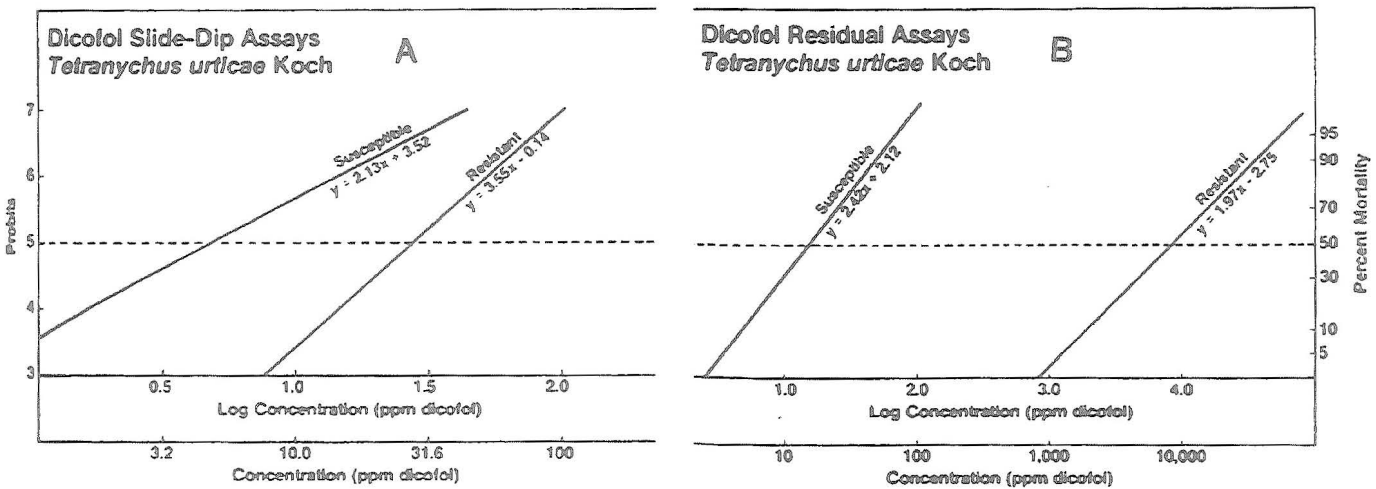

Figure 4. Probit data of dicofol (Kelthane®) on twospotted spider mites collected from cotton fields in central California (From Dennehy *et al.,* 1983). The slide-dip assays (A) and the residual assays (B) gave very different results. The LD_{50} values (horizontal dashed line) between laboratory (susceptible) and field collected (resistant) strains assayed by the residual assay were almost three orders of magnitude different indicating huge resistance to dicofol residues. The topical assay showed a much smaller difference.

To monitor resistance of adult pink bollworm, the Delta trap was purchased without sticky adhesive. Cards trimmed to fit in the bottom of the Delta trap were smeared with a mixture of sticky material (Tangle-Trap®, Tanglefoot Co., Grand Rapids, Michigan) and an insecticide to be tested. Each card had a different concentration of insecticide and a series of three to five concentrations was prepared besides a control without insecticide. The series of doses were replicated for each insecticide at least once.

Modified Delta traps with dosed cards were placed in cotton fields overnight. The traps were collected in the morning before sunrise. The cards with their trapped adult male pink bollworms were removed and stored at room temperature (70F). After two days the number of dead moths and the total number per card were determined. These data together with the mortality of controls for each insecticide were analyzed by probit analysis (See earlier section in this chapter for discussion of probit analysis) program (Raymond, 1986).

Protocols for conducting attracticide tests in the field were described in two papers (Haynes *et al.*, 1986, 1987). It was observed that control mortality was reduced if sticky cards were scraped before use to eliminate blobs of stickum. The attracticide method has been used for pink bollworm resistance monitoring programs in Arizona, Texas, Mexico and China as well as in California.

Data from the laboratory of Dr. Wen-gu Li in Shanghai, China shows the mortality of adult male pink bollworm over time on sticky cards (Figure 5). Similar data on the treatment of third instar tobacco budworm larvae by cypermethrin (Ammo®, Cymbush®) gave remarkably similar results (Firko and Wolfenbarger, 1991) (Figure 6). These results demonstrate clearly the need for a specific incubation period following dosing in order for reliable toxicity values to be obtained. In this case, two days are needed before toxicity data become stable. All studies using bioassay of insecticides require calibration charts such as that of Dr. Li for each species and insecticide category tested.

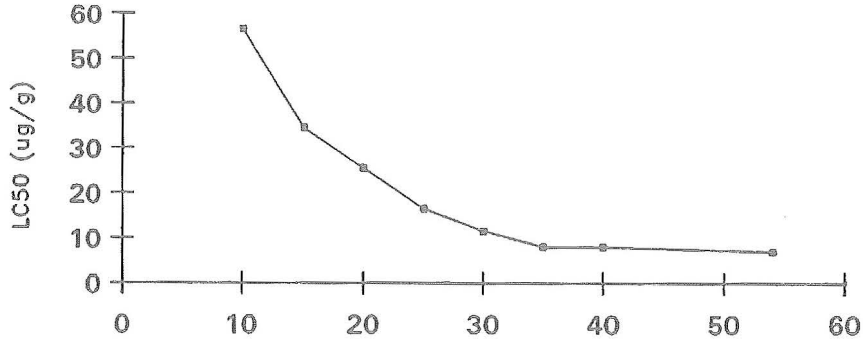

Figure 5. Change in toxicity following attracticide dosing of adult male pink bollworm by fenvalerate (Pydrin®). (From Dr. Wen-gu Li, Shanghai Institute of Entomology, unpublished data, 1991.)

The attracticide method or modifications have been adapted to monitor resistance in codling moth, *Cydia pomonella* (Linnaeus), citrus thrips, leaf miner, whitefly, oriental fruit moth, *Grapholita molesta* (Busck), german cockroach, *Blattella germanica* (Linnaeus) and peach twig borer. Major advantages of the use of insecticide and stickum mixtures are that any formulated insecticide may be used and the mixtures survive cold storage well.

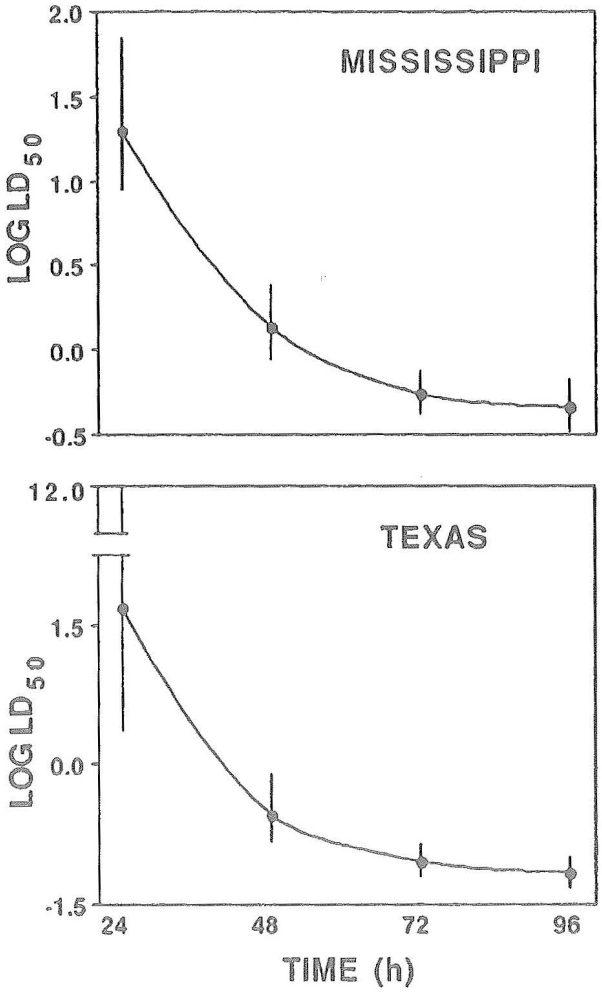

Figure 6. Estimated LD_{50}s (with 95% CI) based on mortality observations 24, 48, 72 and 96 hours after treatment with cypermethrin (Ammo®, Cymbush®) of third instar tobacco budworm larvae from Mississippi and Texas. (From Firko and Wolfenbarger, 1991.)

Vial Residue Assay Method — An alternative method for resistance monitoring was perfected by Plapp (see Plapp *et al.*, 1990; Kanga and Plapp, 1992, and Kanga *et al.*, 1993 for materials and methods). A given amount of insecticide dissolved in solvent is placed in a glass vial (20 ml scintillation vials are perfect for this). The solvent evaporates as the vial is rotated mechanically. When dry, a uniform coating of the insecticide is left on the inside of the vial. Live adult or larval insects are placed in the vial and kept at room temperature usually for 24 hours, before mortality is determined.

Although the vial assay was originally designed for use with tobacco budworm adults as part of a field monitoring program, it is suitable also for testing discriminating doses on adult pink bollworm. The pink bollworm is not nearly so sensitive to temperature in the vial assay as in the attracticide assay method (Schouest and Miller, 1988).

In addition, the vial assay has been adopted for resistance monitoring of the whitefly (Staetz *et al.*, 1992) with one very important modification. It is conducted for only three hours instead of 24 hours as used for pink bollworm and tobacco budworm. The shorter time is needed because there is significant mortality of adult whitefly when held longer than six hours (Figure 7). The immediacy of the whitefly resistance problem is such that the vial assay was adopted quickly despite the obvious drawbacks of assessing mortality after such a short time. This would not reflect fully the metabolic component as demonstrated by Busvine's (1951) results (Table 3) and the von Keyserlingk deltamethrin results (Figure 2). Strictly speaking, the short assay period would make the whitefly results a knockdown assay, not a toxicity or mortality assay, and should be reported as such to avoid confusion.

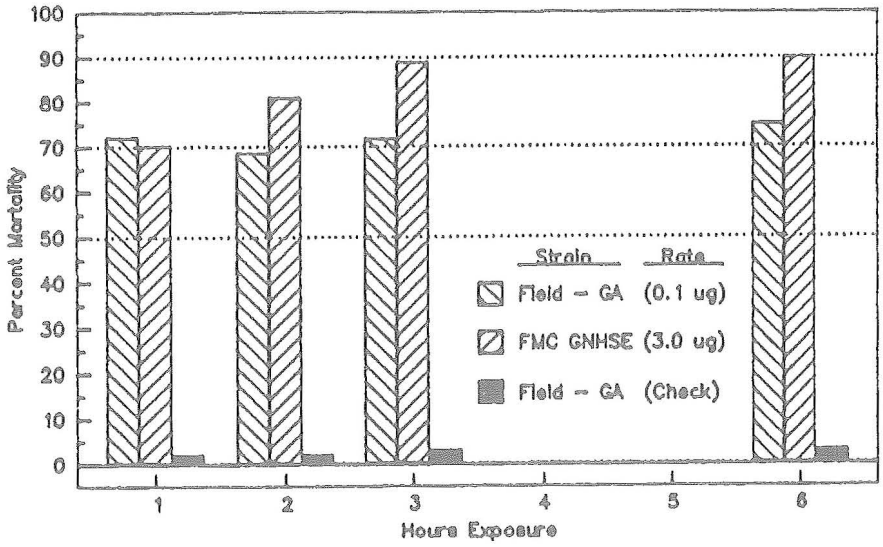

Figure 7. Bifenthrin (Capture®) knockdown of adult whitefly over a six hour period following exposure to treated glass vials. (From Staetz *et al.*, 1992.)

Originally the vial assay was used to monitor only cypermethrin toxicity. Although cypermethrin was selected for a number of sound reasons, no other pyrethroids were monitored. Other categories of carbamate and organophosphorus insecticides were not stable enough on the glass surface to withstand storage or shipment. One way around this would be to make up the vials immediately before use (Personal communication, D. A. Wolfenbarger, USDA, ARS, Weslaco, TX).

Recently, it was learned that organophosphorus insecticides can be adopted for use in the vial assay if care it taken to ensure the stability of the chemical on the glass surface (Kanga *et al*, 1992). If the glass vials are treated with benzoic acid, the insecticide film (residue) deposited on the glass vial becomes far more stable (Figure 8). Still, it

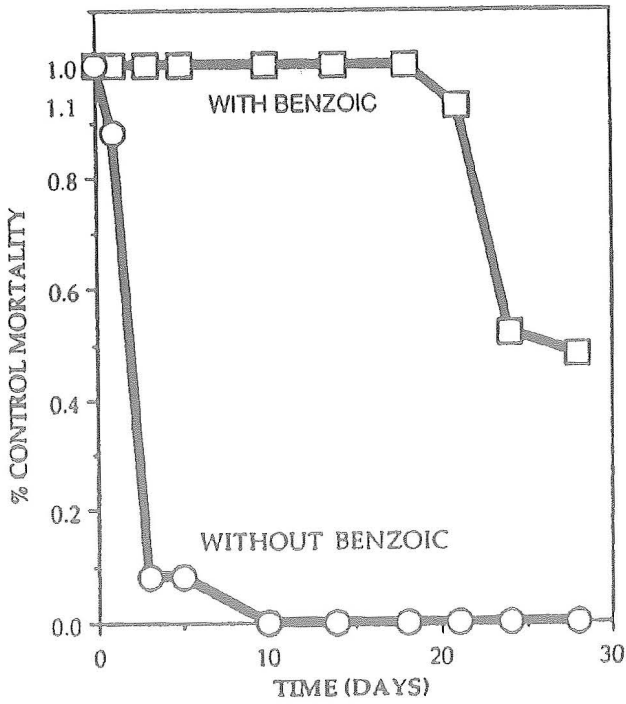

Figure 8. Toxicity of profenofos (Curacron®) to adult house flies confined to glass vials treated with 3 *mg*/vial with or without benzoic acid to stabilize the organophosphate (profenofos). (From Kanga *et al.*, 1993). Note that without the benzoic acid, the toxicity of profenofos drops off rapidly starting immediately after the vials are coated; whereas, with benzoic acid the vials remain effective for bioassay testing for over two weeks.

is a good practice to use organophosphorus insecticide-treated vials quickly, and to be aware of possible degradation upon storage.

FIELD INCUBATION

One of the best methods of saving time during resistance monitoring was to employ on-site incubation. We learned early on that carrying insects from the field to constant temperature chambers for incubation was awkward and time consuming. Yet insects had to be held at constant temperature to insure accurate data and to keep control mortality down to acceptable levels.

Control experiments showed that a hole in the ground maintained a constant temperature sufficient for incubation of field-collected insects (Figure 9). The depth of the hole had to be at least six inches (15.2 cm), but was very stable and convenient at a depth of 28 to 39 inches (70 to 100cm) (Schouest and Miller, 1991).

Data from pink bollworm adults held in the ground in vials, or stuck on attracticide cards was very similar to insects held in environmental chambers with the temperature constantly controlled. This meant that resistance tests could be conducted all on site in rural areas and eliminated the need to carry insects from field collection sites into a laboratory or other special facility.

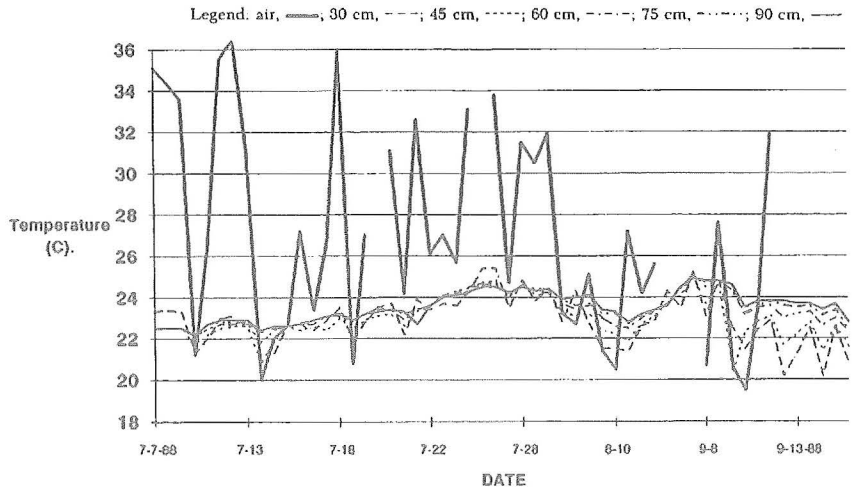

Figure 9. Air temperatures recorded at various depths in a one-meter hole in the ground from July to mid-September at Riverside, California. (From Schouest and Miller, 1991.)

DEVELOPMENT OF RESISTANCE

Resistance management strategies depend on factors that influence the development of insecticide resistance. The list of these conditions is in Table 4 and is modified from Sparks *et al.* (1985).

Insects that just begin to develop resistance show poor viability compared to susceptible strains. Kelly and Watson (1987) confirmed this for laboratory-pressured tobacco budworm. Fitt (1984) reported extensive growth and survival data from strains of *Helicoverpa armigera* that showed this trend. Recently Plapp and his coworkers obtained similar data for the pyrethroid resistance strains of tobacco budworm collected from cotton fields (Table 5).

Table 4. Conditions conducive to rapid development of resistance. (From Sparks *et al.*, 1985.)

1. Prolonged exposure to a single insecticide.
2. Every generation of the insect treated (selected).
3. Selection pressure high (high doses).
4. No insects escape treatment.
5. Large geographic area treated.
6. Selection occurs prior to mating.
7. Insecticide related to one used earlier.
8. Treatment triggered by low numbers of pest insects.
9. Insecticide inherently irritating and/or repellent.
10. No gene flow between insect populations (no migration between populations).
11. Pest insects monophagous (feed mainly on one kind of plant).
12. Short generation time (short life cycle).
13. Numerous offspring per generation.
14. Insects highly mobile.
15. Insecticide has long residue life.

Table 5. Growth, development and reproductive data for susceptible and resistant tobacco budworm males and females. (From Campanhola *et al.*, 1991.)

Characteristics	Susceptible strain		Resistant strain	
	? (male)	/ (female)	? (male)	/ (female)
Mean pupal developmental period, days	15.1	13.5	14.9	13.3
Mean pupal weight, milligrams	324.1	318.8	328.6	315.4
Mortality at pupal stage, percent	1.8	2.4	1.9	2.1
Mean hatching to adult development period, days	31.3	29.8	32.81	31.0[1]

Mean fecundity, number of eggs per female	—	2,552.8	—	1,270.0[1]
Mean fertility (number of females producing eggs), percent	—	93.5	—	62.5[1]
Mean hatchability (hatched eggs), percent	—	74.8	—	71.5
Mean adult longevity, days	21.2	17.4[2]	23.5	14.2[2]
Intrinsic rate of increase, r[3]	—	0.12	—	0.10

[1]Significantly different from the susceptible strain ($P<0.05$; t test).

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

[3]$r = (\log_e R_0)/T$, where R_0 is the net replacement rate (number of daughters/female) and T is the mean generation time.

Note: For information on number of individuals tested and statistical confidence limits of results, reader is referred to the paper cited above.

Fitness data are not spectacular because the lack of viability in insects that are just in the process of developing resistance is sometimes a matter of degrees and changes are subtle. Plapp and his co-workers show, for example, that resistant budworm larvae grow more slowly and weigh slightly less than susceptible strains. Adults of resistant strains are less responsive to pheromone than adults of susceptible stains. Females of resistant strains produce less pheromone, lay fewer eggs and have a slightly higher mortality than susceptible strains.

Taken individually, these parameters of growth, development, fecundity and reproduction are not impressive. In fact, some are barely discernable by good statistical comparisons. However, when taken together, they represent a distinct advantage for the susceptible populations providing there is not a continuing selection pressure from the continued use of the same insecticide. If spraying continues, then the selection process continues, swinging the chances of survival decidedly back in favor of the slightly less viable resistant strains.

Muggleton (1984) termed these processes "selective disadvantages," and a few studies have given them quantitative values from 34-56 percent. He concluded that resistance genes have appeared and disappeared spontaneously in all insects and have been doing so all along, certainly prior to the introduction of insecticides.

A recent genetic study of resistance confirmed these trends of fitness disadvantage in the development of resistance (Clarke and McKenzie, 1987). The important feature described by the latter study, however, is that once resistance is selected for several generations, the resistance remains and viability returns. Empirical results confirm this. This explains part of the "rachet up" effect mentioned above which leads to gradually increasing tolerance (the "creep" up of resistance) as insecticide selective pressure is maintained year after year.

Although laboratory selection is said to produce polygenic resistance (Roush and McKenzie, 1987), laboratory colonies under selection sometimes develop an initial resistance followed by reversion to susceptibility for one or two generations before resistance develops (Brown, 1981). Reversion in the field can have two causes: (a) fitness disadvantages to the resistant individuals or (b) dilution of the resistance frequency by susceptible migrants (Personal Communication, R. T. Roush, Cornell University, Ithaca, NY), or reversion of the resistant trait.

Results of laboratory selection may be misleading if inbreeding depression is mistaken for lack of viability (Roush, 1986). Selection in the field will cause inbreeding depression as well if the numbers of insects remaining in any general location is kept low by constant insecticide treatments. Indeed, this is one possible explanation of "selective disadvantage," or reversion.

To understand Condition No. 7 in Table 4, "Insecticides related to one used earlier", one must realize that some similarity exists between the mode of action of pyrethroids and DDT. Both of these categories of insecticides are thought to poison insects by acting on nerve membranes. Although the nature of the interaction may vary with each different chemical in this class, the lethal property of these compounds appears to be their ability to render the nerve membranes permeable to sodium ions over a long period of time. The dissertation work of Vincent Salgado (Salgado *et al.*, 1983a,b) made it clear that neuromuscular blockage could be produced by a prolonged membrane depolarization of only a few millivolts caused by a prolonged increase in sodium permeability.

DDT and the pyrethroids share another property that sets them apart as a class of insecticide. Many members of this group have a negative temperature coefficient of toxicity with some important exceptions. This means that DDT and some pyrethroids are more toxic at lower temperatures and less toxic at higher temperatures. The relationship between temperature and toxicity is a continuous one meaning there is no specific temperature above which DDT is non-toxic.

Furthermore, each insect pest has a different temperature-toxicity relationship with members of this class, and very few of these relationships have been measured and studied. Permethrin (Ambush®, Pounce®), for example, is known to be 10 times less toxic to tobacco budworm larvae at 86F than at 52F (Sparks *et al.*, 1982, 1983; Toth and Sparks, 1988), but fenvalerate (Pydrin®) is equally toxic at the same two temperatures. Because these temperature relationships vary from one species to another, laboratory data may not be a good indicator of field efficacy. Therefore, it is important that field rates are determined empirically.

The pyrethroids and DDT share another property. Both can induce kdr- resistance (knock down resistance) in insects. If kdr-resistance were previously induced by DDT use at some point during the past 40 years, and if some of the genes responsible were still present, then it will be somewhat easier for insects to adapt to pyrethroid sprays by developing kdr-resistance. There is evidence that the budworm resistance to pyrethroids discovered in 1985 includes a kdr-like component (Sparks *et al.*, 1989).

Plans for the management of resistance must take into account the 15 factors listed in Table 4 and also the practices that exist for controlling insects in cotton. As an exam-

ple, the biology of the pink bollworm is described below in relation to the list of factors affecting the development of resistance.

RESISTANCE DEVELOPMENT IN PINK BOLLWORM

Ironically, the current best method of controlling the pink bollworm is use of cultural practices (Bottrell and Adkisson, 1977). A simple early harvest strategy has been shown repeatedly to deny the pink bollworm the time necessary to produce a diapause or overwintering generation. Despite this knowledge and proven strategy, the cotton growers in southern California and Arizona have stubbornly refused to use the short season strategy—at least until 1989, when the Imperial Valley growers finally began to use the strategy.

The pink bollworm, a microlepidopteran, is a selective and endemic infestor of cotton in a circumscribed growing region. Therefore, its presence is much more predictable than other cotton pests. The insistence of the growers in southern desert valleys of the United States on using chemical control has guaranteed the presence and pest status of the pink bollworm for an extended period. This made it the subject of a valuable case study of the onset of resistance to the newly introduced pyrethroid insecticides starting about 1980.

There were several advantages to this study of the pink bollworm over studies of most other cotton pests, except mites and aphids. The pink bollworm was predictable. Its yearly appearance was regular and populations were very large. The pink bollworm pheromone had been described years before and use of pheromone traps was routine which helped to describe and define the population fluxes.

The attracticide monitoring method was created early on. This meant that actual probit values for toxicity could be obtained, instead of the incomplete discriminating dose data that was the hallmark of resistance monitoring of the bollworm and tobacco budworm problems in the Mid-South and Australia. In addition, all insecticide categories could be tested. Resistance monitoring was aimed at adults, the same stage treated commercially. Larval stages were not under selective pressure since they were inaccessible to treatments inside the cotton bolls.

The pink bollworm is a moth in the family Gelechiidae that, in addition to cotton, attacks other plants in the malvaceous group including *Hibiscus* sp. and okra. While they can be found rarely on *Hibiscus* sp., they may be considered essentially monophagous (Condition No. 11 in Table 4) on cotton in the desert valleys of the southwestern United States.

The pink bollworm can have five generations in one year, especially in hot desert conditions (Anonymous, 1984; Noble, 1969; Graham, 1980). Except for early and late season when migration is more likely to occur (Stern, 1979), pink bollworm usually remain in a cotton crop once hostable fruiting bodies or flowers are present. The adults fly a short distance from any given field and thus do not strictly satisfy Condition No. 10, (Table 4) "no migration between populations". However, the total mixing within a field population is relatively low, thus encouraging the development of resistance (Condition No. 10, Table 4).

Yield Loss Due to the Pink Bollworm — Insecticide treatment for pink bollworm can start in June and continue until September. Estimates of the average seasonal cost for chemical control vary between one and three hundred dollars per acre assuming an individual treatment to cost $10 - $15 per acre. Average yield losses to pink bollworm in the Imperial Valley of California for 5-year periods between 1961-1985 are shown in Table 6. After the arrival of the pink bollworm in 1965-1966, the average yield dropped dramatically by more than one bale per acre.

Similarly, the costs for controlling the pink bollworm and pests that arise as a direct result of chemical treatments for pink bollworm were given by Burrows *et al.* (1982). These show a dramatic increase and have remained unacceptably high compared to 1966 and previous years. Unusually high costs for 1977 were due to storms that created a one time climate for explosive insect growth and an inability to get into the field for control measures.

Table 6. Average yield and value of cotton produced in the Imperial Valley of California before and after the arrival of the pink bollworm. (From unpublished data, R. T. Staten, USDA, APHIS Methods Development, Phoenix, AZ.)

Years[1]	Average yield	Average value[2]
	(bales/acre)	($)
1961-65	3.43	1029
1966-70	2.25	675
1971-75	2.13	639
1976-80	2.23	669
1981-85	2.53	760

[1]The pink bollworm was established by 1966.
[2]Based on $0.60 per pound of lint.

Control Methods for Pink Bollworm — Despite the clear success of the technology, there has been spotty acceptance by cotton pruodcers in the use of pheromones—the so-called mating confusion technique—to control the pink bollworm. This method is selective, does not affect other insects, especially beneficials, and fits ideally into a pest management program.

The newer Mitsubishi Rope pheromone technique for pink bollworm control has been studied. Results show that pheromone technology must be applied with care, but can greatly reduce pink bollworm populations when treatments are conducted in large blocks with area cooperation (Natwick and Staten, 1986; Staten, 1987).

The more traditional method of controlling pink bollworm with chemical insecticides is shown in Table 7. This example is at one extreme in that it relies on 12 treatments of one product, the pyrethroid insecticide, Pydrin® (fenvalerate). However, it does illustrate how one can come close to satisfying many of the conditions for rapid development of resistance under existing pest control practices in the desert growing regions.

The field represented in Table 7 has exposed pink bollworm to a single insecticide (Condition No. 1, Table 4). Nearly every generation was selected (Condition No. 2). The commercial dosage (high dose) was used presumably (Condition No. 3). The entire population in this field was treated (Condition No. 4). Treatment was delivered, at least part of the time, before mating (partial Condition No. 6). The pyrethroids are similar in their mode of action to DDT, therefore Condition No. 7 (Table 4) was satisfied. Treatment was probably based on pheromone trap catches thus satisfying Condition No. 8. The pyrethroid insecticides are known to be irritable to most insects pests (Condition No. 9). The commercial compound used, Pydrin® (fenvalerate), is photostable and has a residue life of at least several days in the field, therefore Condition No. 15 was considered satisfied.

The pink bollworm tends to be locally infesting insect in the middle part of the season (Condition No. 10). The pink bollworm, being monophagous on cotton, satisfy Condition No. 11. Five generations per season partially satisfies Condition No. 12. Each female can deposit at least 200 eggs, satisfying Condition No. 13. The adult pink bollworm are highly mobile, satisfying Condition No. 14.

Thus, out of the 15 conditions (Table 4) that are conducive to rapid development of resistance, the field discussed above met, at least in part, 14 of them. Condition No. 5 was the

Table 7. An example of chemical use on a cotton field (144 acres) in the Imperial Valley of California in 1984.

Date		Materials
April	22	Azodrin® + Fertilizer
May	5	Kelthane® + Fertilizer
June	19	Orthene® + Fertilizer + PIX®
	30	Orthene® + Fertilizer + Supracide®
July	6	Guthion® + COTE®
	13	Pydrin®
	18	Pydrin® + Galecron® + COTE®
	21	Pydrin® + COTE®
	23	Pydrin®
	29	Pydrin® + Galecron® + COTE®
August	4	Pydrin®
	8	DEF® + Isobac®
	11	Pydrin®
	16	Pydrin® + Comite®
	22	Pydrin® + PIX®
	28	Pydrin® + Galecron® + COTE®
September	2	Pydrin® + Comite® + COTE®
	10	Pydrin®
	19	Bolstar® + Galecron®

only one that was not met. A treatment schedule as presented in Table 7, is an example of one most likely to produce resistance. Obviously, in a growing area as large as the California Imperial Valley, normal pest control practices will vary from one farm to another. Reliance almost entirely on chemical control has led to extremely high costs (Table 8). The cost of resistance and cost to the environment are not included in the calculation.

It has been learned from resistance monitoring that, especially with pink bollworm, resistance likelihood increases for every year the same plot of ground is planted back to cotton. This is true because pink bollworm overwinters in the same field it infests if it is allowed to diapause in the fall (if cotton in infested areas is allowed to grow past September).

Table 8. Cost for control of Imperial Valley pink bollworm pest complex, 1966 to 1980. (From Burrows *et al.*, 1982.)

Year	Total costs	Total cost/acre	Percent of crop value
	($	($)	(%)
1966	4,219,339	120.33	8.04
1967	5,751,033	168.26	9.99
1968	9,247,736	248.36	12.37
1969	7,250,476	167.06	15.74
1970	22,895,979	651.10	56.64
1971	18,489,124	592.60	51.10
1972	10,853,798	332.94	23.09
1973	13,485,458	363.00	21.17
1974	28,365,110	326.04	26.29
1975	12,517,718	291.11	27.78
1976	10,303,364	153.78	14.15
1977	67,251,863	487.33	79.59
1978	10,060,773	150.16	12.26
1979	15,046,694	156.74	13.27
1980	18,058,080	205.21	16.48

Results of Resistance Monitoring of Pink Bollworm — All results from the first two years of resistance monitoring on pink bollworm confirm what one would expect

from the analysis given above. In fact, one growing area in the Imperial Valley (the area around Westmoreland, Figure 10) appeared from the monitoring data to have sat-

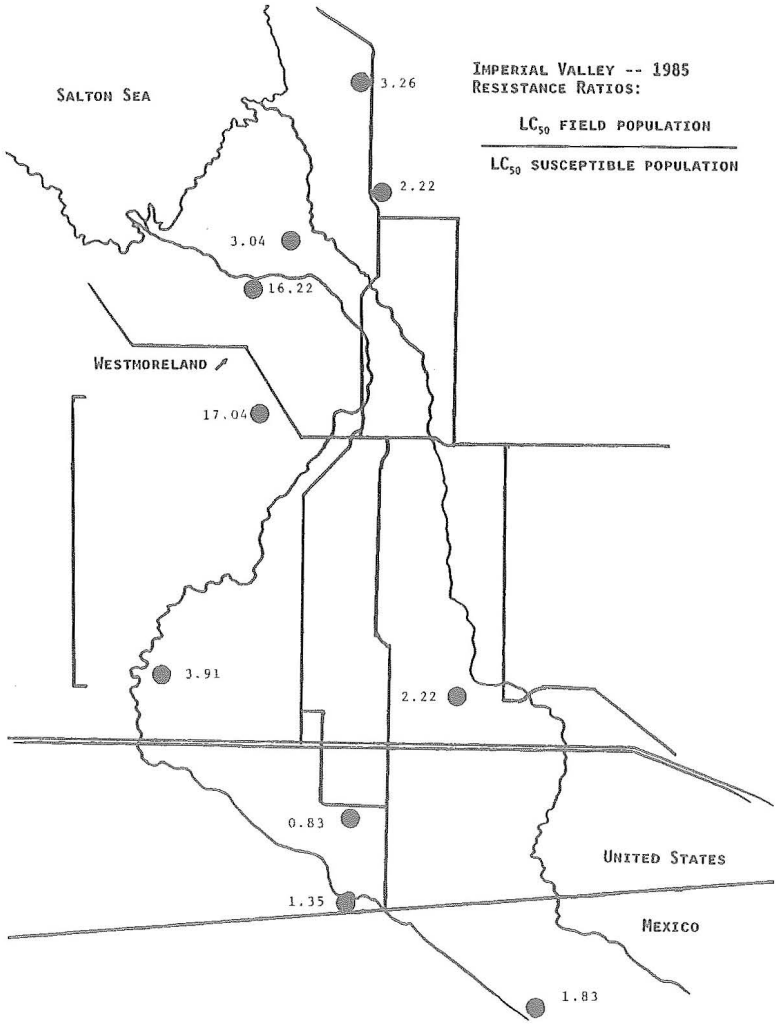

Figure 10. Resistance ratios of fenvalerate (Pydrin®) toxicity measured in the cotton fields indicated on this map of the Imperial Valley, California. The Salton Sea is in the upper left hand corner and the United States-Mexican border is shown by a dot-dashed line. The vertical calibration mark on the left indicates 15 miles or about 24 kilometers. (Tom Miller, unpublished data.)

isfied conditions for development of resistance in 1985 and 1986. The Westmoreland area was known to contain a number of growers who traditionally relied heavily on insecticide spraying, especially pyrethroids, for protecting cotton and they had been complaining of reduced efficacy for some time.

Correlation Between Pink Bollworm Resistance and Insecticide Use — The nature of pink bollworm infestation and the resistance monitoring technique allow plotting the resistance of a specific population of pink bollworm against a given insecticide versus a measure of use of the same insecticide to control the same population of pink bollworms in a given field. Such a plotting was done and is shown as Figure 11. The data show clearly that the more a pyrethroid such as fenvalerate (Pydrin®) was used, the greater the resistance became.

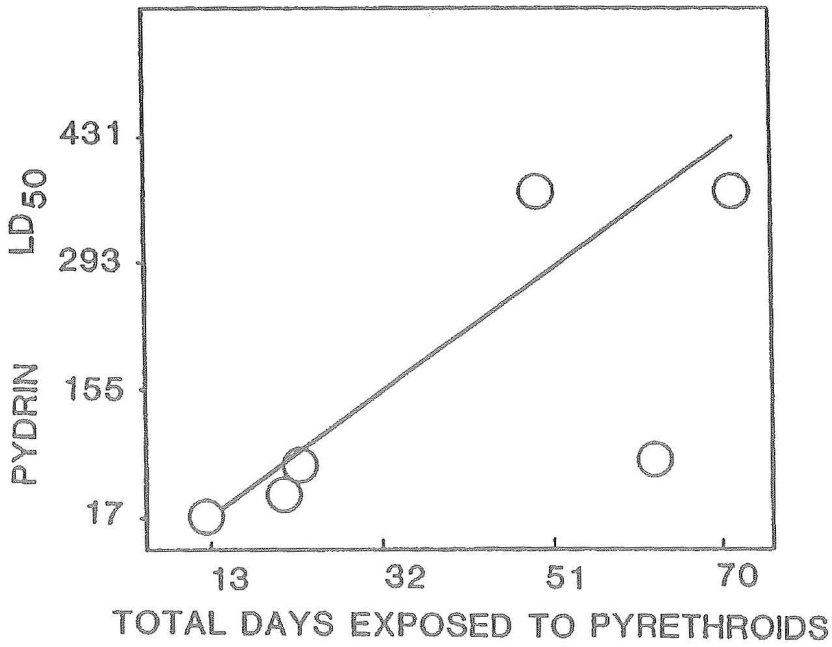

Figure 11. Toxicity of fenvalerate (Pydrin®) to adult pink bollworms plotted against the total number of days Pydrin® was used in a given cotton field. (Tom Miller, unpublished data). All of the data were taken from populations in cotton fields. The cotton fields are shown on the map in Figure 10. As a rule, the longer the population was treated with fenvalerate, the greater the resistance with one exception (the point in the lower right corner). This was from the field with a resistance ratio of 3.91 (on the left side of the map in Figure 10) which was close enough to Mexico to allow an influx of susceptible pink bollworms to dilute the expected resistance.

The Figure 11 plot also shows that resistance was not a discrete increase. Instead, the populations represented showed a gradual increase or "creep" upwards towards greater tolerance in direct proportion to the amount of insecticide used. There is one significant exception, the point in the lower right hand corner of the plot. It represents a cotton field about five miles from the Mexican border. Obviously, the resistance in that field was not proportional to the use of pyrethroid insecticide. In fact, all values of pyrethroid resistance, regardless of its use in controlling pink bollworm, were low near the Mexican border.

We suspected this has happened because pink bollworms tended to bleed across the border at a low but steady extent from Mexico to cotton fields in the United States. Since the Mexican cotton fields were not treated with pyrethroid insecticides at the time these studies were done, they would seem to have served as a source of susceptible pink bollworms to dilute the developing tolerance on the American side of the border.

Mathematicians call this a "boundary effect." As long as conditions of no pyrethroids used in Mexico and extensive use in the American cotton fields are maintained, groups of susceptible populations would be maintained only in American fields close enough to be influenced by the influx of populations from Mexico. The resistance map of Figure 10 gives a fascinating glimpse of how far from the border this influx of susceptible populations penetrated the native pink bollworm populations. Apparently 15 miles was sufficient to negate the effect since resistance was chronically building around Westmoreland.

RESISTANCE DEVELOPMENT IN TOBACCO BUDWORM

Larval stages of insects may have a greater variety of resources to call upon with which to develop resistance compared to adults of the same species because of differences in metabolic requirements in digesting plant material (Brattsten, 1987a,b). The tobacco bollworm, in particular, is more prone to insecticide resistance development than either the pink bollworm or the boll weevil because the larvae stage is accessible to spray treatments, and therefore, is under selective pressure. The pink bollworm and boll weevil are only accessible as adults to commercial spray treatments. The larval stages are encased inside the cotton boll for the balance of their development time and are therefore not under selective pressure.

This generality is important because after the introduction of transgenic cotton plants, presumably the larval stage of the pink bollworm will come under pressure from the B.t. endotoxin for the first time in commercial cotton production. We can only assume that the larval stage of the pink bollworm, like the larvae of the tobacco budworm, will be capable of developing a wider variety of resistance mechanisms than the adult.

Being polyphagous (feeds on many kinds of food), the tobacco budworm has a much stronger mixed function oxidase system with which to overcome toxicants compared to boll weevil or pink bollworm. Therefore it is much more readily able to generate resistive responses to insecticides (Devonshire and Field, 1991; Ronis and Hodgson, 1989). The tobacco budworm is a multihost pest, thereby violating one of the conditions conducive to rapid development of resistance. However, the polyphagous nature of tobacco budworm presents another type of problem when this

pest is treated on cotton then moves to other crops and is treated again, or vice versa. When the same pest is treated on all of its hosts by essentially the same insecticides, then the conditions for the development of resistance are satisfied.

Gene flow studies of tobacco budworm indicate a local population has an average diameter of five miles in mid-season with random mating. Some 13 enzyme loci were studied electrophoretically. Allele frequencies and genotypic proportioning suggested large numbers of insects with high mobility. In conducting these studies, sampling sites were located around the north-western rim of the Gulf of Mexico from south Texas through the middle of the delta states (Louisiana, Arkansas and Mississippi) to one site in Georgia (skipping Alabama) (Korman *et al.*, 1993).

One thing still not clear from these genetic studies is why tobacco budworm represents a resistance threat in the United States while bollworm does not (Clower *et al.*, 1992; Mallet *et al.*, 1993). Both presumably occupy the same niche, and both are treated with insecticides in cotton. Yet traditionally the tobacco budworm has been the greater resistance threat. The answer must lie in the host selection behavior, or details of host preference. This question is in need of further study.

Ironically, the first measurements of resistance in the tobacco budworm to pyrethroid insecticides were conducted on insects collected in the western cotton fields of southern California and in Arizona (Twine and Reynolds, 1980; Martinez-Carrillo and Reynolds, 1983; Kelly and Watson, 1987; Crowder *et al.*, 1979; Watson and Kelly, 1991; Unpublished data, J. Leeper, DuPont Chemical Co., Wilmington, Delaware). Despite these measurements of pyrethroid resistance, and the clear warnings and calls for action (Twine and Reynolds, 1980), nothing was done in terms of organizing a concerted effort to develop a resistance management plan until after resistance was apparent.

RESISTANCE DEVELOPMENT IN WHITEFLY AND APHID

Sweetpotato whitefly— named *Bemisia tabaci*, but suspected of existing as a number of strains, perhaps many (Perring *et al.*, 1993)—has been referred to as a tropical aphid (Byrne and Bellows, 1991). This designation is most helpful because it lumps aphids and whiteflies together when considering Homoptera in general as cotton pests. This is especially true since aphids and whiteflies are normally kept under good biological control by a number of parasites and predators. As a result, these Homoptera would be prime candidates as insecticide-induced pests, and both are already resistant to a wide variety of insecticides.

The problem is complicated by the fact that whiteflies and aphids contaminate the cotton with honeydew (Toscano *et al.*, 1992), and whiteflies pose a virus transmission threat to alternate hosts such as lettuce and melons. This threat is more serious because the result can be loss of entire alternate host crops.

More recently, the B strain of sweetpotato whitefly, or renamed silverleaf whitefly, has been defoliating cotton plants in mid season from Texas to southern California, and including adjacent regions in Mexico. This much more immediate problem transcends resistance problems because of the need for instant control due to the unusual virulence of this strain of the whitefly.

There is abundant evidence that the whitefly has become a pest insect everywhere in the tropics and subtropics. A critical role is assigned to insecticide overuse in this pest emergence (Byrne *et al.*, 1992), and the development of resistance in the whitefly is indicated as being the primary inducement (Dittrich *et al.*, 1985).

Mallet and Luttrell (1991) categorized three types of cotton insect pests. The whitefly and aphid belong to the first category of insect pests which have very high natural rates of population increase (known by ecologists as "r-strategists"), and are capable of readily reinvading a crop once treated. These insects are said to be prone to developing resistance quickly. The use of insecticides exacerbates the increases in population by removing predators and parasites, and increasing rates of reproduction in the pests, thereby increasing the probability of control failure. Experience shows that this type of insect pest tends to be difficult to control with insecticides.

A second category of insect pests may evolve a type of resistance to insecticides that is relatively ineffective, and have a normally low rate of population increase or crop invasion behavior. Boll weevil is considered a good example of this type of insect, and they have been controlled successfully for many years with organophosphorus unsecticides by treating the adult stages.

The third category of insect pests in cotton, according to Mallet and Luttrell's classification, is intermediate between the first two. As long as this pest is susceptible, insecticides control them well; however, as soon as resistance is present in any form, the populations tend to increase more rapidly and the pest response is more similar to the first type of insect pest. The tobacco budworm and bollworm complex is thought to be a good example of this third type of insect with respect to the development of resistance.

Mallet and Luttrell (1991) pointed out that the boll weevil is moved from the second type to the third type of pest insect merely by switching from organophosphorus insecticides to chlorinated hydrocarbons. The adult boll weevil developed resistance to chlorinated hydrocarbons and would, therefore, be prone to have this resistance selected under insecticide treatment pressure. This would lead to population explosions of resistant insects. They concluded, that as a member of the third category of cotton pests, the tobacco budworm is probably the ideal candidate for resistance management approaches.

STRATEGIES FOR INSECTICIDE USE

HOW INSECTICIDE RESISTANCE TRAITS ARE PRODUCED

The theoretical bases for resistance management tactics and strategies all hinge more or less on how the resistance traits are inherited. The question of where resistance genes come from generally has not been addressed.

Natural Mutations — Muggleton (1984) points out that a natural mutation rate is considered to be somewhere around 1 in 100,000 cell divisions. Based merely on the observation that resistance developed fairly rapidly after the postwar introduction and widespread use of organic insecticides, mutations to resistance genes is thought by Muggleton to be a fairly common event. Natural genetic mutations of bacteria are also

thought to be in this same range (Ames, 1989a,b).

Fortunately, there are abundant examples of mutations occurring in the field of medicine. Resistance is a continuing problem with bacteria in hospitals. Indeed, the pathology laboratory of every major hospital operates a routine screening of bacterial samples from patients for resistance to antibiotics. In addition, the treatment of cancer encounters a similar problem in that cells develop resistance to chemotherapeutic agents, and this problem must be dealt with constantly.

New recessive, loss-of-function mutations occur spontaneously in fruit flies at the rate of about 10^{-4} to 10^{-5} per gene per individual, depending on the gene (Strickberger, 1968). Gain-of-function mutations that express a qualitatively new function (neomorphs) generally occur at a much lower rate. The rate is so low, in fact, that it is difficult to measure accurately in fruit flies.

In bacteria, on the other hand, the recessive loss-of-function mutation to arabinose dependence occurs at the rate of 2×10^{-6}, while the dominant, neomorphic, gain-of-function mutation to streptomycin resistance occurs at the rate of 4×10^{-10} (Fristrom and Clegg, 1988). The difference between gain- and loss-of-function mutations is based on mathematical probability. Many different changes in the DNA base sequence of a gene can eliminate its function, but there are far fewer possible ways of producing a new novel function. Classical genetic studies have suggested that the majority of genetically dominant mutations are neomorphs (Park and Horvitz, 1986).

The process of how mutations produce insecticide resistance is not completely understood, but several general points are well established. Insecticide resistance genes often have an altered function, as for example the altered juvenile hormone receptor that produces methoprene (Altosid®) resistance in fruit flies (Wilson and Fabian, 1986), or the alteration of glutathione-S-transferase to catalyze the glutathione-dependent dehydrochlorination of DDT in house flies (Clark and Shamaan, 1984).

Such mutations would be expected to occur at a very low frequency. They would also be expected to show an incompletely dominant pattern of inheritance, and this has been confirmed by genetic studies of many specific insecticide resistance genes (Roush and Daly, 1990; Watson and Kelly, 1991a,b; Payne *et al.*, 1988). This incomplete dominance is of some economic importance, because genetic selection favoring rare dominant (or incompletely dominant) genes is much more effective than selection favoring rare recessive genes (Fristrom and Clegg, 1988). Dominant selection is most effective because all individuals that carry the dominant gene have a selective advantage.

The effectiveness of dominant selection undoubtedly contributes to the difficulty of managing insecticide resistance. One response to this problem has been to treat alternate generations with different insecticides, which should at least reduce the selective advantage of heterozygotes. Another strategy has been to increase the dose of insecticide, to levels theoretically high enough to kill the heterozygotes, so that resistance might become effectively "recessive" (Roush and Daly, 1990). However, this strategy has high economic and environmental costs.

The killing of insects by any insecticide is probalistic rather that all- or-none (even in the laboratory where uniform doses can be applied). An applied dose in the field will

decrease with time (Luttrell *et al.*, 1991) and at the edges of the field, so that some heterozygotes are likely to survive such a treatment and retain a net selective advantage. That is, treatment with insecticides probably always produces selection with some degree of effective genetic dominance (Mallet and Luttrell, 1991).

The apparently very low spontaneous mutation rate to neomorphic mutations (such as DDTase in house flies) may appear to be a minor risk. However, such alleles can accumulate spontaneously over time in unselected populations, so that the natural frequency is much higher than the mutation rate (Fristrom and Clegg, 1988). The extent to which this occurs in insecticide resistance would depend on the selective disadvantage of each resistance trait in the field. This is likely to vary considerably from one type of resistance to the next and is difficult to measure accurately.

The accumulation of rare unselected dominant mutations is best understood in humans. For example, new dominant mutations that cause the human disease called "Huntington's chorea" occur at the rate of about 10^{-7} per individual (actually, this could still be an overestimate, because of the difficulty of proving paternity after the death of the parents). However, the frequency of humans with Huntington's chorea is much greater (about 10^{-4}), so that the vast majority of all individuals with the mutant gene have inherited it from their parents (Hayden, 1981).

Considering the large number of insects per acre, the large number of acres that are treated with insecticides, and the large number of insect generations over which this selection has continued, it becomes clear that the eventual development of neomorphic mutations in localized insect populations is inevitable.

Gene amplification — Insecticide resistance can also be caused by another type of dominant, gain-of-function mutation, known as "gene amplification" (Devonshire and Field, 1991). Gene amplification, also called "hypermorphic mutation" by classical geneticists, refers to the genetic duplication of a normal gene. Multiple copies of a gene, when activated, result in an increased synthesis of the corresponding messenger RNA, an increased synthesis of the corresponding protein, and a net increase in the total enzymatic activity of the protein.

In the green peach aphid, resistance to organophosphates, carbamates and pyrethroids is caused by amplification of the gene encoding the E4 type esterase enzyme; the degree of amplification is about 64-fold (Field *et al.*, 1988). In other strains of resistant aphids, the FE4 type esterase gene is amplified (Devonshire and Field, 1991).

The mechanism of the initial gene duplication mutation is not well understood, although such events are thought to have occurred frequently in evolutionary history (Devonshire and Field, 1991). Also, gene amplification can be reproducible induced by drug selection in cell culture (Schimke, 1986). Genetic duplication events probably occur more frequently than neomorphic mutations.

Once a single gene duplication has become homozygous, subsequent additional duplications occur at a much higher frequency. There are many examples of this in the literature, including resistant aphids (Devonshire and Sawicki, 1979) and cell lines (Schimke, 1986). It is apparently characteristic of gene amplification in general.

Additional gene duplications are thought to occur by a process called "unequal crossing over", which is essentially an normal type of meiotic recombination between duplicated genes. Unequal crossovers occur at the rate of 10^{-3} per duplicated gene per generation in normal humans (Nathans *et al.*, 1986).

The ability of duplicated genes to be repeatedly amplified at a high frequency undoubtedly explains the ability of many resistant insect strains to progressively increase their resistance in every selected generation. This has been shown directly in the case of resistant aphids (Devonshire and Sawicki, 1979), and has been observed during chemotherapy for human cancers.

Treatment of tumors with the cytotoxic drug methotrexate selects for progressive amplification of the gene that encodes dihydrofolate reductase, the target of the drug methotrexate (Schimke, 1986). This results in methotrexate-resistant tumor cells, which many physicians had previously treated by progressively increasing the dose of methotrexate. The strategy was dropped when it was shown to provide an optimal selective pressure for repeated rounds of gene amplification (Schimke, 1986).

Unequal crossing over can produce genetic deletions as well as duplications. By the mechanism of selecting for the deletions, gene amplifications tend naturally to revert to normal at a relatively high frequency. This means that if the selective pressure is removed for a prolonged period of time, insecticide resistance may decline through new spontaneous reversion mutation. This has been reported in aphids (ffrench-Constant *et al.*, 1988a,b; Devonshire and Field, 1991).

Gradually increasing doses of insecticides to counteract gradually increasing resistance as viewed from these mechanisms would probably ensure the accelerated development of resistance. Rotation or other resistance management strategies would have to be employed before any of the insecticides became ineffective.

MIXTURES OF INSECTICIDES VERSUS ROTATION OF INSECTICIDES

The development of resistance to insecticides depends very much on the insect and the insecticide. Rotation of pyrethroid insecticides or acaricides in the Australian and Zimbabwe resistance management strategies respectively was designed to remove one or more generation of pests from selective pressure by a given class of compound. This was also one of the guiding principles behind the Tri-state resistance management scheme; however, the latter suggested, even urged, depending on how one reads the descriptions, the use of mixtures of insecticides, whereas, the former two did not. All three of these strategies/schemes are discussed in an earlier section of this chapter.

Mathematical models of the selective pressure exerted by using mixtures of insecticides rely on modifying the relative fitness values in the fundamental equation of population genetics. Sawicki and Denholm (1987) dismissed such models as being of little practical use because detailed studies of each case were necessary for the best understanding. Others have cautioned that models are exactly that and not meant to replace the much more complex field situation (Tabashnik, 1986; Mallet and Luttrell, 1991). Muggleton (1984) disagreed with Denholm, arguing that such case-by-case studies were themselves impractical because of a lack of resources.

Two studies of the inheritance of insecticide resistance using theoretical models have concluded that mixtures of one or more insecticides are superior to rotation from one category of insecticide to another provided certain conditions are met (Curtis, 1985; Mani, 1985). The models were analyzed using certain assumptions to simplify calculations. Mani (1985) in particular was careful to heavily qualify his conclusions because of these assumptions. He also pointed out that theoretical model studies could provide only a guide to further experiments employing actual field examples, and that resistance management decisions should only come out of practical studies. In this, he appears to support Denholm's conclusion.

One of the assumptions in model studies is that resistance genes are rare. The argument for mixtures of insecticides follows the simple logic that if resistance genes are rare, traits that confer resistance to two different insecticides would be exceptionally rare to occur in the same individual. Indeed, the counter arguments against using mixtures warn that when resistance is already present, even at low frequency, using mixtures would be an inappropriate strategy (MacDonald *et al.*, 1983; Wood and Mani, 1981).

The qualifications and conditions for using mixtures were explained and dealt with at length by Mani (1985) who also points out that the choice of chemicals for the mixture has to satisfy various constraints, not the least of which is that the combination should not readily evoke cross-resistance. He cautions, as an example, against combining organophosphates with carbamates because of the chance of evoking an altered cholinesterase resistance that might confer cross-resistance to both classes of insecticides.

For this reason, the description of E4 esterase resistance traits recently described in green peach aphid, *Myzus persicae* (Sulzer), that confer cross- resistance to carbamates, organohosphates and pyrethroids (ffrench-Constant *et al.*, 1988a,b) would seem to mute the argument for mixtures of insecticides. Obviously the presence of this resistance gene would outflank a mixture including most of the existing major neurotoxic insecticides currently available for cotton pest control.

There is a danger of placing too great a reliance on theoretical models. Mani (1985) was very careful to point out the need for practical tests in normal field situations. The problem here is one that has nagged resistance management efforts from the beginning; it is difficult to design an experiment that would duplicate or even approach commercial cotton production in such a manner that would account for all of the consequences of insecticide use and give guidance in choosing the best insecticide use strategy.

One particularly troublesome part of the Mani (1985) model is the dose used. "The dosage of insecticide applied is assumed to be large enough to kill all susceptible homozygotes a fraction ... of the heterozygotes ... but no resistant homozygotes." A little reflection and reference to the papers that have been published on this subject (Denholm *et al.*, 1983; ffrench-Constant *et al.*, 1988a,b; Watson *et al.*, 1991) suggests that after an insecticide spray treatment has been made, the residual chemical gradually decreases in amount over a matter of days. This means that once a spray treatment is made, the operator completely loses control of what happens next. In effect, the dose used to treat the insects is changed, not constant (Mallet and Luttrell, 1991).

All too often, thinking about selective insecticide pressure focuses on the single

spray treatment in time and ignores what happens for several days following the treatment. The decreasing dose of a sprayed chemical means that very quickly the conditions imposed by Mani in his model study are changed. For one thing, all of the heterozygotes might very well survive and none of the susceptible insects may survive as suggested by Watson *et al.* (1991) from single treatments by permethrin, particularly if the resistance trait(s) is (are) incompletely dominant, which seems to be commonly the case (Watson and Kelly, 1991a,b; Payne *et al.*, 1988).

The effect of insecticide persistence and mixtures on resistance development was addressed by Luttrell *et al.* (1991) in one of the few studies of this kind. They argued that growers often treat a complex of insects, not just one pest, and tank mixes are therefore, "convenient." Aside from the overall effectiveness of chlordimeform (no longer available to agriculture) in delaying resistance (Liu and Plapp, 1992; Watson *et al.*, 1991), the study found that often two insecticides applied together aged at different rates, leaving one material to act as the selector.

Many side effects of insecticide use are subtle and little understood. Pyrethroid insecticides were known to induce a repellency in insects and mites almost from their introduction (Penman *et al.*, 1986). Repellency affects the overall response of pest insects including those that treatments were not intended to control.

The intriguing side-effect of increasing the reproductive capacity of aphids was documented in response to organophosphorus residues (ffrench-Constant *et al.*, 1988a,b). In addition, Kerns and Gaylor (1991) speculated that somehow sulprofos (Bolstar®) treatments were improving the cotton plant as a host for the cotton aphid leading to population explosions. They noted that cotton plants in sulprofos treated plots continued to grow after the plants in cypermethrin (Ammo®, Cymbush®) treated plots had cut-out. Mathematical models of resistance management fail to take these and other consequences into account. These subtle effects of pest control in cotton would seem to justify Mani's caution.

The observation that cotton aphid susceptibility to insecticides depended on the time of treatment was also intriguing (Grafton-Cardwell, 1991). Instead of just being a change in tolerance, this phenomenon appears to be related at least in part to the physiological state of the aphid itself. Alatiform (winged) nymphs were significantly more tolerant to five insecticides tested compared to apterous (wingless) adults. The phenomenon appeared to be general since the insecticides tested, oxydemeton-methyl (Metasystox-R®), chlorpyrifos (Lorsban®), dicrotophos (Bidrin®), biphenate and endosulfan (Thiodan®), included organophosphorus, pyrethroid and cylcodiene types, each with a distinct mode of action. In addition, survival of treatments appeared to decline as the season progressed, signalling possibly another physiological change in the aphid.

A field evaluation of insecticide rotation versus mixtures for control of citrus thrips, *Scirtothrips citri* (Moulton), found that insecticide rotation was superior in retarding resistance at half the recommended rates of each insecticide (Immaraju *et al.*, 1990). Formetanate (Carzol®), a carbamate, and fluvalinate (Mavrik®), a pyrethroid, were used. In addition, in the absence of selective pressure, fluvalinate resistance regressed to levels before selection after one year.

Tests of mixtures of profenofos (Curacron®) plus cyhalothrin (Karate®) applied at full, half or quarter rates were compared to each compound separately in field plots. Results suggested that the lower rates of the mixtures reduced selection pressure for resistance to both compounds (Kostroun and Plapp, 1992). Thus another qualification for the use of mixtures would urge that they be used only at low rates.

USE OF HIGH VERSUS LOW INSECTICIDE RATES

The use of low rates in the report described above conflicts with the theoretical argument in favor of the use of high rates to control heterozygous hybrid insects with intermediate resistance to insecticides that arise from the first mating of resistance individuals with susceptible insects, the so-called management by "saturation." Again the logic here is deceptively simple. If a resistant adult moth somehow manages to appear in a field population, this individual by itself would not represent a threat, only a potential threat. True to the concept of dilution, if there were sufficient susceptible insects around, the successful mating of two resistant individuals would be unlikely.

If however, the single resistant individual mated with any of the presumed freely available susceptible mates then the off-spring would be heterozygous for resistance. Since most resistances are due to incompletely dominant genes, the heterozygous offspring would not express the full resistance, but would be somewhat intermediate in response to insecticides. As the logic goes, if a given spray treatment is sufficiently high, it would still be high enough to control the heterozygous resistant individual forcing true resistance to require that two fully resistance individuals mate before a fully resistance survivor could be produced.

Although paraphrased and simplified, the argument given above is essentially the one for high doses as a resistance management tactic (Wood and Mani, 1981). Being theoretically sound, this certainly would work. It might be practical if some way were found to decrease the residual dose of insecticide from full effective rate to zero at some point after spraying.

Perhaps the most pertinent model study of the rate of development of resistance is that of Mallet and Luttrell (1991) who put the subject and interpretation into the context of the cotton industry. They reached some very important, even startling, conclusions making the arguments of rotation of insecticides versus mixtures of insecticides and low versus high doses somewhat academic.

Their first point was that tobacco budworm was not a pest before DDT began to be used for cotton pest control. Indeed, there is little or no use of insecticides in the Central Valley of California and there are no key pests of cotton there outside of occasional mite problems. Perhaps most pertinent of all, the tobacco budworm is present, on alternate hosts, but is not a cotton pest.

Their second point was that many cotton pest insects may not be amenable to insecticide control. In this category they include the cotton aphid and whitefly, both homopterans and both subject to population explosions. Indeed, a number of experiences suggest that spraying causes population increases, not decreases since one negative side effect of spraying is to actually increase the reproductive rate leading to population explosions.

In a useful analysis of the use of low and high doses, Mallet and Luttrell (1991) report that while theoretical analyses are usually based on laboratory data, field dosage-mortality responses are likely to be shallow with genotypes overlapping. Low dose rates would increase heterozygote survival, and high dose rates would eliminate more susceptibles assisted, if anything by shallow dose responses. Either strategy delays resistance, but does not eliminate it. Despite model studies of resistance gene inheritance, in the end, resistance is correlated with insecticide use; the more insecticides are used, the greater will be the probability of developing resistance. Put another way, one cannot develop resistance without using insecticides frequently, i.e., without selective pressure.

NATURAL ELIMINATION OF RESISTANCE

Monitoring the efficacy of a number of insecticides not only tells which compounds show tolerance, it also shows which compounds are still effective, and gives the toxicologist some hint as to the type of tolerance developing. Such monitoring also tends to make resistance management a more natural procedure.

We inadvertently came across natural resistance management operating in the Palo Verde Valley in the spring of 1987. Six insecticides were being surveyed for resistance by the attracticide method at four sites that were selected by Dr. C. A. Beasley of the California Cooperative Extension Service. The tests were conducted by Mr. Richard Wellman, a local commercial pest control advisor. Because of the cost involved, not all compounds were tested at every field. The tests were conducted very early in the season (June 1) before fruiting bodies were present.

The results (Figure 12A) showed that one field (Wuertz) contained a pink bollworm population that showed excessively high tolerance to fenvalerate (Pydrin®). The adults in the Wuertz field were 400-fold more resistant to fenvalerate compared to our susceptible laboratory strain. At the same time all other tests gave results that were considerably lower. Indeed, to even see the various results of the other tests, the data point for fenvalerate at the Wuertz field had to be omitted. When replotted, the results showed a widespread low level resistance to all compounds tested (Figure 12B).

Fortunately, we also tested Cymbush® (cypermethrin) and Guthion® (azinphos-methyl) on the same field at the same time. While the resistance to fenvalerate was high, the same population showed a sensitivity to cypermethrin and azinphos-methyl. This suggested that the resistance to fenvalerate was specific and showed no cross-resistance to another pyrethroid nor to organophosphorus insecticides. Thus the resistance was not likely to be site insensitivity (not kdr-like). The pest control advisor used this information to begin the season using organophosphorus insecticides to control the pink bollworm. When pyrethroids were used a month later, no pyrethroid resistance remained.

The fenvalerate resistance in the Wuertz field was specific to that site. The Chaffin 49 and Chaffin 25 cotton fields nearby showed no such tolerance even though they were within two miles of the Wuertz field. The Wuertz field had been planted to cotton successively for five years. This suggested that pink bollworm populations remained endemic and built up tolerance to the given regime of insecticides, with little gene flow to or from nearby fields.

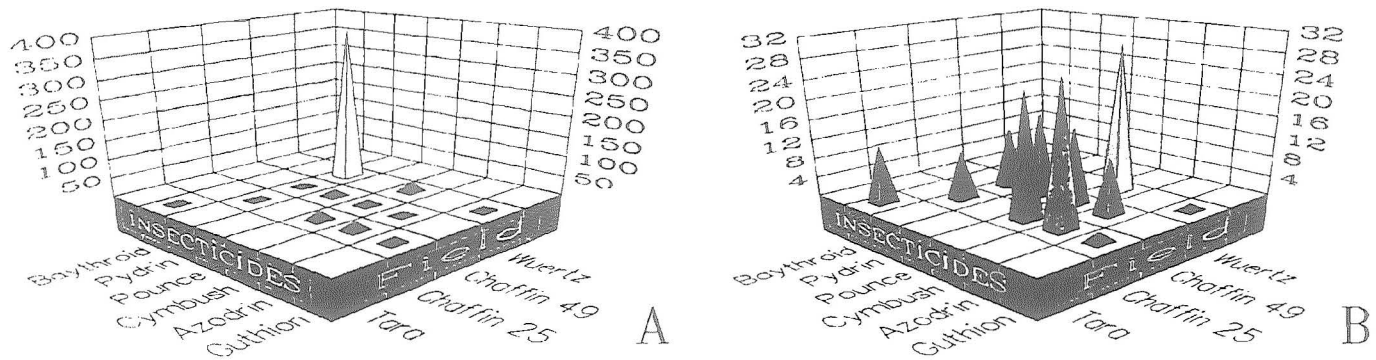

Figure 12. Resistance ratios of six insecticides on adult pink bollworms measured at four cotton fields in the Palo Verde Valley of California in June of 1987 by the attracticide resistance monitoring method. (Tom Miller, unpublished data.) All of the data is shown in A. In B, the large data point from the Wuertz farm was removed to get a better perspective on the remaining values. The Wuertz and Chaffin fields are separated by a few miles in the Valley. The one value of 400-fold resistance to fenvalerate (Pydrin®) was the highest value recorded in field testing. The grower began the season a week later by treating the Wuertz field with organophosphorus insecticides and the fenvalerate resistance was gone a month later. Note the partial cross-resistance to cypermethrin (Cymbush®) at the same Wuertz field, and lack of azinphos-methyl (Guthion®) resistance.

We now suspect that for the past 20 years, following the introduction of pyrethroid insecticides, phenomena, such as that documented in the Wuertz field in 1987, have been widespread. Extensive resistance monitoring in pink bollworm was never established, and the resistance monitoring program was not funded for very long after the development of the attracticide resistance monitoring method.

The commercial pest control advisor, Richard Wellman, who was monitoring the Wuertz field in the spring of 1987, was thinking of switching to organophosphorus insecticides even before the resistance monitoring showed the problem with fenvalerate. He based this "feeling" on the general ineffectiveness of the pyrethroids the previous fall in the same field. Thus insight and practical experience in noticing the efficacy of insecticides in ordinary pest control practices can be as effective as an extensive and expensive resistance monitoring program. The value of resistance monitoring was in establishing exactly what the resistance was immediately and eliminating guesswork in remedying the problem.

SUMMARY AND PERSPECTIVE

The existence of insecticide resistance is now familiar and well established. The factors that confer resistance are generally understood, even if the genetic bases for all of them have not been identified, nor the ecological factors appreciated. The development of resistance to insecticides and acaricides is something that can be dealt with in a rational manner. There are now several successful examples showing that, with a concerted effort, the development of resistance is not nearly as inevitable as once thought.

Fundamental research into the mode of action of insecticides has provided useful tools and information for measuring and understanding insecticide resistance, and designing resistance monitoring protocols. There exists, however, a nationwide trend away from research on insect toxicology. This is occurring at the same time as, but is less well appreciated, the obvious contraction in agrochemical industry. To dismantle the research effort in insect toxicology as one consequence of national concern over the use of pesticides in agriculture, seems neither well thought out nor wise.

One danger in the focus on resistance to insecticides and the effort to seek remedy has been that these approaches and efforts tend to formalize or lock in chemical control methods. Tom Brown touched on this subject over ten years ago (Brown, 1981) when he wrote in the very first paragraph of his review on resistance countermeasures: "In confronting the insecticide resistance problem the most important countermeasure is good pest management practice... to minimize selection pressure from chemical insecticides." More recently, Mallet and Luttrell (1991) reached the same conclusion. The surest way to reduce selection pressure is to use fewer insecticide treatments.

In light of Brown's comment, it is clear why the growers in Texas adopted the short season strategy to circumvent boll weevil and pink bollworm problems very early on, but less clear why the California and Arizona cotton growers on the whole resisted the strategy for years.

It is further remarkable that the cotton growers of California's Imperial Valley, after contending with the pink bollworm as a key pest for some 23 years almost to the point of bankruptcy, finally adopted the short season strategy in 1989. This strategy worked as predicted, quickly relegating the pink bollworm to minor pest status. Several years of insecticide resistance monitoring have revealed that, while valid cases of high resistance to pyrethroid insecticides were found, there were no widespread and obvious failures of insecticides on the same scale found in Texas and Australia in bollworm and tobacco budworm.

This is the main reason why growers in California and Arizona have never pushed for nor sought a resistance management plan or program. In other words, a genuine crisis must occur in order to provoke an effort to actually do something beyond providing token support to a few entomologists in agricultural experiment stations to conduct some measurements. The bigger the crisis, the greater the attention it attracts.

The cultural control of pink bollworm was not the only noninsecticide control tactic available for the past 25 years. Pheromone control applied in the early season now has been shown on several occasions to cause a steady decline in pink bollworm numbers, and has been adopted by a few individual growers. The most common complaint from growers who tried and did not continue with pheromone control was that it worked, but the cost was the same as chemical control. Unmentioned was the fact that pheromone control is so much more compatible with integrated pest management and good resistance management. It is also much less likely that pink bollworms will become resistant to their own pheromone.

Unfortunately, the pheromone system works best to suppress pink bollworm populations when employed over a large contiguous area of cotton. This approach was adopted in California or Arizona in only a few programs because the areawide approach requires cooperation on an unprecedented scale.

Given the will, it is clear that remedial measures preventing or delaying insecticide resistance can be taken. Probably the best that one can expect from a pragmatic standpoint is that resistance management becomes a routine habit rather than something that one reverts to in a crisis atmosphere. How one encourages good integrated pest management, however, is something else.

In 1983, *Helicoverpa armigera* developed resistance to pyrethroid insecticides in Emerald, Australia. The Australians voluntarily instituted a resistance management strategy that has been an integral part of their pest management program ever since. They have supported the monitoring of insecticide resistance and consider that a crucial part of their cotton industry.

The United States had no resistance management program nor plans until 1986 when widespread resistance in tobacco budworms to pyrethroid insecticides appeared at Uvalde, Texas. As a direct result of that incident, Texans began widespread resistance monitoring using the Plapp vial assay method. This method was adopted across the entire southern portion of the Cotton Belt by Arkansas, Louisiana and Mississippi as well as Texas. Monitoring reports have been a common feature of the Annual Cotton Insect Research and Control Conferences in the United States.

Today it is possible to monitor resistance to insecticides not only in the pink bollworm and tobacco budworm, but in almost any pest insect. Such monitoring can be done rapidly and entirely on site in the field, and, in some cases, before the crop is mature enough to be attacked by pests.

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