Resistant Pest Management

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Table of Contents

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Letter from the Editor

Did you miss us?

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Both of us have been through some major shifts in our professional appointments at Michigan State University over the last year. This took up a lot of time and consequently the Spring issue of the Newsletter never got put together. Some of you even noticed it's absence. Now we are back on track, but it is not certain for how long. Putting together the Newsletter has always been a hand-to-mouth business financially. We have had great support from IRAC and this continues, but the availability of the additional funding for printing and mailing the Newsletter is in doubt. We have the resources to maintain the publication in its electronic form (you can find it at (www.cips.msu.edu/resistance/rpmnews) and the IRAC website (http://plantprotection.org/irac)) and we will continue to put out the printed copy as long as possible. If the disappearance of the print version would create a problem for you, please let us know by e-mail (our addresses are on the cover page) or by mailing your comments using the tear-off form on the back cover. If we don't hear from at least a few people we will assume that the printed version is not too valuable. In fact we would love to hear from you about your comments, ideas and most of all, articles and views for publication. If you have any bright ideas for funding to continue the printed version, do let us know. We are reluctant to start charging for the Newsletter since that complicates our lives by putting us in the accounting and banking business.

On another note, the preliminary web page detailing arthropod resistance globally is also up on the web (www.cips.msu.edu/resistance/rmdb). The database is based on an up-to-date literature search of over 5,000 articles. This database will be developed in more detail as time and resources allow. If you are interested in a particular custom relational analysis of the data please contact Mark Whalon.

Resistance Around the Globe - Fungicides

Resistance of *Botryosphaeria dothidea* from Pistachio to Iprodione

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Botryosphaeria panicle and shoot blight, caused by *Botryosphaeria dothidea*, is a yield devastating disease throughout California. A number of fungicides have been tested and proved to provide only marginal control. The dicarboximide fungicide iprodione (Rovral®), however, has showed good efficacy in controlling Botryosphaeria panicle and shoot blight of pistachio. Many reports have demonstrated that plant pathogens easily develop resistance to iprodione in laboratory and in the field. The objectives of this study were to survey the sensitivity of *B. dothidea* to iprodione and to assess the resistance risk of *B. dothidea* to iprodione.

Seventy-nine isolates, collected from 8 California pistachio orchards from 1997 to 1999, were used to assess the sensitivity of *B. dothidea* to iprodione. Only one out of these isolates was low resistant (LR) to iprodione (EC_{50} = 2.726 µg/ml), whereas all the other isolates were sensitive (EC_{50} < 1µg/ ml). *B. dothidea* isolates readily developed resistance to iprodione in laboratory. Furthermore, these laboratory-derived iprodione-resistant (IR) isolates retained high virulence to pistachio. Iprodione resistance significantly declined when these IR isolates were propagated on pistachio leaves in the absence of the fungicide. IR isolates were also resistant to vinclozolin, another dicarboximide fungicide, but sensitive to tebuconazole and benomyl. Applications of iprodione at 500 µg/ml were effective against naturally sensitive isolates, but failed to control disease caused by IR isolates in both the laboratory and greenhouse. The results indicate that, although naturally occurring IR isolates of *B. dothidea* may be rare in California pistachio orchards, the fungus readily develops resistance to iprodione in laboratory and, most importantly, retains high levels of virulence on pistachio. Alternating applications of tebuconazole, benomyl, and iprodione thus may delay iprodione resistant development in pistachio *B. dothidea* isolates.

Resistance Around the Globe - Herbicides

Common Sunflower (*Helianthus annuus* L.) Resistance to Acetolactate Synthase Inhibiting Herbicides

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Herbicides that inhibit acetolactate synthase (ALS; EC 4.1.3.18) have become important tools in a number of crops since being introduced in 1982. ALS is a plant enzyme involved in the biosynthesis of the branch chain amino acids, valine, leucine and isoleucine (Saari *et al*. 1994). ALS herbicides demonstrate wide crop selectivity, high efficacy, low use rates, and low mammalian toxicity. Currently, ALS inhibitor herbicides are comprised of over 30 active ingredients for selective use in at least 10 different crops. A total of 15 chemical classes have been identified as inhibitors of ALS, but not all have been commercially developed (Saari *et al*. 1994).

Although ALS inhibiting herbicides have excellent herbicidal and environmental properties, weed resistance to ALS inhibitor herbicides has become a major concern in many crops around the world. Typically, weeds that formerly were controlled by a herbicide now do not respond, even to high rates of the herbicide. Generally, the resistant population developed after multiple applications of the same herbicide, and thus is different from the natural tolerance that weeds have for many herbicides (Saari *et al*. 1994, Wrubel and Gressel 1994).

The occurrence of herbicide resistance in weeds has been accelerating in recent years. Heap

(1997) reported that 188 different weed species in 42 countries resistant to specific herbicides had been discovered. He also states that since 1978, an average of nine new cases of herbicide resistance are reported each year. ALS inhibitor herbicides play a major role in these statistics by contributing 38 species to the total reported cases of herbicide resistance.

In 1996, ALS resistance was discovered in common sunflower (*Helianthus annuus*) (Heap 1997). Common sunflower is a summer annual found over much of the North Central United States and thrives in almost any environment including cultivated fields, pastures, and waste land (Wax *et al*. 1981).

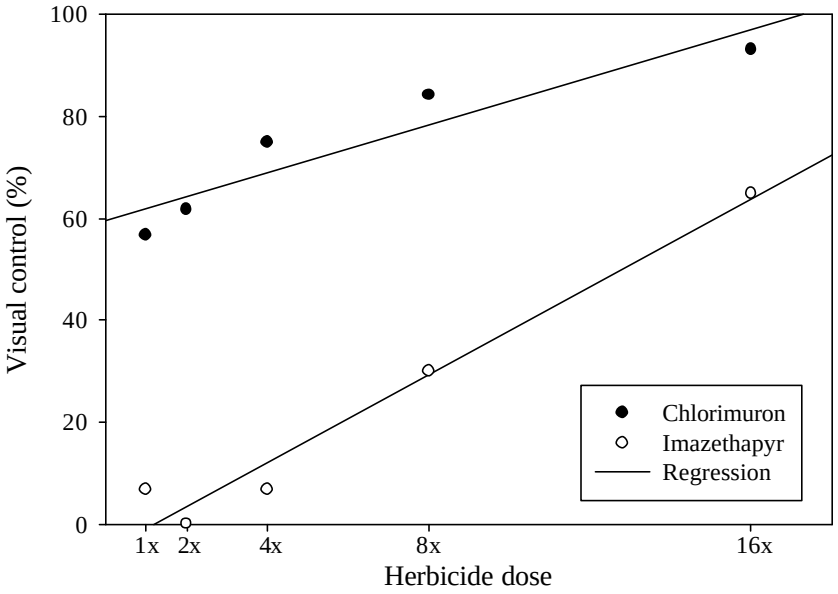

Figure 1. Dose response curve for resistant common sunflower treated with imazethapyr or chlorimuron 21DAT. The susceptible biotype was controlled at the normal use rate and data was not included. (The 1x application rates for imazethapyr and chlorimuron were 70 and 13 g/ha, respectively.)

The ALS inhibitors chlorimuron, thifensulfuron and imazethapyr are labeled to control common sunflower (Anonymous 1997). In 1996, common sunflowers from Howard, South Dakota were suspected to be cross-resistant to these ALS inhibitor herbicides. ALS herbicide use at this site began in 1988 in soybeans (Table 1). In 1989 the field was rotated to corn with no ALS herbicide applied and in 1990 the field was planted back to soybeans and ALS products were applied for weed control. That year, the field was resprayed to control escaped sunflowers. Chlorimuron and thifensulfuron were used as the primary herbicide treatment until 1992 when imazethapyr was added to the herbicide program. After the 1992 application of imazethapyr, a respray with chlorimuron was performed.

The soybean and corn rotation has continued from 1988 until the present time and has not reduced the problem. ALS inhibitor herbicides were not used during years

Table 1. Grower field history in Howard, South Dakota from 1988 to 1996.

Year	Crop	Tillage Practice	Herbicide Program[a]	Respray Treatment[a]
1988	Soybean	Conventional	138g trifluralin PPI 4.4g thifensulfuron + 5.8g chlorimuron POST	None
1989	Corn	No-till	2.2kg cyanazine PRE 1.3kg cyanazine + 419g atrazine (premix) + 560g dicamba POST	None
1990	Soybean	Conventional	138g trifluralin PPI 4.4g thifenslufuron + 5.8g chlorimuron POST	8.8g chlorimuron
1991	Corn	No-till	2.2kg cyanazine PRE 1.3kg cyanazine + 419g atrazine (premix) POST	None
1992	Soybean	Conventional	70g imazethapyr POST	5.8g chlorimuron
1993[b]	Corn	No-till	2.2kg metolachlor PRE 2.0kg acetachlor PRE 1.6kg dimethenamid PRE 560g atrazine + 560g dicamba POST	None
1994	Soybean	No-till	1.1kg glyphosate BURNDOWN 70g imazethapyr POST	13.1g chlorimuron
1995	Corn	No-till	2.2kg cyanazine PRE 1.3kg cyanazine + 419g atrazine (premix) + 560g dicamba POST	None
1996	Soybean	No-till	1.1kg glyphosate + 420g metribuzin BURNDOWN 70g imazethapyr + 6.6g imazaquin POST	36.8g imazaquin

[a] Herbicide applied per hectare.

[b] Divided into thirds for demonstration plots. POST treatment was applied to the entire area.

when corn was planted. The need for respray in 1990 suggests that resistant weeds may have been prevalent after the second application of an ALS herbicide.

ALS-inhibitor resistance was confirmed with a whole plant assay developed by Hinz and Owen (1997). A confirmed sensitive biotype of common sunflower from Ames, Iowa was used for comparison. The I_{50} values for imazethapyr were 200.0 and 5.2 µM for the resistant and sensitive biotypes, respectively. Chlorimuron I_{50} values are 10.5 and 1.2 nM for the resistant and sensitive biotypes, respectively (Table 2). The I_{50} values indicate the resistant population requires 39 and 9 times more imazethapyr and chlorimuron, respectively, to obtain the same level of enzyme inhibition compared to the sensitive biotype. Although resistance to both herbicides has been confirmed, the level of cross-resistance to chlorimuron is limited and requires further evaluation.

Dose response (Figure 1) and biomass data (not shown) support the whole plant enzyme assay data. The 1X rates were 70 and 13 g/ha for chlorimuron and imazethapyr, respectively. A visual estimate of control (<90%) was not achieved with less than an the 8X application rate (560 g/ha) of chlorimuron, whereas control with imazethapyr was poor even at the 16X application rate (208 g/ha). It is likely that this population of common sunflower was initially sensitive to both imazethapyr and chlorimuron. However, through limited selection, this biotype is now functionally resistant to imazethapyr and chlorimuron.

Table 2. Whole plant ALS I_{50} values for resistant and susceptible biotypes of common sunflower.

	I_{50} values[b]	
Herbicide	Resistant	Susceptible
Imazethapyr (µM)	30.0 (15 to 45)[a]	1.5 (0.7 to 2.2)
Chlorimuron (nM)	105.0 (55 to 160)	5.2 (2.5 to 7.7)

[a]Values in parentheses are 95% confidence intervals.
[b]I_{50}: Inhibition of 50% of the enzyme activity.

ALS resistance can also include increased metabolism of the herbicide by the resistant biotypes and by limited uptake and translocation of the herbicide (Saari *et al*. 1994). Our conclusion is that the mechanism of resistance in common sunflower is an altered site of herbicide action. Further evaluations of rapid herbicide metabolism and differential uptake and translocation are needed to confirm this conclusion.

Herbicide resistance is a problem which continues to grow with respect to common sunflower. Preventative measures must be taken to slow the onset or expansion of a resistance problem. These measures involve using herbicides with different modes of action, rotating crops, and using more mechanical practices in crop production.

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Glyphosate Resistance in Another Plant

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At a recent meeting Monsanto scientists described to Midwestern weed scientists and agronomists the discovery and state of knowledge about a biotype of goosegrass (*Eleusine indica*) resistant to glyphosate. The problem appeared in 1997 in oil palm plantations of Malaysia where as many as eight annual applications of glyphosate have been made for the past 10 years. Unlike the resistance in rigid ryegrass (the other annual grass with glyphosate resistance) in which the specific mechanism of resistance still remains a mystery, the resistance in goosegrass is already known to be due to an altered binding site. In susceptible plants, glyphosate binds with the EPSPS enzyme thereby preventing the formation of key aromatic amino acids required to build proteins needed to sustain the plant's life. In the resistant goosegrass, the EPSPS enzyme has a single amino acid substitution (proline to serine change) in the region of the EPSPS enzyme

where glyphosate normally binds, thus preventing glyphosate from binding. Thus, the formation of the amino acids continues normally and plants do not die. The mechanism of glyphosate resistance in rigid ryegrass is unknown, but it is believed to be due to multi-genetic differences and is not the result of an altered binding site.

Depending upon the method of measuring the level of resistance, the resistant biotype is two to five times more tolerant of glyphosate than the susceptible strains. The impact of the resistant ryegrass in the field is quite small in both areas where it has been confirmed (Australia and California) and the problem in these areas has not spread. This is somewhat similar to the triazine resistant velvetleaf in Wisconsin: only some farms have the problem and it basically has not moved to new sites nor developed on other farms. In contrast, the glyphosate resistant goosegrass has appeared on four oil palm plantations and already infests approximately 12,500 acres. Additionally, the infestations are reportedly similar to what we would see in fields with triazine resistant lambsquarters in Wisconsin: a large and aggressive population of a single species.

Herbicide resistant biotypes of goosegrass have previously been found in several regions of the world. A biotype resistant to trifluralin (Treflan) was documented in 1973 in the southern U.S. after many years of using this and perhaps related products in soybean and cotton production. Biotypes of goosegrass resistant to imazapyr (Arsenal) in Costa Rica, fluazifop (Fusilade) and paraquat (Gramoxone) in Malaysia have also been documented in 1989 and 1990. Goosegrass is one of the major annual grass weeds in the tropical and subtropical regions of the world. It is considered among the 20 worst weeds in the world by the authors of The World's Worst Weeds (Holm *et al*., 1977). Single plants can produce 40,000 seeds so an infestation could produce 2,000,000 seeds per acre. If uncontrolled, it causes economic losses in crops and orchards in the southern and western regions of the United States. It is described on page 24 of the Weeds of the North Central States book and the map indicates it is abundant in the southern regions of Illinois, Missouri, Indiana and Ohio and that it is present in most of the central and northern areas of these states.

More non-target site herbicide cross-resistance in *Echinochloa* spp. in rice

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The grass weeds of wheat, barley, and rice have shown a penchant to evolve non-target site cross resistances (Gressel, 2000). Such cross resistances are rare in dicot weeds, and there has been little spread or co-evolution. The problematic grass weeds of these three small grain crops are mainly "camp followers" that have followed the crops around the world from their joint centers of origin. In that respect they have been co-domesticated with the crops. These weeds can hardly exist outside agro-ecosystems away from their centers of origin. Typically they also seem to be mimicking the crops in their evolution of resistance; they up-regulate the same enzyme systems that the crop has been using to selectively detoxify or otherwise become immune to the effects of the herbicides. There is a lesson to be learned - mimics are not clones of the original, the same enzyme (by name) can have slightly different properties in different pecies. Thus, it was documented a decade ago that *Echinochloa crus-galli* (Giannopolitis and Vassiliou 1989) and *E. colona* (Garro *et al.*, 1991; Fischer *et al*., 1993) evolved resistance to propanil (an inhibitor of photosystem II) by up-regulating the herbicide-detoxifying, acylamidases to levels similar to those in rice. *Echinochloa* spp. have subsequently evolved propanil resistance around the world, wherever propanil is heavily used (Heap, 2000). This propanil-resistance can be overcome by adding very small amounts of aniliphos and related herbicides that act as specific synergists (Caseley *et al*., 1996). The acylamidase in the weed is inhibited, but not in rice

Echinochloa has evolved resistance to quinolorac (an auxin type herbicide) in Brazil and USA rice (Heap, 2000), and to fenoxaprop (an inhibitor of acetylCoA carboxylase) in Costa Rica (Riches *et al*., 1996). *Echinochloa* resistance is not limited to rice. It has evolved atrazine (a photosystem II inhibitor) resistance in maize and orchards world wide, and pendimethalin (an inhibitor of tubulin) resistance in Bulgarian orchards (Heap, 2000).

The extensive evolution of butachlor (chloroacetamide) and thiobencarb (thiocarbamate) cross-resistant *Echinochloa crus-galli* in China was reported earlier on these

pages (Huang and Gressel, 1997). The different actions of these herbicides suggest different targets, but the targets are unknown, suggesting more than one target for each. The mode(s) of resistance in both weed and crop resistance is/are still unknown.

A most disturbing report has just been published from California (Fischer *et al*. 2000). Continuous flooding of the paddies was instituted 80 years ago to control *E. crus-galli*. This species was supplanted by the related *E. oryzoides* and *E. phyllopogon*, and they were initially controlled by propanil (dropped for environmental reasons), then molinate and thiobencarb (thiocarbamates) in the 1980s, and fenoxaprop (an inhibitor of acetyl CoA carboxylase) in 1990s. The succession of herbicides was governed by many considerations, but the appearance of resistance was not a cause - until recently, when farmers complained. Fischer *et al*. (2000) tested various accessions of both species and found populations that were cross-resistant (4-20 fold the wild type levels) to thiobencarb, molinate, and fenoxaprop, with a 50% increase in resistance to propanil (which is not resistance at field rates). Even more surprising was that the accessions of both species were cross resistant to the acetolactate synthase inhibitor bispyribac, which has not been introduced as yet into California. The low levels of resistance are sufficient for lack of selective control at economically and physiologically feasible herbicide rates (except for propanil).

The low (but devastating in the field) levels of all the resistances, and their appearing simultaneously argues against multiple target site resistances. Little has been published about how rice metabolizes these herbicides, but a common metabolic mechanism is doubted. This author's best guess would be that a common exclusionary mechanism is active, similar to the multidrug resistances (MDR), as are known to occur in bacteria, fungi and insects. This is foreboding, although some herbicides (glyphosate, glufosinate, pendimethalin, and clomazone) still kill this weed. Thus, if there is MDR, it is not to all herbicides. Glyphosate and glufosinate could only control these *Echinochloa* spp. selectively in transgenic herbicide-resistance rice, otherwise the rice would be killed (Gressel, 2000), and clomazone is not registered for use in rice, leaving pendimethalin as the sole herbicide for control at present. *E. crus-galli* has evolved pendimethalin resistance in Bulgaria (Heap, 2000), indicating that it could do so in California.

It appears that *Echinocloa* spp. may soon surpass *Lolium* spp. in demonstrating a propensity to evolve resistance, by a multitude of mechanisms, to many herbicides around the world.

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Resistance Around the Globe - Insecticides

Adaptive Resistance Management

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An adaptive preemptive resistance management strategy can be used to hedge against failures in the scientific assumptions underlying the high-dose plus refuge strategy for Bt corn. This will require sensitive monitoring coupled with effective adaptive management interventions. Using a theoretical model, we show that resistance alleles must be detected at frequencies of $\leq 5x10^{-3}$ to provide enough time to adapt management. Previous work on the costs of monitoring indicates that an F_2 screen is the most cost effective method for monitoring recessive resistance to Bt corn and an in-field screen is the most cost effective method for monitoring dominant resistance. Both methods can detect and measure resistance at frequencies of $\leq 5x10^{-3}$ for ~$5000. Two types of adaptive responses can be taken, reducing the selective advantage of the resistance allele or modifying the mating system so fewer resistance alleles are passed on to future generations. Assuming there is a 2-year time delay between detecting and measuring the resistance frequency and taking an adaptive response, we found that increasing refuge size to 66% (from 20%) can prolong susceptibility 10 generations, decreasing survival and reproduction of moths from Bt corn fields by 90% can prolong susceptibility by 10 generations. Modification of the mating system by changing movement rates and attracting susceptible males into Bt corn fields could prolong susceptibility for >20 generations. These results suggest that adaptive resistance management could increase the durability of Bt corn.

Azinphosmethyl Resistance in Populations of Male Oriental Fruit Moth (Lepidoptera: Tortricidae) from New Jersey Apple Orchards

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ABSTRACT

The Oriental fruit moth, *Grapholita molesta* (Busck), is a serious orchard pest in many production regions throughout the world including peaches and nectarines in northeastern United States. In eastern Canada, some populations have developed resistance to azinphosmethyl (Kanga *et al*. 1997, Pree *et al.* 1998). While *G. molesta* is usually a stone fruit pest in Mid-Atlantic States, larvae have been found infesting apples during the last few years causing growers economic hardships from lost fruit and additional control costs.

Azinphosmethyl has been sprayed extensively on apples for more than thirty years because of its effectiveness against many apple insect pests and because of its low toxicity to predators of European red mite, *Panonychus ulmi* (Koch). From 1995 to present, azinphosmethyl control failures have been reported in parts of New Jersey. In some locations, both the application rate and the number of applications per season have increased as a result of reduced product efficacy. Many growers are considering replacing azinphosmethyl with synthetic pyrethroids whose continuous use could cause outbreaks of European red mite, and possibly the development of pyrethroid resistance. In response to this particular situation, a project was initiated to determine if *G. molesta* populations were becoming less susceptible to azinphosmethyl in New Jersey apple orchards.

Toxicological responses to azinphosmethyl of male Oriental fruit moth from five commercial orchards in which control failures had occurred were examined for azinphosmethyl resistance and for potential resistance mechanisms using topical pheromone trap bioassay (Riedl *et al*. 1985, Shearer *et al.* 1994, Varela *et al*. 1997). These responses were compared with a reference population that had no history of control failure and received little selection pressure. Results from a field survey conducted in 1998 indicated moderate level of

resistance to azinphosmethyl (2.7 to 4.1 fold) in *G. molesta* and slopes of regressions lines (2.5 - 2.8) indicated genetically heterogeneous populations. A 2-fold decline was observed between fourth flight of 1998 and first flight of 1999, suggesting that resistance was unstable in moths collected from these study sites. Field surveys conducted in 1999 indicated lower level of resistance to azinphosmethyl. The resistance ratios were ranged from 1.2 to 1.9 during first flight of 1999 and 1.2 to 2.6 during fourth flight of 1999. Steep slopes of regression lines during 1999 season indicating the presence of genetically homogeneous populations with the exception of site five population. A 1.5 to 2.0 fold increase in LC_{50}s was observed between first and fourth *G. molesta* flights in1999, indicating that resistance can build up during a growing season. DEF, but not Piperonyl butoxide, significantly enhanced the toxicity of azinphosmethyl, suggesting that enhanced metabolism by esterases were involved in the resistance of azinphosmethyl in moths collected form these study sties. Our data also provided a basis for suggestion that nonmetabolic (target sites) mechanisms could be involved in the increased resistance of azinphosmethyl.

Results of our study suggest that problems with *G. molesta* in apple orchards can be avoided by making timed applications of insecticides based upon degree-days and using rotational strategies of different chemistries to control *G. molesta* in apples. Given that resistance to azinphosmethyl was unstable in these study orchards, we recommend a rotational strategy of effective insecticides to reduce the development of resistance against azinphosmethyl. A high label rate of azinphosmethyl might be used to suppress the first heavy flight of *G. molesta* in spring. A carbamate, methomyl or carbaryl could be applied during second and third *G. molesta* flights when the pest pressure is low. During the fourth and sometimes partial fifth flight of *G. molesta*, when the pest pressure is typically high, pyrethroid applications would reduce population levels and avoid outbreaks of European red mite at this stage of apple development. Mating disruption of *G. molesta* may be another control option in apple orchards because it is not harmful to natural enemies when compared with pyrethroids and carbamates.

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Baseline toxicity of spinosad on the cotton bollworm, *Helicoverpa armigera* (Hub.), in India

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ABSTRACT

The baseline toxicity of spinosad on a reference susceptible strain and nine field populations of *Helicoverpa armigera* was determined through a standard topical application method. The toxicity of spinosad (Table 2) was relatively less variable falling within a range of an LD_{50} of 0.023 to 0.24 µg/larva and an LD_{90} of 0.27 to 4.33 µg/larva. The data from all assays were pooled together and subjected to probit analysis to obtain a cumulative log dose probit response. The LD_{50} and LD_{99} deduced from the data were 0.058 and 9.85 µg/larva respectively. This data would be useful to consider the LD_{50} of 0.058 as the baseline susceptibility index for resistance monitoring through the conventional log dose probit assays and the LD_{99} of 10.0 µg/larva as diagnostic dose for routine monitoring of resistance to spinosad through diagnostic dose assays.

INTRODUCTION

Spinosad belongs to a class of novel insecticides, being introduced for the control of lepidopteran pests of cotton. Spinosad which was introduced in 1997 as Tracer® by DowElanco, is a mixture of two naturally occurring active components, 85 % spinosyn A and 15 % spinosin D, produced by the soil actinomycetes *Saccharopolyspora spinosa.* Structurally, these compounds are macrolides based on a unique tetracyclic ring system to which two different sugars are attached (Salgado *et. al.*, 1997). The

insecticidal activity of spinosad on a range of lepidopteran pests has been demonstrated (Sparks *et. al.*, 1995). Spinosyns have been reported to effect g-aminobutyric acid or glutamate receptors (Duce *et al.*, 1995) or cause excitation and persistent activation of nicotinic acetylcholine receptors. They were also reported to cause prolongation of acetylcholine responses in isolated neurons by a novel mechanism that distinguishes this group of insecticides from all other nicotinic agonists (Salgado *et al.*, 1997). This novel mechanism of action is perceived to be of great interest from the stand point of resistance management, through the facilitation of rotation of insecticides with different chemistries and mode of action in attempts to delay resistance. Resistance to other insecticides in the diamondback moth has not been reported to confer cross-resistance to spinosad (Sparks *et al.*, 1995), and also this class of compounds has not been used earlier for pest management in India. This provides an opportunity to determine baseline toxicity and a diagnostic dose for resistance monitoring of spinosad on field populations of the cotton bollworm, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae), which is a major pest of cotton and pulse crops. The toxicity profile of spinosad on *H. armigera* has not been reported so far and this paper provides information on the response of field populations and of a standard susceptible strain to spinosad to enable deduce the baseline susceptible data as well as determination of a diagnostic dose for resistance monitoring.

MATERIALS AND METHODS

Insects

Helicoverpa armigera larvae were collected from different cotton growing regions in India from cotton fields during the cropping season of 1998-99. The larvae were reared on a chickpea based semi-synthetic diet (Armes *et al.*, 1996) individually in 75 ml cells of 12 well tissue culture plate. Laboratory cultures were established for each population from 200-400 moths and third instar (30-40 mg larvae) of the resulting F1 generation, were used for bioassays. An insecticide susceptible *H. armigera* strain was kindly provided by Dr. Alan McCaffery, The University of Reading, UK, through Dr. Nigel Armes, ICRISAT, India. The 'Reading susceptible' strain, originally collected in Southern Africa had been maintained at The University of Reading for at least 15 years.

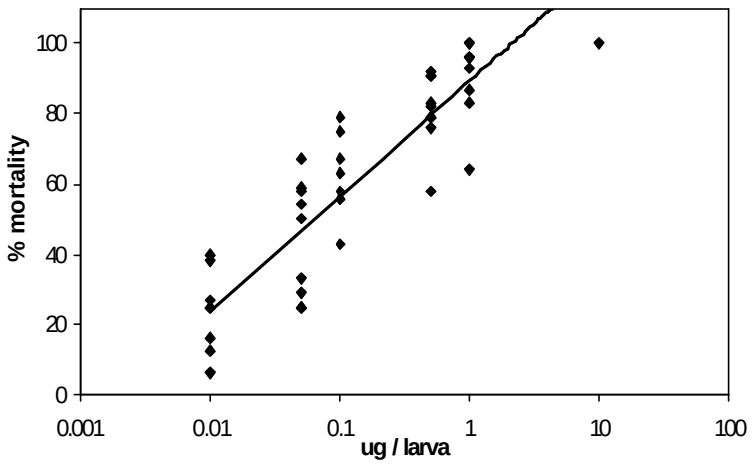
Insecticides and bioassay

Technical grade (99%) spinosad (85% spinosyn A and 15% spinosin D), was kindly provided by De-Nocil, India ltd. The compound was dissolved in analytical grade acetone and used for bioassays. Third instar larvae were randomly assigned to topical application on the dorsal thorax with 1.0 µl of 5-6 serial dilutions of technical insecticide in acetone with a Hamilton repeating dispenser. At least 20-40 larvae were used per concentration. After treatment, larvae were held individually in 12- well tissue culture plates containing semi-synthetic diet, at 25(±2) ^{0}C for 72 hours when mortality was recorded. Larvae were considered dead if they were unable to move in a co-ordinated manner when prodded. All rearing and bioassay operations were carried out at 25(±2) ^{0}C under a 12:12h light: dark regime. Dose-mortality regressions were computed by probit analysis (Finney, 1971). When required, corrections for control mortality were made using Abbott's formula (Snedecor and Cochran., 1989), control mortality never exceeded 2%.

Results and discussion

The results of the standard topical bioassay (Table 1) on the 'Reading susceptible' strain indicated that spinosad has an insecticidal activity that is slightly less than cypermethrin and much better than the conventional insecticides such as endosulfan and quinalphos. The toxicity of spinosad (Table 2) was relatively less variable falling within a range of an LD_{50} of 0.023 to 0.24 µg/larva and an LD_{90} of 0.27 to 4.33 µg/larva. The populations collected from Central and Northern states of India exhibited an LD_{50} of 0.023 to 0.084 µg/larva and an LD_{90} of 0.27 to 1.266 µg/larva. Whereas the populations from Rangareddy and Guntur dis-

Figure 1. Cumulative log dose mortality response of third instar *H. armigera* larvae to spinosad



tricts of South India were slightly less susceptible at an LD_{50} of 0.15 and 0.24 µg/larva and an LD_{90} of 1.59 and 4.33 µg/larva respectively. The reasons for this variation in susceptibility in the South Indian populations are not clear at this stage. The relatively shallow slopes of 0.85 to 1.38 in the field populations as compared to 1.708 in the 'Reading susceptible' strain appear to suggest a minor extent of heterogeneity in the populations with respect to some factor that may be responsible for the slight variation in susceptibility to spinosad. The data from all assays were pooled together and subjected to probit analysis to obtain a cumulative log dose probit response (Fig. 1). The LD_{50} and LD_{99} deduced from the data were 0.058 and 9.85 µg/larva respectively. This data would be useful to consider the LD_{50} of 0.058 as the baseline susceptibility index for resistance monitoring through the conventional log dose probit assays and the LD_{99} of 10.0 µg/larva as diagnostic dose for routine monitoring of resistance to spinosad through diagnostic dose assays.

The cotton bollworm, *H. armigera* is the major pest of cotton and legumes in India. Annual yield losses attributable to this pest in India alone are estimated to be of the order of US$ 290-350 million (King, 1994). It is estimated that insecticides worth about Rs 1800 crores are used in agriculture in India, of which nearly 50% is used for cotton crop protection. In recent years, management of this pest has become increasingly difficult due to its development of resistance to most chemical classes of insecticides commonly used in the country (Armes *et al*., 1996). Pyrethroid resistance levels are particularly high, in India (upto 6500-fold) (Armes *et al*., 1996), significantly decreasing the efficacy of this group of insecticides against *H.armigera*. In India, an intensive countrywide resistance monitoring programme has demonstrated that pyrethroid resistance is now widespread across the country and significant levels of cyclodiene and organophosphate resistance is also present in most populations. Of the many novel groups of insecticides

Table 1. Toxicity of topically applied spinosad, cypermethrin and conve tional insecticides on 'Reading susceptible' third instar H.armigera larvae

	LD_{50} (95 % F.L)	LD_{90}	LD_{99}	Slope + S.E	$\chi 2$	n	d.f
Reading (s)							
Cypermethrin	0.005 (0.003-0.007)	0.018	0.05	2.246 ± 0.37	2.87	140	3
Endosulfan	0.551 (0.382-0.759)	1.98	5.63	2.304 ± 0.37	0.92	120	3
Quinalphos	0.113 (0.086-0.151)	0.282	0.59	3.240 ± 0.54	1.72	143	3
Spinosad	0.062 (0.039-0.091)	0.347	1.42	1.708 ± 0.26	2.55	123	3

Table 2. Toxicity of topically applied spinosad on different F1 populations of third instar H.armigera larvae collected from various cotton growing regions of India

	LD_{50} (95 % F.L)	LD_{90}	LD_{99}	Slope ± S.E	$\chi 2$	n	d.f
North India							
Bhatinda	0.027 (0.011-0.047)	0.267	1.72	1.294 ± 0.25	1.49	116	3
Haryana	0.042 (0.018-0.069)	0.448	3.10	1.244 ± 0.25	2.16	116	3
Central India							
Nanded	0.037 (0.007-0.076)	1.158	19.13	0.857 ± 0.22	2.88	114	3
Parbhani	0.042 (0.013-0.078)	0.839	9.64	0.985 + 0.22	1.77	116	3
Wardha	0.084 (0.047-0.132)	0.707	4.02	1.384 ± 0.25	1.89	108	3
Nagpur	0.054 (0.023-0.099)	1.266	16.41	0.939 ± 0.17	0.16	132	3
Akola	0.023 (0.006-0.046)	0.522	6.619	0.947 ± 0.21	1.35	116	3
South India							
Rangareddy	0.154 (0.091-0.260)	1.594	10.72	1.262 ± 0.23	0.25	107	3
Guntur	0.245 (0.138-0.430)	4.33	45.06	1.028 ± 0.17	3.52	139	4

LD_{50}, LD_{90} and LD_{99} are expressed as µg/larva

Abbreviations used : LD = lethal dose, S.E = standard error, n = total number of larvae, d.f = degrees of freedom.

that have been recently introduced for lepidopteran pest management in cotton, spinosad is one of the few most promising chemicals, which has a favourable mammalian toxicity and environmental profile (Sparks *et al*., 1995). The availability of a novel chemical group, with a mode of action that is different from the insecticides in current use, is an asset to insecticide resistance management programmes. Especially in countries like India, where management of the cotton bollworm, *H. armigera*, has become difficult with the available chemistries on account of resistance.

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Comparison of Bio Assay Techniques for Resistance Monitoring in *Plutella Xylostella* Linn

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Insecticide resistance is becoming a major concern in insect pest management in our country. The insect pest with most severe resistance problems for cruciferous crops in India is diamondback moth (DBM), *Plutella xylostella* (Linn.). Resistance monitoring is essential for resistance management programms. To monitor the development of true pesticide resistance, base line values and suitable method for detection of insecticide resistance are prerequisite. Various workers use direct spraying in potters tower, filter paper impregnation, topical application and leaf- residue bio assay methods.

Since baseline values of various insecticides are not available against DBM, two commonly used methods viz., topical application and leaf residue bioassay methods were tested to develop an accurate, suitable, reliable, and economical method to measure the resistance of *P. xylostella*.

To assess the methods, larvae were collected from eight different locations in and around Hyderabad, Andhra Pradesh state and were tested against eight insecticides. The details of village(s) and chemicals tested are mentioned in Table 1.

In each bioassay treatment, ten insects were used with three replications. As described by Leibee and Savage (1990), for topical application, 0.05 µ1 drop let of each insecticide were applied with micro syringe to the mid dorsum of an anaesthetized early fourth instar larvae. After topical application of the larvae were placed in petriplates containing the cabbage leaves for feeding.

In leaf residue bioassay methods the cabbage leaf disc (5cm cut from fully expanded leaves grown in insecticide free field) were immersed in test solutions for 10 seconds, drained on filter paper and dried for one hour at room temperature (Fauziah *et al*., 1990). The mortalities were recorded at 24, 48 and 72 hrs. The data was subjected to probit analysis (Finney, 1952), resistant ratios were computed using formula of FOA 1979. The resistant ratios were calculated using LC_{50} values of susceptible strain to various insecticides as obtained by Sannaveerappanavar (1995). To determine the suitability of different bioassay techniques, the slope values obtained in both the techniques were compared using paired 't' test and are only presented (Tabashnik and Cushing, 1989), since the slope of a probit regression line is inversely proportional to the standard deviation of log tolerances. The present study on two different methods of bio assay with eight insecticides for eight field propulations of *P. xylostella* revealed that field collected DBM larvae differed significantly in their susceptibility to insecticides (Table 1). Resistant ratios calculated taking Bangalore strain as standard indicated higher degree of resistance to fenvalerate followed by cypermethrin in both the methods employed. Results indicated that among the eight insecticides tested there was significance difference in 't' values as was observed with

monocrotophos, acephate, carbaryl, cartap, cypermethion. Similar results were also obtained by Tabashnik and Cushing (1987) who reported that when different methods are employed, it leads to comparable resistance ratios. The results amply proved that leaf residue bioassay has clear advantage over topical application, as it is time and labour saving method.

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Table 1. Comparision of Different Methods for Monitoring Resistance in Diamondback Moth

Name of the Chemical	Average LC_{50}		Average Resistance Ratios		T-Value
	Topical Bioassay	Leaf Residue Bioassay	Topical Bioassay	Leaf Residue Bioassay	
Endosulfan	0.3603	0.0739	32.22	6.61	1.12
Monocrotophos	0.2721	0.1000	66.03	24.14	3.89
Malathion	0.2098	0.0446	24.13	5.14	0.32
Acephate	0.2429	0.0719	27.62	8.18	2.57
Carbaryl	0.4476	0.0519	28.21	3.27	4.95
Cartap	0.0792	0.0353	5.94	2.65	36.61
Cypermethrin	0.0828	0.0266	613.7	197.49	7.79
Fenvalerate	0.1345	0.0171	4576.23	590.09	1.47

**t-Value significant at 0.05 percent probability

Cypermethrin Pharmacokinetics in Laboratory and Field Strains of *Helicoverpa zea* (Lepidoptera: Noctuidae)

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INTRODUCTION

Helicoverpa zea (Boddie), the bollworm, is a major pest of corn and cotton in the United States and elsewhere. *H. zea* control has been widely accomplished by insecticides, and resistance to some organochlorines, organophosphates and carbamates has been reported (Spark 1981). Resistance to pyrethroids has been reported in several insect species (Sawicki 1985). *H. zea*, however, are still susceptible to pyrethroids, and only in the last decade occasional control difficulties with pyrethroids have been reported. Hsu and Yu (1991) reported a three-fold resistance to permethrin to a population of *H. zea* larvae collected from a corn field in Florida during spring 1990. *H. zea* collected from the fields of cotton and sweet corn in Illinois and Arkansas during 1991 where control difficulties with pyrethroids were experienced possessed low levels of resistance to several pyrethroids, with resistance ratios at the LD_{90} ranging from 2.1 to 18.0 (Abd-Elghafar *et al*. 1993). These results are believed to constitute the first unequivocal demonstration of pyrethroid resistance in *H. zea*. Kanga *et al*. (1996) observed three-fold difference in the toxicity of cypermethrin between laboratory and field strain of *H. zea* males. Brown *et al*. (1997) reported that adults of *H. zea* collected from the fields of cotton in Estill, South Carolina during 1996 where control

difficulties with pyrethroids were experienced possessed low levels of resistance to cypermethrin (2.5 µg/vial gave 17.6% and 92% mortality in field and laboratory strains, respectively) and cyhlothrin (2.5 µg/vial gave 6.6% and 100% mortality in field and laboratory strains, respectively).

Cypermethrin is not only widely used pyrethroid to control *H. zea* but also commonly used in resistance monitoring programs. To better understand cypermethrin pharmacokinetics in *H. zea*, we examined the penetration and metabolism of cypermethrin in larvae of a composite *H. zea* strain that had been selected with cypermethrin and compared with that in larvae of pyrethroid-susceptible laboratory strain of *H. zea*.

MATERIALS AND METHODS

Insect Strains

A composite strain of *H. zea*, referred to hereinafter as HZC, was begun in February 1993 and was composed mainly of the Arkansas strain with additions of field collections from Illinois and Missouri. Details regarding these strains and a pyrethroid-susceptible strain of *H. zea* (BCIRL-S) have been reported (Abd-Elghafar *et al*. 1993, Abd-Elghafar and Knowles 1996). Fourth instars of the multiple generations (F_{1-10}, F_{19}, F_{21-23}) were treated topically with cypermethrin; the dosage usually was 0.3 µg per larva, but 0.5 or 0.6 µg per larva was used occasionally. Toxicity studies were conducted with third instars from generations F_{17} and F_{28}. A virus seriously impacted the colony at generation F_{13-14} and by a rearing chamber malfunction at generation F_{20}. At generation F_{20} eggs and larvae were received from the Arkansas field and mixed with the HZC larvae. The LD_{50} and LD_{90} values (95% CL) of cypermethrin to generation F_{17} HZC *H. zea* third instars were 6.67 (4.46-13.71) and 58.56 (23.36-514.12) µg per gram, respectively. For generation F_{28} third instars the LD_{50} and LD_{90} values were 2.84 (2.04-4.07) and 19.06 (10.56-60.71) µg per gram, respectively. When compared with susceptible strain, resistance ratios (95% CL) were 8.89 (3.89-34.63) and 24.61 (5.93-102.12) for the LD_{50} and LD_{90}, respectively, for larvae from generation F_{17}. For generation F_{28} larvae, resistant ratios were 3.80 (2.50-5.77) and 8.01 (7.12-9.59) for the LD_{50} and LD_{90}, respectively. Pharmacokinetic studies were conducted with HZC third instars from generation F_{29}. *H. zea* were reared as described by Abd-Elghafar *et al*. (1993).

Chemicals

Samples of technical-grade cypermethrin (94%; 38:62 *cis-trans*) and two samples of cypermethrin-^{14}C radiolabeled at C-1 of the cyclopropyl ring of the acid moiety were provided by FMC (Princeton, NJ). Separation of geometrical isomers of cypermethrin-^{14}C was accomplished by thin-layer chromatography (TLC) on silica gel-coated glass plates (250 µm, LK6DF, Whatman) with *n*-hexane:ethyl:ether (10:1, three developments) (McKee and Knowles 1985). The radiochemical purity of the (*1RS*)-*trans* (specific activity 56 mCi/mmol, R_f 0.50) isomer was greater than 98.0%.

Pharmacokinetic Studies

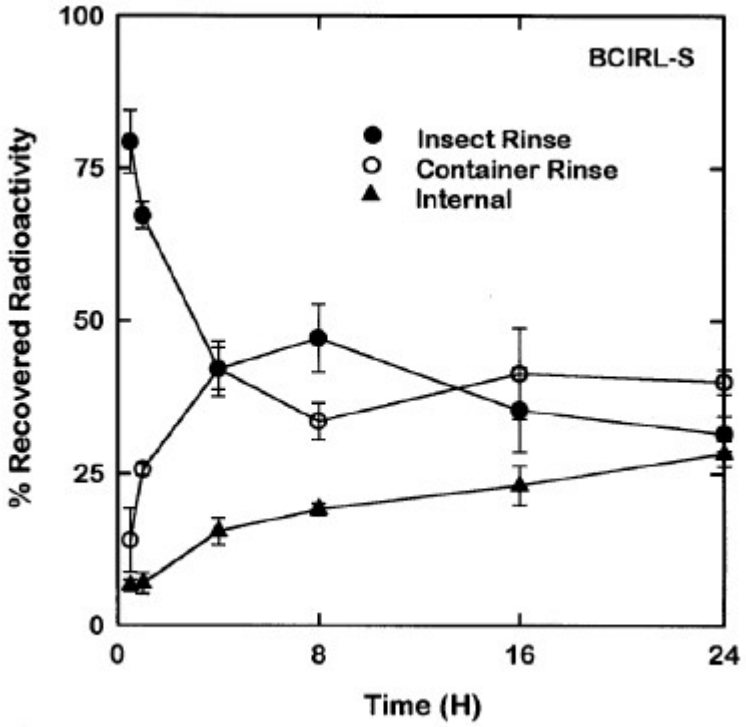
Third instars from each strain were starved for three hours and were each treated topically on thoracic dorsum with 1.0 µl of acetone solution containing trans-cypermethrin-^{14}C (0.007 µg). Treated larvae were kept individually in glass vials (20 ml) for intervals of 0.5, 1, 4, 8, 16, or 24 hours at 25°C. Three replicates of 20 to 30 live larvae per replicate were analyzed. Larvae were rinsed 3 times with 4 ml of acetone. Rinses were combined, concentrated with nitrogen, and an aliquot was radioassayed in 10 ml of ScintiVerse BD (Fisher, St. Louis, MO) with a Beckman LS 7500 liquid scintillation counter to yield the total radiocarbon in the insect rinse. Larvae were extracted by homogenizing 3 times in 5 ml of methanol, and extracts were pooled, concentrated with rotary evaporator, and an aliquot was radioassayed to determine total radiocarbon. Tissue residue remaining after extraction was solubilized with ScintiGest (Fisher) and radioassayed to determine unextractable radiocarbon. Radiocarbon in the methanol extract and unextractable fraction represented total internal radiocarbon. Holding containers were rinsed 3 times with 4 ml of acetone, and an aliquot of combined rinses was radioassayed to determine total radiocarbon. Aliquots of the insect rinse, internal extract and container rinse were subjected to TLC and radiocarbon on TLC plates was quantitated by scanning on a Rita-3200 TLC Analyzer (Raytest, Wilmington, DE).

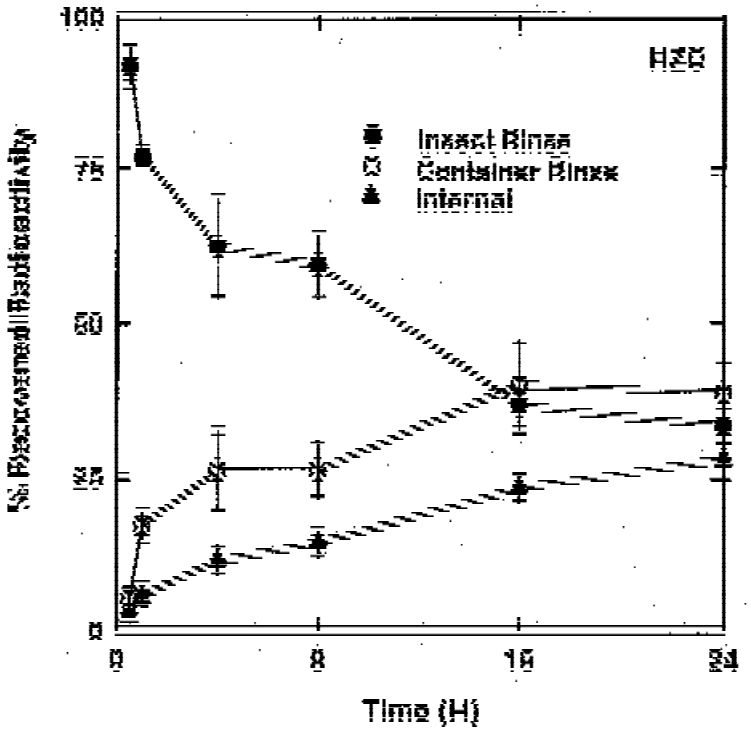
RESULTS AND DISCUSSION

Distribution of radiocarbon following topical treatment of BCIRL-S and HZC third instars with trans-cypermethrin-14^C is given in Fig. 1. Radiocarbon levels in the insect rinses decreased with time in each case. With BCIRL-S larvae, the initial rate of decrease was considerably faster when compared with HZC larvae. Half-times for disappearance of radiocarbon in the insect rinses of BC 0IRL-S and HZC larvae were 3 and 11 h, respectively. The decreases in radiocarbon levels in the insect rinses were accompanied by increases in radiocarbon levels in the internal

fraction. With BCIRL-S and HZC larvae, internal levels increased slowly throughout the test period comprising about 29% and 28% at 24 h, respectively. Levels of radiocarbon in the container rinse of HZC larvae were generally lower than those in BCIRL-S larvae at the early sampling intervals and similar at 16 and 24 h.

The nature and amounts of radiocarbon after topical treatment of BCIRL-S and HZC third instars with trans-cypermethrin-^{14}C are presented in Table 1. Although there were several minor exceptions, levels of parent compound generally were higher than those of combined metabolites in the insect rinses of BCIRL-S and HZC larvae, while reverse was the case in the container rinses. With the internal fraction, levels of the parent compound reached a maximum of 5.8% at 8 h and 7.6% at 16 h in BCIRL-S and HZC larvae, respectively. In both experiments, internal levels of combined metabolites exceed levels of parent compound at least two-fold at 16 and 24 h. At 24 h, the levels of metabolites in the container and insect rinses and internal fraction totaled 71.1% and 67.5% in BCIRL-S and HZC larvae, respectively. Level of metabolites in the container and insect rinses at 24 h totaled 47.5% and 46.8% in BCIRL-S and HZC larvae, respectively. At least five metabolites were detected in most rinses and extracts.





With HZC third instars, *trans*-cypermethrin penetration was considerably slower than that observed with BCIRL-S third instars. Half-times for *trans*-cypermethrin penetration based on actual disappearance of total radiocarbon from the insect rinses were about 3.67 times slower in HZC strain when compared with BCIRL-S strain. However, no marked differences in rates of *trans*-cypermethrin metabolism and excretion between the two strains were detected. Thus, the decreased rate of penetration probably contributed to cypermethrin resistance in HZC larvae. In their study of the pharmacokinetics of fenvalerate using the same two strains, Abd-Elghafar and Knowles (1996) found that the pyrethroid penetrated at somewhat lower rate into HZC *H. zea* third instars (F_{13-14}) than into pyrethroid-susceptible *H. zea*. Fenvalerate was also metabolized at slightly higher rate in resistant larvae than in susceptible larvae. The mechanism responsible for the slower penetration of pyrethroids

in HZC third instars as compared with BCIRL-S larvae is not known. However, reduced penetration has been found in several resistant strains of insects, and the phenomenon has been intensively examined in house flies (Oppenoorth 1985). Farnham (1973) has shown that house flies resistant to pyrethrins I have a penetration factor, *Pen*, on the same autosome as *kdr*. By retarding penetration, *Pen* presumably enables detoxification mechanisms to keep parent compound levels from achieving lethal concentrations.

These baseline pharmacokinetic investigations of cypermethrin in the larvae of laboratory and field strains of *H. zea* will be useful in future mechanistic studies in the case of higher levels of resistance to this pyrethroid are observed.

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Table 1. Nature and relative amount of radiocarbon after topical treatment of *H. zea* third instarts with trans-cypermethrin-14C

Nature and relative amount of radiocarbon	% Recovered radiocarbon at indicated hours after treatment[a]					
	0.5	1	4	8	16	24
BCIRL-S Strain						
Container rinse						
trans-Cypermethrin	2.8 ± 1.7	2.6 ± 0.1	17.3 ± 0.3	6.0 ± 0.1	8.4 ± 2.4	9.3 ± 0.9
Metabolites	11.3 ± 3.5	23.1 ± 0.8	24.9 ± 3.1	27.6 ± 1.9	33.0 ± 5.0	30.8 ± 1.1
Insect rinse						
trans-Cypermethrin	71.8 ± 4.8	55.2 ± 0.5	27.7 ± 2.1	26.5 ± 2.6	17.3 ± 3.7	14.9 ± 1.0
Metabolites	7.5 ± 0.4	12.1 ± 1.7	14.5 ± 2.4	20.7 ± 2.9	18.2 ± 3.2	16.7 ± 1.9
Internal						
trans-Cypermethrin	3.9 ± 0.3	3.4 ± 1.1	5.7 ± 1.1	5.8 ± 0.4	4.8 ± 1.0	4.5 ± 1.4
Metabolites	2.6 ± 0.7	3.5 ± 0.6	9.7 ± 1.1	13.3 ± 0.5	18.1 ± 2.1	23.6 ± 0.7
Unextractable	0.1	0.1	0.2	0.1	0.2 ± 0.1	0.2
HZC Strain						
Container rinse						
trans-Cypermethrin	3.7 ± 1.6	7.4 ± 0.7	9.6 ± 3.1	6.4 ± 2.0	9.7 ± 1.2	6.0 ± 2.5
Metabolites	1.4 ± 0.3	9.7 ± 1.5	16.5 ± 3.3	19.8 ± 2.5	30.3 ± 6.1	32.7 ± 3.2
Insect rinse						
trans-Cypermethrin	89.6 ± 2.6	73.0 ± 0.9	54.9 ± 7.9	47.2 ± 4.0	26.0 ± 3.7	19.2 ± 1.7
Metabolites	2.3 ± 0.5	4.1 ± 0.5	7.5 ± 0.1	12.3 ± 1.0	10.7 ± 0.6	14.1 ± 0.7
Internal						
trans-Cypermethrin	2.2 ± 0.7	4.1 ± 1.2	5.9 ± 1.7	5.6 ± 0.6	7.6 ± 0.9	6.9 ± 1.6
Metabolites	0.8 ± 0.1	1.6 ± 0.4	5.5 ± 0.5	8.5 ± 1.4	15.4 ± 1.3	20.7 ± 1.3
Unextractable	0	0.1	0.1	0.2	0.3	0.4

[a]Values are means ± SD, n = 3.SD not given when < 0.1

Determination of pathways of resistance in a pyrethroid resistant horn fly colony

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INTRODUCTION

The metabolic mechanisms of resistance were examined by the use of selective synergists in a super resistant *in vivo* strain of horn flies. The super resistant strain was originally collected from steers at the Red River Research Station in Shreveport, Louisiana, after 4-5 years of exposure to pyrethroid ear tags. Byford *et al*. (1995) described the colony as resistant to multiple pyrethroid insecticides and DDT. This colony was maintained on steers in enclosed fly rearing facilities at New Mexico State University where it was selected with a permethrin spray formulation applied to the backs of the stanchioned steers.

In 1990, Sparks *et al*. showed that the pyrethroid resistance in this colony was due to decreased penetration and increased metabolism of ^{14}C-permethrin. A filter paper bioassay of these flies indicated that they had an LC_{50} estimate of 20.5 $\mu g/cm^2$, with a resistance ratio of 17, however, they were susceptible to diazinon.

In August 1992, a colony of these flies was established at the Knipling-Bushland U.S. Livestock Insect Research Laboratory (KBUSLIRL) in Kerrville, Texas. At the KBUSLIRL, selection with cyhalothrin greatly increased the LC_{50} estimate. The current LC_{50} estimate is 2500 $\mu g/cm^2$, with a resistance ratio of 1100. This colony is still susceptible to diazinon.

Guerrero *et al*. (1998) determined through PCR screening that the level of resistance in the super resistant strain is mainly due to *kdr* and super-*kdr* mutations of the sodium channel. However, because increased ^{14}C-permethrin metabolism was described by Sparks, synergists were used in this study to determine the involvement that esterase, cytochrome P-450, and glutathion s-transferase may have in the pyrethroid resistance of this super resistant strain of horn flies.

MATERIALS AND METHODS

Three to five day old horn flies from the susceptible and super resistant horn fly strains maintained at the KBUSLIRL were bioassayed using the treated filter paper technique as described in Sheppard and Hinkle (1987).

The role of metabolic enzymes in pyrethroid resistance was determined with the use of synergists. Synergists used in this study were piperonyl butoxide (PBO), an inhibitor of cytochrome P450; triphenylphosphate (TPP), an inhibitor of esterase; and diethyl maleate (DEM), an inhibitor of glutathion S-transferase (GST). These synergists were added to the permethrin formulations at a constant rate of 10%,

Table 1. Horn fly strains treated with permethrin and synergists using a treated filter paper bioassay

Strain	Synergist	n	Slope ± SE	LC_{50} (95% CL)	χ^2	RR (95% CI)	SR (95% CI)
Susceptible	-	975	7.495 ± 0.420	2.002 (1.857-2.153)	55.9*		*
	PBO	1275	4.735 ± 0.269	0.697 (0.639-0.751)	49.3*		2.873 (2.641-3.125)
Super Resistant	-	1800	1.964 ± 0.129	2883.497 (2327.57-3987.20)	79.5*	1441.731 (1283.09-1619.99)	*
	PBO	1575	2.660 ± 0.149	159.702 (138.136-180.713)	72.6*	229.285 (205.98-255.23)	18.064 (15.795-20.659)
Susceptible	-	975	6.037 ± 0.391	2.366 (2.145-2.685)	71.5*		*
	TPP	1125	5.525 ± 0.326	2.143 (1.990-2.332)	50.6*		1.104 (1.049-1.162)
Super Resistant	-	1350	2.337 ± 0.194	3073.856 (2469.41-4591.16)	66.9*	1299.505 (1157.09-1459.45)	*
	TPP	1125	2.523 ± 0.234	2942.249 (12324.36-5091.77)	79.5*	1372.300 (1182.07-1593.15)	1.045 (0.872-1.254)
Susceptible	-	665	4.561 ± 0.419	1.663 (1.288-1.935)	55.9*		*
	DEM	1045	2.011 ± 0.333	2.628 (2.219-3.791)	3.9*		-
Super Resistant	-	1500	1.955 ± 0.134	2459.189 (1980.23-3345.08)	54.8*	1479.135 (1286.14-1700.07)	*
	DEM	1500	2.244 ± 0.111	889.164 (749.82-1060.9)	84.2*	338.904 (293.11-391.86)	2.762 (2.371-3.216)

*Indicates significance (P>0.05)

10% and 0.5% respectively (the highest rate that would not cause mortality in *Haematobia irritans* when applied alone, (unpublished data).

Probit analysis was run on bioassay results using POLO-PC (LeOra Software 1987). Resistance and synergism ratios were calculated by taking into account the variance and covariance of the slope and intercept of each regression line at the LC_{50} for the comparison in question using the method of Robertson and Preisler (1991). Resistance ratios were calculated relative to the susceptible strain and synergism ratios were calculated relative to the formulation that contained permethrin only. Significance of each comparison was determined only if 1 was not included in the confidence interval (Robertson and Preisler 1991).

RESULTS AND DISCUSSION

The super resistant strain demonstrated a synergism ratio of 18 when exposed to PBO in the presence of the pyrethroid permethrin, compared to the susceptible strain's synergist ratio of 2 (Table 1, Figure 1 and 2). The addition of TPP produced no significant change in the synergist ratios of either the super resistant or susceptible strain (Table 1, figure 3 and 4). DEM demonstrated no synergism in the susceptible strain, but slight synergism of 2X occurred in the super resistant strain (Table 1, Figure 5 and 6).

These results indicate that increased cytochrome P450 and, to a lesser extent, glutathione s-transferase activity in the super resistant strain contributes to the high resistance level. This resistance is likely a result of rapid metabolism or sequestration of pesticide prior to it reaching the target site. There is no indication that esterases are involved in pyrethroid resistance for this strain.

The metabolic mechanisms of resistance in the super resistant strain discussed here are likely to be secondary to the *kdr* or super-*kdr* sodium channel mutations described earlier by Guerrero *et al*. (1998). Guerrero determined through PCR screening that horn flies with *kdr* and super-*kdr* mutations of the sodium channel were much more resistant to pyrethroid insecticides than flies that did not have these mutations. Given that super resistant flies exposed to permethrin and synergists still have a resistance ratio of at least 229 (Table 1) to permethrin than susceptible flies exposed to synergists indicates that the sodium channel mutations are the main source for resistance in this strain. The increased metabolic activity of this strain may be a result of secondary mutations in response to the continuous selection of cyhalothrin in the laboratory. It could also be a holdover of intermediate resistance mechanisms that were selected for in the field (Red River Research Station in Shreveport, Louisiana) or the laboratory (New Mexico State University) prior to the selection for *kdr* and super-*kdr* mutations (KBUSLIRL).

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Figure 1. Susceptible - PBO

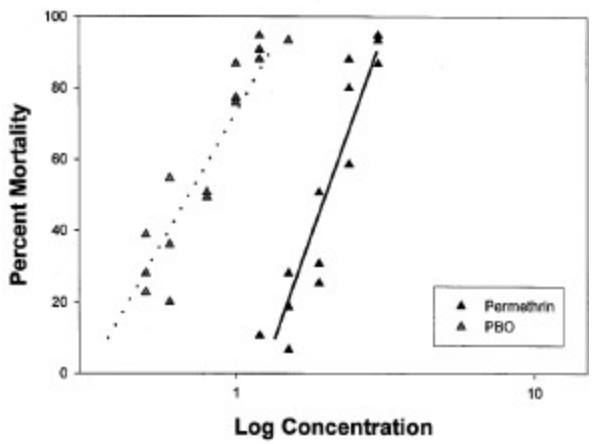
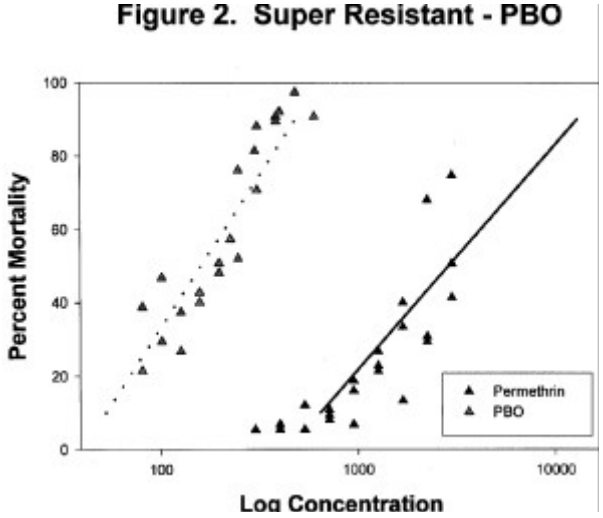
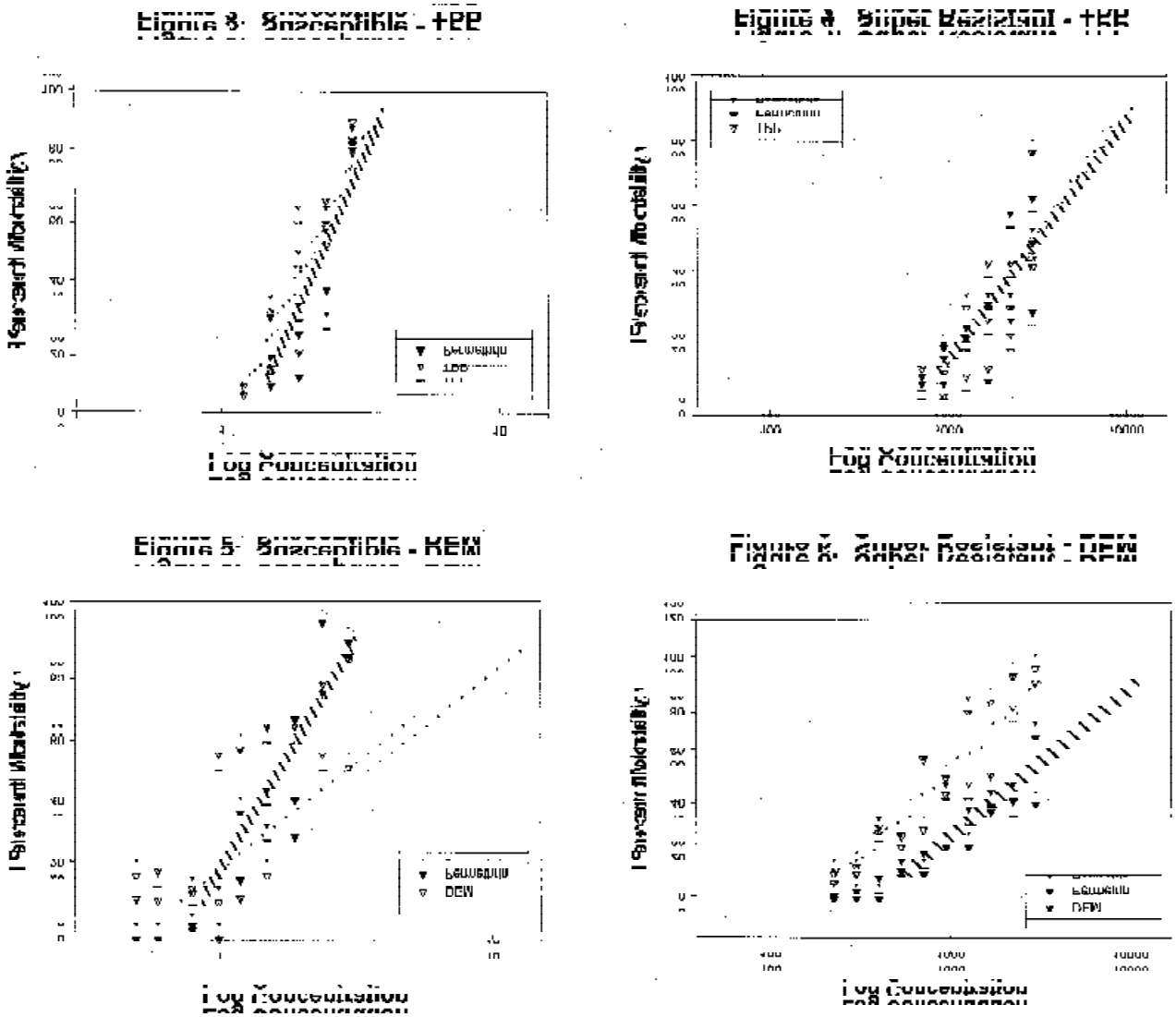


Figure 2. Super Resistant - PBO





Effects on Reproduction of Silverleaf Whitefly, *Bemisia argentifolii* due to Bifenthrin Resistance

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Management of insecticide resistance is critical for controlling silverleaf whitefly *Bemisia argentifolii* Bellows & Perring (Tan *et al.* 1996, and Riley *et al.* 1999). Resistant populations of this insect have been observed to rapidly increase in cotton, vegetable, and ornamental crops following insecticide application, possibly due to variation of reproduction and/or development linked to resistance to insecticides. Variation of reproduction due to direct insecticide stimulation (hormoligosis) has been reported for thrips, flies and aphids (Morse and Zareh 1991, Bouchard and Wilson 1987, Halpern and Morton 1986, and Rider and Wilde 1998, respectively). We found that inheritance of bifenthrin resistance can be associated with reproduction changes in whiteflies (Riley 1997). However, we observed an indirect stimulation of population growth in silverleaf whitefly associated insecticide resistance selection. Because of this pest's ability to reproduce in high numbers and its devastating impact on crops, understanding whitefly reproduction associated with insecticide resistance has become an important component of our research project.

Reproduction and development experiments were conducted with bifenthrin-resistant, susceptible, F_1 cross and backcross populations of silverleaf whitefly in the lab. Responses (LC_{50}) of resistant and susceptible populations to bifenthrin

were 0.005µg/vial and 143.79µg/vial, respectively (Staetz *et al.* 1992). One female from a bifenthrin-resistant population and one male from a susceptible population were placed in a gelatin capsule on a fully expanded leaf of a semi-mature cotton plant. Seven to nine pairs of adults per cross and backcross were monitored daily for oviposition and survivorship of egg clutches. Pairs were moved every 24 hours on the leaf and each consecutive clutch of eggs was monitored for development. The resulting F_1 hybrid progenies were then backcrossed to resistant parents. The results showed that net reproduction (Ro) rates ranged from 9 to 26 and significant differences among these populations were demonstrated. A two-fold increase in reproduction was observed in the backcross population compared with the parent lines (Figure 1). There were distinct patterns in the progeny that developed from these populations. Whitefly adult emergence in some of the backcross populations was higher than that in their parental populations (approximately two fold). Duration of oviposition and mean egg production by whiteflies varied significantly across resistant, susceptible, F_1 cross and backcross populations, but were not positively correlated with net reproduction. Figure 1 shows the variation in oviposition, reproduction and development of resistant, susceptible, F_1 cross and backcross populations of whitefly. The important result was that the backcross to the resistant parent resulted in a two-fold increase in net reproduction.

The shift in oviposition patterns was not associated with direct chemical stress, but occurred as a result of some selection process. It was hypothesized that selection for insecticide resistance was associated with changes in oviposition patterns in *Bemisia argentifolii*, either as an effect linked to bifenthrin resistance or coincidentally occurring with the population crosses. The oviposition patterns appear to be heritable and can affect the progeny's ability to reproduce. The genetics of such a mechanism is unclear, however, data that we are currently developing provide strong evidence for heterosis.

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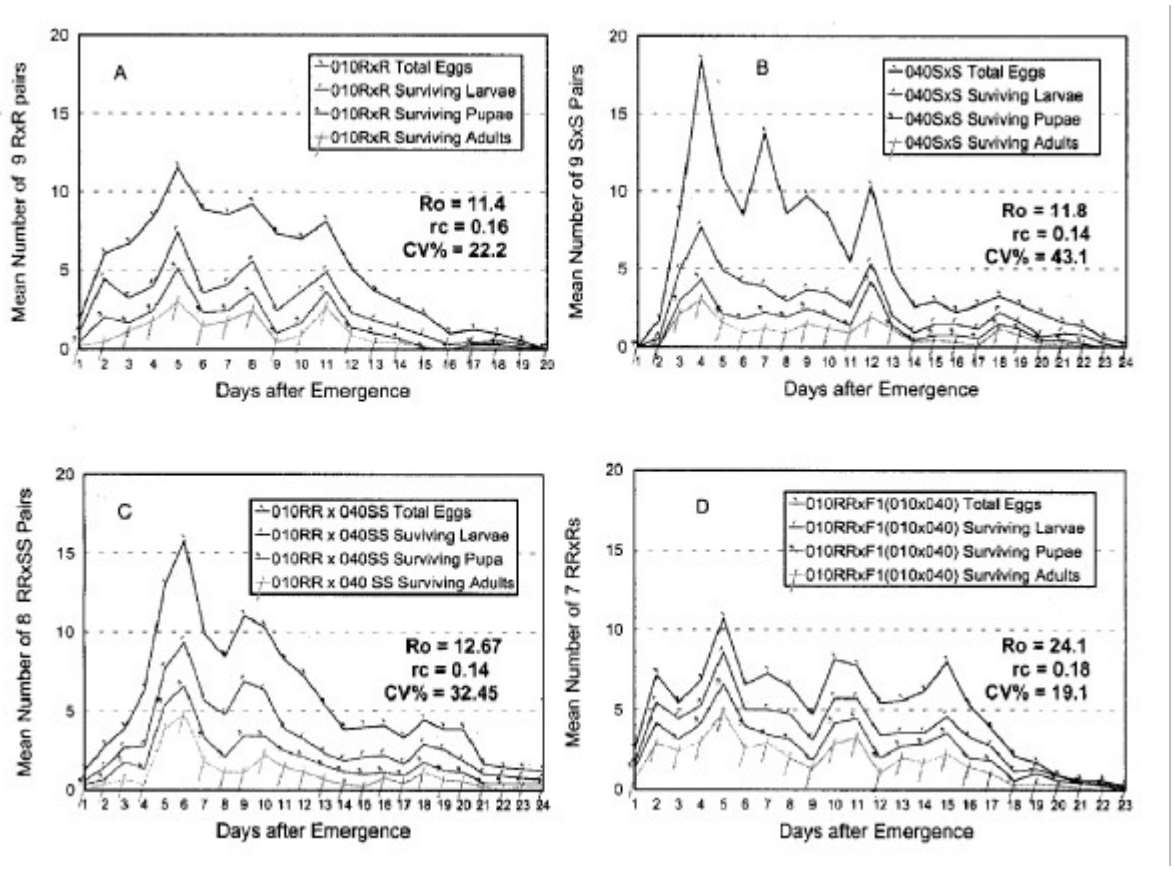

Fig. 1 Variation of oviposition in the resistant (010RR), susceptible (040SS) F_1 cross (010RRx040SS) and backcross (010RRxF1(010x040) populations of silverleaf whitefly *Bemisia argentifolii*. A: resistant population, B: susceptible population, C: F_1 cross population, and D: backcross population. Ro: net reproduction. rc: the capacity for increase. CV: coefficient of variability

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Evidence of Antibiotic Resistant Development on *Nosema* spp infecting Diamondback Moth Larvae

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INTRODUCTION

The diamondback Moth (DBM), *Pluetella xylostella* L., is the major pests of crucifers and it has been the subject of numerous researches because of its economic impact on the cabbage industry. The usual method employed in controlling this pest is by spraying insecticide including the biological one, *Bacillus thuringiensis* (Bt) (Talekar & Shelton 1993). Lately, the use of natural enemies has been emphasized as a result of pesticides resistant problem developed by DBM (Cheng, 1988; Tabahsnik *et al.*, 1990). The use of parasitoids, *Diadegma* spp. and *Cotesia* spp., has shown a promising result in controlling DBM in Guatemala (Biever *et al.*, 1994). The use of insect pathogens including microspordia (example - *Nosema* spp.) in controlling DBM has yet been reported, as it still in the laboratory studies (Wilding, 1986). However, Idris and Grafius (1999) reported that infection by *Nosema* sp. (most probably *N. bombycis*) has caused major concern in the rearing of DBM and its parasitoids in Malaysia and United States. The use of predatory arthropods in biological control necessitates that the predators are pathogen-free to avoid the risk of introduction of pathogens that could destabilized the natural fauna. Cultures of insects and probably other invertebrates, reared at high density for experimental use, can be decimated if microsporidia are introduced by a few infected individuals. Although many *Nosema*-infected individuals are successfully surviving, the result of any study using them may be invalid and cause misleading the fact. This is because the protozoan may enhance the toxicity effect of Bt and other insecticides on DBM larvae (Manasherob *et al.*, 1994).

The usual techniques being employed in reducing microsporidial infection in the laboratory are heat treatment, selection, pairing of healthy individuals and adding Fumagillin or Fumidil-B into the artificial diet (Steinhaus, 1949; Geden *et al.*, 1995). However, there is a great possibility of resistance to Fumidil-B with prolonged use of this antibiotic in rearing DBM. For example, Idris and Sajap (1999) reported that as high as 70% of DBM egg samples collected from DBM culture of the Malaysian Agriculture Research and Development Institute (MARDI) contained *Nosema* spores. This is in spite of the fact that these samples were raised on artificial diets treated with Fumidil-B. The objective of our study was to investigate resistant development of *Nosema* spp. infecting DBM culture in which their artificial diet was incorporated with fumidil-B, a conventional antibiotic recommended for controlling microsporidian diseases (Steinhaus, 1949; Katznelson & Jamieson, 1952).

MATERIALS AND METHODS

The *Nosema* infected-DBM eggs and untreated artificial diet were provided by MARDI. DBM eggs were allowed to hatch for 3-days. Five different concentrations of Fumidil-B (25, 50, 100, 200 and 400 ppm) were prepared. A slice of 4 cm^2 and 0.1 cm thick artificial diet (prepared normally but without Fumidil-B) but was soaked into a respective

Fumidil-B solution for 15 minutes (to ensure diet being impregnated well by the antibiotic), air dried for 2 h and placed in a 15-cm diameter petri dish. Five first instar DBM larvae (3 h after hatching) were randomly selected and placed in a respective petri dish plus diet (25 larvae per treatment). Diets were changed daily. Larva mortality were recorded every other days started with 2 day after hatching until the day 10 as most surviving larvae started pupating. The untreated diet (treated with distilled water) was used as a control treatment. Each treatment was replicated four times. Percent mortality was calculated as the total number of larvae per replicate minus the accumulated dead larvae and divided by the total larvae x 100. Data were analyzed by one-way ANOVA and the treatment's means were separated by Fisher's Protected LSD test (Abacus Concept, 1991).

RESULTS AND DISCUSSION

There was a significant difference in the percent of larval mortality among the treatments at all day's of data collection. Percent mortality was higher at the lower than at the higher concentrations of Fumidil-B (Table 1). The highest larval mortality (92.5% at day 10) observed when larvae fed untreated diet. There was no significant difference in the percent mortality when larvae fed on diets treated with 100 and 200 ppm antibiotic solutions. This indicates that the 220 ppm Fumidil-B normally used in preparation of DBM artificial diet is unable to contain the development of the disease. Although there was a significant different in percent mortality of DBM larvae fed on diets treated with 200 and 300 ppm Fumidil-B solutions, except on day-6, the accumulated percent mortality was still considerably high (36.3%). Percent mortality was significantly lower when larvae fed diet treated with 300 ppm Fumidil-B solutions on day-8 and 10 than those treated with 200 ppm Fumidil-B. Further increase in Fumidil-B concentration (400 ppm) had significantly reduced the percent mortality, indicating the *Nosema* development within the larvae was suppressed and that only 10% larval mortality occurred. The mortality observed for larvae fed diet treated with 500 ppm was probably due to indirect effect of high concentration of an antibiotic on early instars as the accumulated mortality was similar till the day-10. In addition, there were no *Nosema* spores observed from dead individual of this treatment. Results of this study showed that the *Nosema* spp-infecting colony of DBM at MARDI might have developed resistant toward Fumidil-B and the disease development could only be checked when diet is treated with Fumidil-B at 500 ppm and above. The resistance develeopment may happen elsewhere but it may be unnoticeable. However, *Nosema* problems are always overcome by restart a new colony and/or plus other measured as suggested by Steinhaus (1949). There may be other methods that are easier and/or cheaper. One of the methods is by using other antibiotics. For example, Haque *et al*. (1993) reported that Albendazole is very effective against development of *Nosema bombycis* infecting *Spodoptera frugiferda* and *Heliocoverpa zea* cell in vivo and in vitro, and there was no deleterious effect of this antibiotic on the growth and ability of *H. zea.* This antibiotic is much cheaper and easier to buy from any pharmacy than Fumidil-B that is more expensive and difficult to get. Others antibiotics that could potentially replace Fumidil-B are those commonly used in veterinary medicine to combat protozoan diseases.

Table 1. Percent mortality (accumulative) of diamondback moth larvae fed on artificial diet treated with various concentration of Fumidil-B

Concentration (ppm) of Fumidil-B	Days after treatments (± S.E)				
	2	4	6	8	10
untreated diet	45.4 ± 5.6a	60.5 ± 7.8a	70.3 ± 8.5a	85.4 ± 10.2a	92.5 ± 10.2a
100	20.5 ± 10.5b	25.7 ± 7.3b	38.5 ± 5.6b	52.6 ± 6.3b	72.5 ± 7.6b
200	23.3 ± 11.3b	24.4 ± 6.7b	35.6 ± 4.7b	50.5 ± 7.3b	69.4 ± 8.2b
300	15.5 ± 10.2c	18.3 ± 5.6c	22.4 ± 5.3b	30.5 ± 5.4c	36.3 ± 4.3c
400	1.5 ± 2.4d	2.6 ± 4.2d	3.5 ± 5.6c	6.3 ± 3.3d	10.9 ± 3.8d
500	0d	1.5 ± 1.5d	1.5 ± 1.5c	1.5 ± 1.5d	1.5 ± 1.5d

Means with same letter in column are not significantly different (Fisher's Protected) LDS, P>0.05.

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High levels of resistance and cross-resistance to *Bacillus thuringiensis* Cry1 toxins in *Heliothis virescens* are due to reduced toxin binding and pore formation

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Heliothis virescens is one of the most important insect pests affecting cotton and other crops. For *H. virescens* control, insecticides based on the *Bacillus thuringiensis* (Bt) parasporal pesticidal proteins have been developed. These proteins posses a unique mode of action (Knowles 1994). After ingestion by the susceptible insect, they are solubilized and activated by midgut enzymes to toxic forms. Activated toxins bind to receptors and insert into the membrane of the columnar cells of the midgut epithelium, leading to cell lysis by osmotic shock. In 1996, transgenic cotton plants producing Cry1Ac toxin (the most active Bt toxin against *H. virescens*) were produced and commercialized in the US to control *H. virescens* populations in the field. One of the major concerns regarding the use of Bt transgenic plants is the observation that target insects can become resistant. Generation of transgenic cotton lines expressing different Bt toxins and conservation of refuges for susceptible individuals has been suggested as potential methods to prevent resistance. To assure the efficacy of these approaches as well as the usefulness of Bt toxins, information on the mode of action as well as on the potential mechanisms of resistance is needed.

Resistance to Bt insecticides has been reported to occur in wild populations of certain insect species (Tabashnik *et al*. 1990; Shelton *et al*. 1993) as well as in laboratory selected insects (Gould *et al*. 1992; Whalon et al. 1993; Oppert *et al*. 1994; Gould *et al*. 1995). To date, no incidences of resistance are reported for field populations of *H. virescens*. The YHD2 strain of *H. virescens* was developed in the laboratory from susceptible individuals (strain YDK) by selection against Cry1Ac (Gould *et al*. 1995). The YHD2 strain developed the highest levels of resistance known in this insect to Cry1Ac, and high levels of cross-resistance to other toxins including Cry1Aa, Cry1Ab and Cry1Fa. At the time, cross-resistance to Cry1Fa was unexpected, since this toxin does not share high homology with the Cry1A family of toxins. These results suggested that cross-resistance can be an important pitfall for strategies of Bt toxin pyramiding or alternation in transgenic plants. Binding competition experiments (Jurat-Fuentes and Adang, submitted) suggest that Cry1A and Cry1Fa cross-resistance is due to shared toxin binding sites.

The resistant strain YHD2 has been continuously selected with Cry1Ac toxin since 1995. Presently, the level of Cry1Ac resistance is 40-fold greater than in 1995. In bioassays, neonate YHD2 larvae were more than 230,000-fold resistant to

Cry1Ac, about 2000-fold cross-resistant to Cry1Ab, more than 130-fold to Cry1Fa and more than 20-fold to Cry1Aa. Activity of these toxins against the YDK susceptible strain larvae was high and similar to previous reports (Lee *et al.*, 1995). Cry1Ea was not toxic against either strain.

Although several mechanisms of resistance have been proposed for insect resistance to Bt toxins, the alteration of toxin binding to the specific receptors in the midgut is the best documented (Ferré *et al.* 1991; Lee *et al.* 1995; Van Rie *et al.* 1990).

To study the mechanism of resistance in the YHD2 *H. virescens* strain, binding and pore formation properties of Cry1Aa, Cry1Ab, Cry1Ac and Cry1Fa Bt toxins on brush border membrane vesicles (BBMVs) were measured. BBMV were prepared (Wolfersberger *et al.* 1987) from larval midguts of susceptible (YDK) and resistant (YHD2) *H. virescens* strains.

Purified Cry1A toxins were ^{125}I-labeled (Garczynski *et al.*, 1991) and used in BBMV binding assays. In these assays, quantitative toxin binding values can be obtained. All the ^{125}I-Cry1A toxins tested bound in a specific and saturable manner to BBMV from the susceptible strain, while specific toxin binding to resistant BBMV was not observed. This dramatic decrease in toxin binding was also observed in qualitative binding assays (Western blots) with Cry1A aswell as Cry1Fa toxins.

To study if this decrease in binding is important for toxin action, we measured toxin pore formation with a light scattering technique (Carroll and Ellar, 1993). In this technique, the pore forming activity of Cry1 toxins on a BBMV suspension was monitored as changes in the scattered light due to toxin-induced vesicle permeation in a hyperosmotic medium. Neither Cry1Aa, Cry1Ab, Cry1Ac nor Cry1Fa toxins were able to permeate BBMV from the resistant strain in any of the experiments. These toxins permeated susceptible vesicles to different extents, Cry1Ac being the most active toxin in the light scattering assay. Cry1Ea (not toxic against *H. virescens*) did not affect either susceptible or resistant BBMV, while Nystatin (a pore-forming antibiotic) permeated both BBMV suspensions to the same extent.

These results suggest that the dramatic reduction in toxin binding can account for high levels of resistance and cross-resistance to Cry1A and Cry1Fa toxins observed in the YHD2 strain. Thus, toxin-binding reduction prevents pore formation, which causes larval survival.

To study the transmission of the Cry1Ac resistance trait, binding and pore formation abilities of Cry1Ac to BBMV from larvae of the F1 crosses between susceptible and resistant adults were studied. Similar binding and pore formation values were obtained for reciprocal crosses, suggesting that resistance is not sex-linked. Furthermore, measured values were more similar to the values for susceptible BBMVs, indicating a partially recessive transmission of the resistant trait, as previously suggested from bioassay experiments (Gould *et al.*, 1995).

One of the possible explanations for reduced toxin binding is the absence of specific binding proteins on the resistant BBMV. To study this possibility, ligand-blotting experiments were done (Garczynski *et al.* 1991). The same patterns of binding molecules for all Cry1A and Cry1Fa toxins were detected for blotted susceptible and resistant BBMV proteins. This indicates that toxin-binding molecules are still present in the resistant BBMV, and they are able to bind toxin only under denaturing conditions.

Experiments in our laboratory are presently aimed at elucidating the molecular mechanism by which decreased toxin binding is achieved in the resistant insects. Information obtained from this work will be useful in understanding Bt toxins mode of action and to design resistance prevention strategies.

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Knockdown resistance (*kdr*) to pyrethroid insecticides in the German cockroach

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For decades, pyrethroid insecticides have been used widely to control many insect pests. Due to intensive use, however, many insect pest populations developed resistance to these compounds. One class of resistance mechanism is knockdown resistance (*kdr*). A single amino acid substitution, from leucine to phenylalanine (L993F in the German cockroach), in the Para sodium channel protein has been found to be responsible for *kdr* in several insect species. Recently, we identified two more mutations in the para gene of five field-collected German cockroach strains that exhibit high levels of *kdr*. The two mutations, from glutamic acid (E434) to lysine (K434) and from cysteine (C764) to arginine (R764), respectively, are located in the first intracellular linker connecting domains I and II. Two additional mutations, from aspartic acid (D57) to glycine (G57) and from proline (P1880) to leucine (L1880), respectively, were found in one of the five highly resistant strains. The four mutations co-exist with the previously identified L993F *kdr* mutation and are present only in the highly resistant individuals of a given strain.

To confirm the involvement of E434K and C764R mutations in *kdr*, we expressed wild-type and *kdr* mutant cockroach sodium channels in *Xenopus* oocytes and examined channel sensitivity to a pyrethroid insecticide, deltamethrin. The L993F mutation decreased Para channel sensitivity to deltamethrin by 5-fold. Neither the E434K mutation nor the C764R mutation alone altered channel sensitivity to deltamethrin. However, double mutations (KF or RF) reduced channel sensitivity by 100-fold. The triple mutations (KRF) further reduced Para channel sensitivity to deltamethrin by 500-fold. Thus, two novel *para* mutations, when combined with the previously identified L993F mutation, are responsible for high-level resistance to pyrethroids in the German cockroach.

Although the E434K or C764R mutation alone did not alter channel sensitivity to deltamethrin, sodium current amplitudes from the recombinant Para channel carrying either E434K or C764R alone were much reduced compared to those of the wild-type channel. Furthermore, individuals with sodium channels carrying either E434K or C764R alone were not detected in any populations we surveyed, suggesting that these individuals may have severe fitness problems because of reduced sodium currents. However, the channels carrying either E434K or C764R together with the L993F mutation (KF and RF) or triple mutations (KRF) exhibit normal sodium currents. These results indicated that evolution of knockdown resistance in the German cockroach is achieved by sequential selection of a primary mutation L993F and two secondary mutations E434K and C764R, and concomitant presence of all three mutations confers high levels of resistance to pyrethroids.

Membrane-bound Esterase Involvement in Cypermethrin Resistance in the German Cockroach, Blattella germanica

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Although the overwhelming majority of reports have concluded that multiple resistance mechanisms were responsible for resistance in the German cockroach, esterases have been identified consistently as a major contributor to the resistance level (Hemingway *et al*. 1993, Anspaugh *et al*. 1994, Scharf *et al*. 1997, Valles 1998). Several of these reports have indicated that esterases are functioning to sequester insecticides (especially pyrethroids) thus preventing the toxicant from reaching the intended target site (Prahbakaran and Kamble 1995, Scharf *et al*. 1997).

In a recent survey of field-collected German cockroaches, DEF pretreatment reduced the cypermethrin resistance level in 83% of populations tested (Valles 1998). In addition, general esterase activity (*p*-nitrophenyl acetate hydrolysis) correlated (r=0.78) with cypermethrin resistance magnitude (Valles 1998). These data further implicated widespread esterase in-

volvement in cypermethrin resistance in the German cockroach. Hence, cypermethrin metabolism studies were conducted using a pyrethroid-resistant strain of German cockroach to gain a more complete understanding of the role esterases in resistance.

MATERIALS AND METHODS

Insects

The German cockroach strain(designated the Aves strain) used in this study was collected by vacuum (Valles 1997) from a single family home in Gainesville, Florida, on 11 June 1998. The strain was maintained in 8 liter glass jars with rolled cardboard for use as harborage, water, and #5001 laboratory rodent diet (PMI Feeds, St. Louis, MO). Orlando, the standard insecticide-susceptible German cockroach strain, was used for comparison with the Aves strain in all assays.

Bioassays

Adult males were anesthetized with CO_2 and treated topically with cypermethrin in 1 µl of acetone. The cypermethrin solution was applied to the first abdominal sternite in five concentrations causing >0% and <100% mortality. At least three replications containing 10 cockroaches per dose were conducted. PBO (100 µg per cockroach) or DEF (30 µg per cockroach) was applied to the first abdominal sternite 1 hr before insecticide application. Mortality, the inability of a cockroach to right itself within 10 seconds after being flipped onto its dorsum, was recorded 24 hr after insecticide treatment.

Experimental

General esterase activity was measured with α-naphthyl acetate (α-NA) and *p*-nitrophenyl acetate (PNPA) as substrates. Microsomal and cytosolic subcellular fractions were used in the esterase assays and prepared from the entire cockroach (excluding the head) as described by Valles and Yu (1996).

Qualitative and quantitative *in vitro* metabolism of cypermethrin were studied using a modified version of the method described by Korytko and Scott (1998). Metabolism studies were conducted with the 105,000 g_{Max} supernatant (soluble fraction) and pellet (microsomes) prepared from adult males of the Orlando (S) and Aves (R) German cockroach strains. The 2 ml reaction mixture contained 50 m*M* sodium phosphate buffer, pH 7; 0.5 mg of protein; and 12,000 dpm (0.04 µg) of [^{14}C]cypermethrin in 40 µl of ethylene glycol monomethyl ether. For oxidative metabolism, the reaction mixture was fortified with an NADPH-generating system; esterase activity was inhibited by adding DEF (10^{-4} M) to the reaction mixture. Duplicate incubations were carried out at 28° C in a shaking water bath for 30 minutes. Cypermethrin and its metabolites were extracted with diethyl ether, separated by thin layer chromatography and quantified by liquid scintillation spectrometry.

RESULTS AND DISCUSSION

Topical bioassay data showed that the Aves strain was highly resistant to cypermethrin, exhibiting a resistance ratio of 93-fold. The cypermethrin resistance level was reduced to 18-fold when pretreated with DEF (an esterase inhibitor; Fig. 1). The synergist data implicated enhanced hydrolytic metabolism as a resistance mechanism in the Aves German cockroach strain. This conclusion was further supported by significantly higher hydrolytic detoxification enzyme activities toward surrogate substrates in the Aves strain as compared with Orlando (Fig. 2). Hydrolysis of *p*-nitrophenyl acetate and α-naphthyl acetate by microsomal and cytosolic esterases was 1.6 to 3.6 times faster in the Aves strain compared with Orlando. Surprisingly, metabolism of [^{14}C]cypermethrin by cytosol (soluble esterases) was not different between the Aves (R) and Orlando (S) strains (Fig. 3). However, [^{14}C]cypermethrin metabolism by microsomal esterases was significantly greater in the Aves strain. Therefore, enhanced cypermethrin metabolism catalyzed by microsomal esterases can be considered a major mechanism of cypermethrin resistance in the Aves strain. This is the first report of microsomal esterase involvement in insecticide resistance in the German cockroach.

Additional *in vitro* metabolism experiments were conducted to further characterize the microsomal esterases in Aves German cockroaches. The cypermethrin hydrolyzing enzymes were susceptible to DEF (10^{-4} M), reducing cypermethrin metabolism to 3.8 and 2.1% in the Orlando and Aves strains, respectively (Fig. 3). An examination of different tissue sources revealed that the majority of cypermethrin metabolism occurred in the midgut and integument, with the greatest quantities apparently localized in the latter (Fig. 4). Despite being an important source of detoxification enzymes in insects, no significant differences in cypermethrin metabolism were observed between strains with fat body-derived microsomal esterases. Interestingly, enhanced cypermethrin detoxification by microsomal esterases was observed in additional German cockroach populations (data not shown).

This research demonstrates the involvement of membrane-bound esterases as a mechanism of resistance in the German cockroach (Valles *et al*. 2000). However, elevated cytosolic esterase activities toward surrogate substrates, were also observed, which is often reported for

the German cockroach (Siegfried and Scott 1992, Prabhakaran and Kamble 1995, Valles and Yu 1996, Scharf *et al.* 1997). Although the cytosolic esterases of the Aves strain did not metabolize cypermethrin faster than Orlando cockroaches, they may play a role in sequestration as previously proposed. Continued pursuit of esterase involvement in insecticide resistance in the German cockroach may yield novel methods of control or improved insecticide management strategies.

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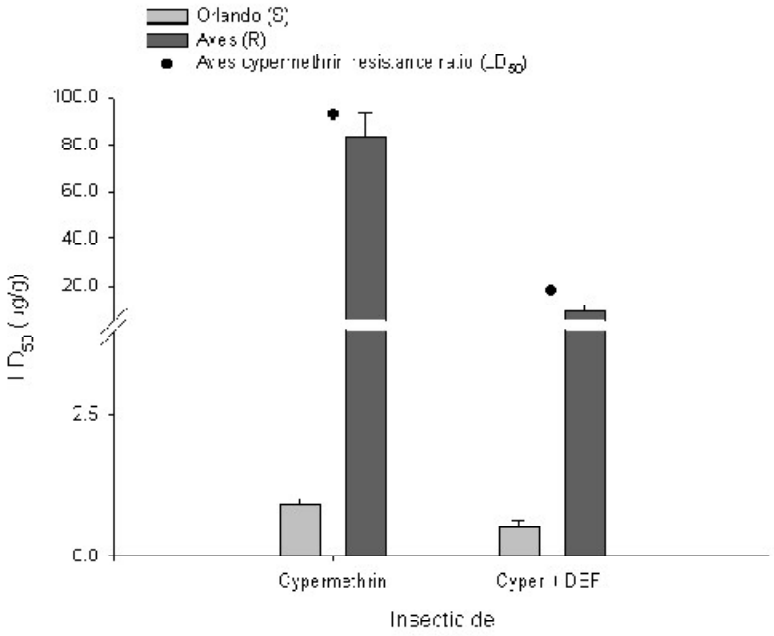

Fig. 1. Topical cypermethrin bioassay results for Aves (R) and Orlando (S) German cockroach strains. Resistance ratio (at the LD_{50}) is denoted with a filled circle (1). Adapted from Valles *et al.* (2000).

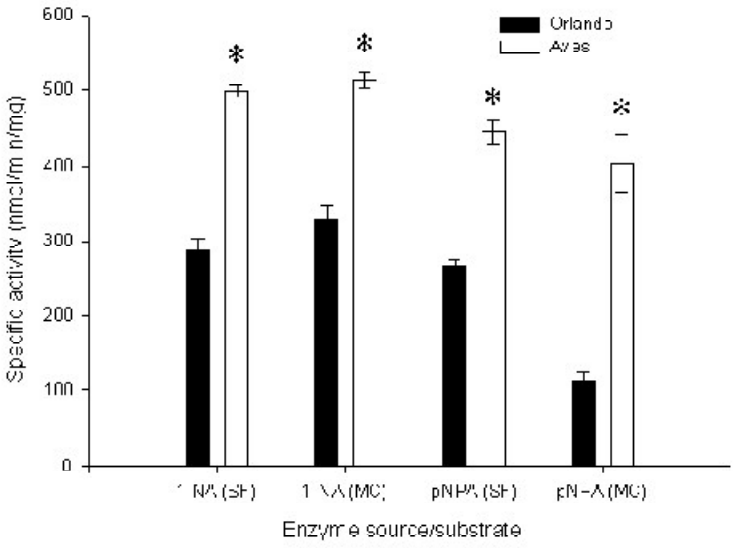

Fig. 2. Specific activities of esterases in the soluble fraction (SF) and microsomes (MC) against α-naphthyl acetate and *p*-nitrophenyl acetate substrates in the Orlando (S) and Aves (R) German cockroach strains. An asterisk denotes significant differences between strains. Adapted from Valles *et al.* (2000).

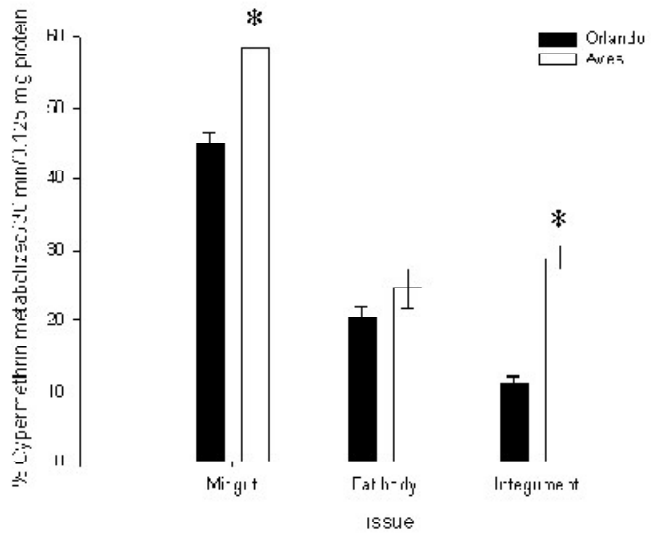

Fig. 3. Cypermethrin metabolism by Aves (R) and Orlando (S) German cockroach strains. SF, soluble fraction; MC, microsomes; MC (+DEF), microsomes in the presence of 10^{-4} M DEF. Adapted from Valles *et al.* (2000).

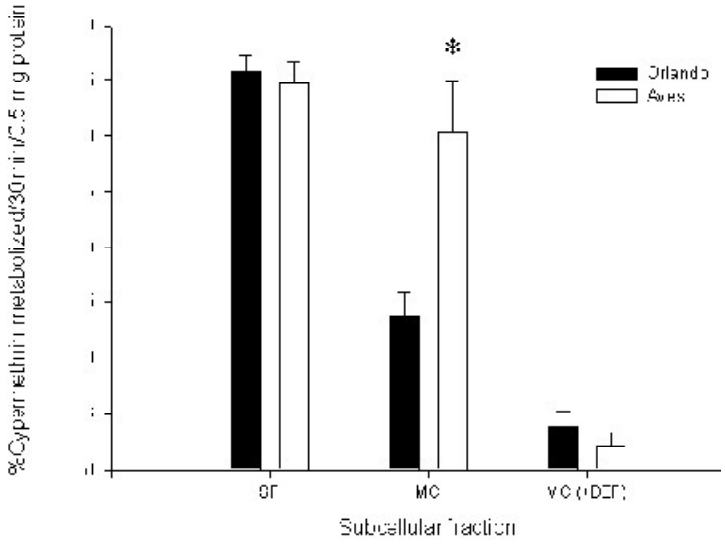

Fig. 4. Cypermethrin metabolism by microsomes prepared from midgut, fat body, and integument in Aves (R) and Orlando (S) cockroaches. Adapted from Valles *et al.* (2000).

Monitoring and Management of Helicoverpa armigera Resistance to Transgenic Bt Cotton in Norntern China

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Helicoverpa armigera Hubner is the most important insect pest of cotton in the Northern China cotton belts (primarily Hebei, Henan and Shangdong provinces). Microbial insecticide formulations from *Bacillus thuringiensis* (Bt) have been widely used in Northern China since the late 1980s for control of *H. armigera*. The acerage of cotton in Northern China significantly decreased after the outbreak of *H. armigera* in 1992 because of high control costs and low profits.

Transgenic Bt cotton entered commerical use in the U.S. in 1996 with success and quick growth tereafter. In 1998, the second largest acreage of Bt cotton in the world was grown in China (Falck-Zepeda *et al*. 1999) after approval of "industiral production" (commercialization by the Ministry of Agriculture (MOA). There were two different sources of Bt cotton expressing Cry1A insecticidal protein, one developed by Monsanto (Perlak *et al*. 1990) an the other developed in China mostly by using the pollen

tube pathway transformation method (Gue 1995, Ni *et al*. 1998). The acerage of Bt cotton in China was bout 80,000 ha. in 1998 and over 0.3 million ha. in 1999. In the Northern China cotton belts, the total cotton acreage in Shangdong and Hebei provices in 2000 was over 0.6 million ha., 90% of which was Bt cotton. The primary target insect pest for Bt cotton is *H. armigera* that has four generations each year.

Although monitoring and management of *H. armigera* resistance to Bt toxin were not mandatory for the commercialization of Bt cotton in China because there is no such requirement by the regulatory authority of MOA (evaluated by the Safety Committee for Agricultural Biological Genetic Engineering), a research program was involved for the deployment of Bt cotton. This is a summary of the research program from 1996 to 1999.

Resistance monitoring

Significant geographic variations of susceptibility of *H. armigera* to a Bt formulation was detected in Northern China (Zhao *et al*. 1995, 1996). Further monitoring tests showed that percent survival of second instar *H. armigera* at the discriminating concentration of Bt formulation (1.0mg/ml of diet, $LC_{99.5}$ of a susceptible strain) was almost zero in four locations of three provinces in Northern China in 1996-1999 (Table 1).

Temporal and spatial dynamics of efficacy of Bt cotton.

Significant variations in insecticidal activity of Bt cotton on *H. armigera* larvae at different times during the growing season and among different organs of Bt cotton plants were observed (Zhao *et al*. 1998, 2000, also see Table 3). After feeding on flowers of Bt cotton as neonates the survivors of *H. armigera* during the season of third and fourth generation (July and August) could feed on boll until pupation and emergence. The flower became the key factor in the interactions between Bt cotton and *H. armigera* in the cotton fields (Zhao *et al*. 1998). High density of large larvae of third and fourth generation *H. armigera* could be found in Bt cotton plants in fields because certain part of Bt cotton plant (flower) did not express a "high dose" of Bt toxin for resistance management, not because of Bt resistance in *H. armigera*. Bt cotton could cause selection on two generations of *H. armigera* (third and fourth in July and August) of *H. armigera* in the field each year in Northern China.

Resistance risk assessment and modeling studies

(1) Resistance selection. The resistance ratio (RR) of *H. armigera* to Cry1Ac based on LC_{50} was 7.1-fold after 17 generations of selection by Bt cotton in the laboratory, with the average corrected mortality of 67.2% each selection (Zhao *et al*. 1998). The insecticidal efficacy of Bt cotton on selected strain was significantly lower than that on the homologous non-selected population, indicating a potential problem with insect resistance, especially in areas with more than 90% Bt cotton. The RR to Cry1Ac was 20.5-fold after 26 generations of selection.

(2) Cross resistance tests. The *H. armigera* strain resistant to Cry1Ac was not cross resistant to Cry1C and Cry2Aa. A mixture of Cry1Ac and Cry1C (Mattch by Mycogen) could overcome the Cry1Ac resistance. The *H. armigera* strain resistant to Cry1Ac was cross resistant to Bt corn expressing Cry1A. The toxicity of the silk of Bt corn on the Cry1A-resistant strain of *H. armigera* was significantly lower than on a non-selected control strain (Table 2).

(3) Modeling studies. A single locus simulation model was developed to predict adaptation of *H. armigera* to Bt cotton. The model involved ecological, biological and operational factors. The temporal dynamics of efficacy of Bt cotton and adults movement between cotton and corn fields were also considered. The model indicated that the resistance allele frequency of *H. armigera* to Bt (Cry1A) will increase from 0.001 to 0.5 after 38 generations or approximately 9 years in a typical cropping system in Hebei province of Northern China where corn fields acted as

Table 1. Resistance monitoring for *H. armigera* to Bt formulation in Northern China

Location[a]	% Survival at discriminating concentration of Bt			
	1996	1997	1998	1999
Qiuxian, HB	0	0	0	0
Jizhou, HB	0	0	0	0
Xihua, HN	0	0	0	0
Gaomi, SD	0	0	0.9	0

[a]HB = Hebei province; HN = Henan province; SD = Shangdong province

a natural refuge for Bt cotton (Fig. 1). If 100% of cotton acreage is Bt cotton, the expected time for field failure of Bt cotton will be only 26 generations (7 years).

(4) Assessment of transgenic plants with two insecticidal genes to delay resistance development of *H. armigera*. Transgenic cotton containing two insecticidal genes, Cry1A and CpTI (cowpea trypsin inhibitor), was developed in China (Guo *et al*. 1999) and the application for commercialization was approved in 1999 for production in four provinces mainly in Northern China. The acreage of the first cultivar of the two-gene cotton ("Shi-Yuan 321") was about 30,000 ha. in 2000 in China. The efficacy of the two-gene cultivar on first instar *H. armigera* was significantly higher than one gene (Bt) cotton in July and August during the third and fourth generation of field population, either on Cry1A selected or non-selected strain (Table 3).

We used transgenic tobacco with Bt or two genes (Bt & CpTI) as model plants to evaluate the effects on resistance development by *H. armigera*. The resistance ratio of *H. armigera* to Cry1Ac of B*t* is 13.1- and 3.02-fold after 18 generations of selection by transgenic Bt tobacco and two-gene tobacco, respectively (Zhao *et al*. 1999). The average corrected mortality for each selection treatment (about 60%) and the level of Cry1Ac expression in either type of tobacco (310-330 ng/g fresh leaf) were similar. This is the first experiment under laboratory conditions that proved transgenic plants with two insecticidal genes could significantly delay resistance development of *H. armigera* compared with one gene plant.

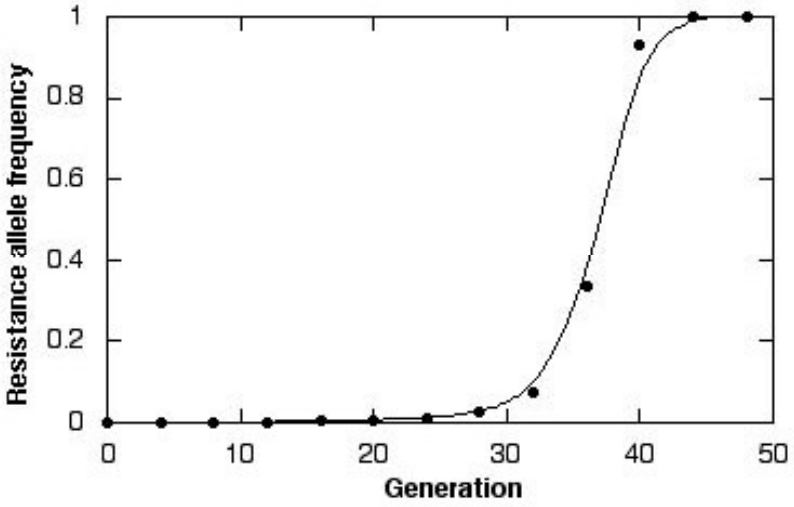

Fig. 1. Resistance development of *H. armigera* to B*t* toxin in the typical cropping system in Hebei province predicted by a simulation model

(5) Resistance management. The lack of "high dose" in current B*t* cotton cultivars for *H. armigera* and the small scale production systems of cotton in Northern China indicated that the "high dose/refuge" strategy widely accepted in USA for resistance management was not feasible for cotton in Northern China. There was also no mandatory requirement for structured refuge by the regulatory authority. We proposed the following steps for resistance management for B*t* cotton primarily based on the situation in Northern China cotton belts (Zhao *et al*. 1998, Zhao 2000).

(a) Improve the regulation of transgenic Bt crops, especially to include resistance management requirements for the commercialization of B*t* crops.

(b) Improve the expression of Bt toxin in cotton plants and the purity of Bt cotton seeds. Some cotton growers prefer to buy Bt cotton seeds grown by their neighbors in small scale and the purity of Bt cotton seeds is not good. Such situation will worsen the resistance problems.

(c) Protect the natural refuge of the non-cotton host plants for *H. armigera* by not to commercialize both B*t* cotton and Bt corn in the same area. The acreage of corn is much larger than cotton in Northern China and there are few insecticide applications in corn fields. The commercialization of Bt corn in Northern China will destroy the most important natural refuge for Bt cotton and *H. armigera* because of cross-resistance.

(d)Use transgenic cotton with two insecticidal genes (Bt and CpTI) as soon as possible to delay resistance development by *H. armigera*.

(e) Monitor resistance frequency of *H. armigera* to Bt toxins in multiple regions.

(f) Use IPM tactics with different mode of action in Bt cotton fields.

Table 2. Efficacy of silk of Bt and non-Bt corn on different strain of *H. armigera*

Strain of *H. armigera*	% Mortality feeding on silk of corn	
	Bt corn	Non-Bt corn
Cry1A resistanat	48.2	46.7
Non-selected control	93.5	35.0

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Table 3. Temporal dynamics of efficacy of transgenic two-gene or one-gene cotton on first instars of H. armigera 6 days after treatment

Month	*H. armigera* strain	% Corrected mortality (±SEM) on transgenic cotton	
		Two genes (Bt&CpTI)	One gene (Bt)
6	Cry1A selected	100	100
	Non-selected	100	100
7	Cry1A selected	88.0 ± 6.8	58.0 ± 4.2
	Non-selected	96.7 ± 3.3	76.9 ± 4.6
8	Cry1A selected	56.7 ± 8.1	18.8 ± 2.0
	Non-selected	66.1 ± 6.9	39.1 ± 5.0

Resistance of Colorado Potato Beetle to imidacloprid

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ABSTRACT

Five field populations of the Colorado potato beetle were collected in Long Island, NY, one population was collected in Michigan and another was collected in Hudson, NY in the summer of 1999 and submitted to topical bioassays with a single dose of imidacloprid alone (3.16 µg of A.I./beetle) or in mix with 5 µg of piperonyl butoxide (PBO). Resistance levels were calculated by using knock down 50 after treatment, and knockdown beetles (dead beetles + knock down beetles) 10 days after treatment. Use of knock down50s differentiate susceptible Colorado potato beetle populations from those that are resistant. Long Island populations expressed higher levels of resistance in comparison with the susceptible, Michigan and Hudson populations. The addition of PBO to some extent suppressed the resistance, suggesting that the P450 complex would be involved in the mechanism of resistance of CPB to imidacloprid. A similar situation was found in the knockdown beetles 10 days after treatment. Some percent of beetles from Long Island populations, especially in the Wells, Rutkoski and Zilverton populations survived to the imidacloprid treatment. An addition of PBO partially suppressed the resistance in these populations, but other factors may also play an important role on the resistance. We are in process of conducting further research with oxidative metabolism, synergists, and receptor binding sites to get more insights about the role of P450 and other resistance mechanism.

INTODUCTION

Imidacloprid is a neonicotinoid insecticide that acts at the insect nAChR (Bai *et al*. 1991). It was registered for use on potatoes in 1995 and soon became the primary means to control organophosphate, pyrethroid and carbamate-resistant CPB in Michigan and other places in the US (Grafius 1997). However, in 1997 low levels of resistance were detected in a Michigan population. In the same year a population from Long Island, NY expressed 100 resistance levels (Zhao *et al*. 2000). Reduced levels of control have been

observed by growers in many potato fields on Long Island and field efficacy indicated that the efficacy of imidacloprid was declining (Moyer et al. 1998). A year later similar and higher levels of resistance in four populations from Long Island were detected (unpublished data). However, these populations expressed very low levels of cross resistance to a new neonicotinoid, thiamethoxam, and no cross-resistance to bensultap, a compound with a similar mode of action. This low or lack of cross-resistance may be due to differences in oxidative metabolism and receptor binding sites.

Some important aspects in the strategies of resistance management include the determination of the mechanism of resistance and the use of a bioassay method to get quick results. Imidacloprid is a compound that kills insects slowly. Detection of resistance by the use of bioassays usually takes from 7 to 10 days because some beetles treated with imidacloprid recover from the effect of the insecticide in a period of time from 3 to 10 days; so that getting faster data about levels of resistance from field populations could be compromised due to the long time to evaluate mortality. Use of knock down $_{50}$ could be a useful tool to get faster data.

OBJECTIVES

a) Monitoring for the resistance levels of field populations of CPB in locations of heavy use of imidacloprid by using the knock down time 50 (KD_{50})

b) Evaluate the effect of piperonyl butoxide (PBO) in the suppression of resistance of imidacloprid to resistant populations of CPB.

METHODOLOGY

Insects

Six adult field populations of Colorado Potato Beetle were collected in Long Island, NY. The Suffolk population was collected in July, 1997 (Zhao *et al*. 2000). The other 5 strains were collected at different locations from Long Island in August, 1999. Two other populations were collected from Hudson, NY and Midland, MI in 1999. The susceptible colony (S69), was collected in Michigan potato fields and has been reared in the laboratory at MSU for 10 years without exposure to insecticides.

Insecticides

Imidacloprid (98.7%, technical grade) was provided by Bayer (Kansas City MO), piperonyl butoxide (90%, technical grade) was supplied by Aldrich Chemical Company, Inc.

Bioassays

Topical bioassays were used to assay adult resistance. Technical-grade insecticide was diluted with acetone. A dose of 3.16 µg/beetle was tested. Ten beetles (1 to 2 weeks old in the case of the susceptible S69 and unknown ages for the field populations) were treated with 1 µl of each solution on the ventral area of the abdomen. After the treatment, beetles were placed in Petri dishes and were fed potato leaves. The control beetles were treated with acetone only. In the case of PBO, previous experiments showed that 5 µg/beetle did not cause mortality or adverse effect on the feeding behavior of the susceptible and resistant populations. PBO was applied an hour before the insecticide treatment. Three replications were performed with both treatments: a single doses of imidacloprid or in mix with PBO. After treatment, beetles were placed in Petri dishes and fed potato leaves and kept at 28° C, 50% relative humidity, photoperiod of 16:8 (L:D). Knockdown beetles were counted periodically for 24 hours after the treatment. In addition, mortality and knockdown were assessed at 24 hours, 3, 5, 7, and 10 days after treatment. Beetles that were unable to stand on their legs and walk a distance corresponding to their own body length were counted as knock downs. Beetles that did not exhibit any movement and which exhibited a progressive darkening were recorded as dead.

Analysis of results

Data were analyzed by probit analysis (SAS Institute, 1988). Knock down (KD_{50}), fiducial limits, and slope were determined. The resistance ratios were calculated as KD_{50} value of resistance colony

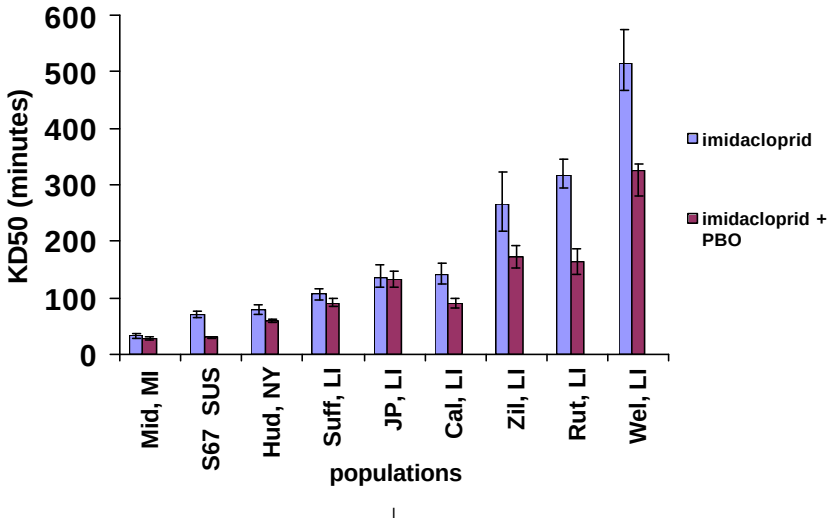

Figure 1. Knockdown (KD50) of several strains of CPB by imidacloprid and imidacloprid + PBO.

(Long Island) / susceptible colony (S69). To analyze the data for knock down beetles 10 days after treatment, an arcsine transformation was used and the experimental design was factorial where populations (nine) and insecticide (alone or mixed) were the factors. We used the PROC GLM procedure of SAS.

RESULTS AND DISCUSSION

Knock down time ratios of Long Island populations ranged from 1.5 to 7.5 (Table 1 and Figure 1). The levels of resistance using knock down ratios are lower in comparison with topical bioassays in which we used at least 5 doses that caused more than 0% and less than 100% percent of mortality and evaluated 7 to 10 days after treatment (unpublished data). Results of previous bioassays indicated that a resistant population from Long Island expressed up to 155 fold levels of resistance. However, use of a single dose to determine knock down$_{50}$s is a quick way of diagnosing resistance. In less than 24 hours it is possible to differentiate resistant beetles from susceptible ones. For instance, resistant populations from Long Island are significantly different from the susceptible laboratory population and from the susceptible field populations (Midland, MI and Hudson, NY). Rapid results of the resistance levels of Colorado potato beetle to imidacloprid would be very important, particularly in areas where foliar treatments of imidacloprid (Provado) are used to control Colorado potato beetle. Use of knock down$_{50}$s would help in the decision of separate susceptible population from the resistant ones.

The highest levels of Colorado potato beetle resistance were found at the Wells, Rutkoski, and Zilverton locations where the populations experienced eight or more imidacloprid treatments since 1995 (Admire and/or Provado). The Hudson, NY and Midland, MI population exhibited lower levels of resistance than the Long Island populations. Growers with the highest resistant populations used Provado as a foliar application the first year or two (1995,1996) without using Admire in-furrow compared to the Jamesport site that used Admire in-furrow all four years, which had the lowest resistant value. The amount of active ingredient present in potato plants is higher and lasts longer for the Admire in-furrow treatments than in the Provado treatments. Maybe this condition prevents the survival of some heterozygous resistant beetles. In contrast, Provado treatments have an initially good efficacy. However, decay of the residues is faster allowing the survival of heterozygous beetles. In addition, foliage treatments of Provado do not cover 100% of the plant. The levels of resistance using knock down ratios seem to be lower in comparison with bioassays evaluated 7 to 10 days after treatments (results of previous bioassays indicated up to 150 fold levels of resistance). However, KD$_{50}$ is a quick way of diagnosing resistance.

The mix of imidacloprid and PBO reduced the time for knock down in most of the populations except in the Jamesport, Suffolk and Midland populations (Table 1 and figure 1). Despite the reduction in the knock down time in the highest resistant Long Island populations, PBO only partially suppressed resistance. Evaluation of the knockdown and mortality 10 days after the treatment showed that imidacloprid caused a high percent of response in the susceptible, Midland, MI, and Hudson, NY. However, there were survivors in the Long Island populations. Treatments using imidacloprid mixed with PBO suppressed the resistance in most of the populations indicating that P450 complex is a factor in the resistance to imidacloprid. However,

Table 1. The knockdown response of several strains of CPB to imidacloprid and imidacloprid + PBO

population	[a]KD$_{50}$	slope ± SE	[b]RR	[a]KD$_{50}$	slope ± SE	[b]RR
Midland, MI	32 (28, 36)	3.0 ± 0.3	0.46	27 (25, 30)	5.0 ± 0.5	1
Susceptible	68 (63, 75)	4.0 ± 0.3	1	28 (26, 31)	4.5 ± 0.5	1
Hudson, NY	77 (69, 85)	3.3 ± 0.3	1.1	58 (54, 61)	7.1 ± 0.6	2
Suffolk, LI	106 (96, 116)	3.9 ± 0.3	1.5	91 (84, 98)	6.8 ± 0.9	3.2
Jamesport, LI	135 (117, 157)	2.1 ± 0.1	2	132 (120,146)	4.1 ± 0.3	4.6
Calverton, LI	142 (125, 160)	3.1 ± 0.2	2.1	89 (81, 99)	3.9 ± 0.3	3.1
Zilverton, LI	266 (216, 322)	2.8 ± 0.3	3.9	172 (152,193)	2.8 ± 0.2	6
Rutkoski, LI	318 (293, 344)	3.4 ± 0.2	4.6	164 (141,185)	2.7 ± 0.3	5.7
Wells, LI	515 (466, 574)	3.2 ± 0.4	7.5	325 (281, 337)	2.7 ± 0.3	11

[a]**KD$_{50}$ = Knock down fifty (minutes)**

[b]**RR= Ratio of resistance. KD$_{50}$ of field population / KD$_{50}$ susceptible population**

in the Wells and Zilverton populations there were only a partial suppression of the resistance suggesting that other mechanisms of resistance may play an important role. We are in process of conducting further research with oxidative metabolism, synergists, binding sites to get more insights about the role of P450 or other resistance mechanism of CPB to imidacloprid.

A history of resistance of CPB to many classes of insecticides in Long Island could have selected many mechanisms of resistance including monooxygenases. However, oxidases are a big family of enzymes and many P450s would be involved. In insecticide resistance (Scott 1996). Therefore a specific enzyme may be responsible for the detoxification of imidacloprid. This specificity may explain the low or non-existant cross-resistance to bensultap and thiamethoxam (unpublished data). Different binding sites on the nicotinic acetylcholine receptors may also explain mechanism of resistance. However, in other imidacloprid resistant insects *Myzus persicae* and *Myzus nicotiana* no differences in target site binding were demonstrated with binding assay using (+3H) (Nauen *et al.* 1996)

Until recently, imidacloprid was the only insecticide registered to be effective for Colorado potato beetle control in many potato growing regions in the US. Field failure on Long Island, together with increasing levels of resistance in Michigan field populations, should be a warning of major problems in the near future. This is a very important reason to design strategies that do not rely exclusively on the sole use of imidacloprid. Growers should readopt resistance management strategies for imidacloprid and other second generation neonicotinoids. Insecticide rotation, crop rotation, propane flamers, and ditch traps were abandoned by many growers with the introduction of imidacloprid. These were very important strategies and tactics implemented in the past to mitigate resistance.

CONCLUSIONS

a) Use of knock down time allows the differentiation of susceptible and resistant Colorado potato beetle populations. The Long Island Colorado potato beetle populations continue with similar trend in the resistance to imidacloprid.

b) PBO suppressed partially the resistance to imidacloprid. However, other factors would be involved in the resistance of Colorado potato beetle to imidacloprid.

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A Resistance Management Program for the German Cockroach in a Low-Income Housing Project

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INTRODUCTION

Many low-income housing projects have high German cockroach populations (Bennett & Runstrom 1980, Wood 1980). Faulty constructions, inadequate maintenance, general clutter, poor sanitation and lack of effective pest management programs are some of the factors that favor the development of high cockroach populations in these housing projects. Spraying insecticides has been the primary method of controlling these cockroach infestations. Because of their relative effectiveness, low cost, low odor and flushing characteristics, pyrethroid insecticides have been the insecticide of choice, used extensively, on fixed-spray schedules (Reid and Bennett 1989). Development of pyrethroid resistance by these cockroach populations is an inevitable consequence of the extensive use of these insecticides.

Strategies that have been suggested for managing resistant German cockroach populations emphasize multiple tactics that include sanitation (Adams 1993), insecticide mixtures (Kaakeh *et al*. 1997), trapping and vacuuming (Kaakeh and Bennett 1997).

MATERIALS & METHODS

This study was conducted in a multi-family apartment complex managed by the Muncie Housing

Authority, Muncie, Indiana. Records obtained from the Housing Authority indicated that its pest control department has been using Demon® Wettable Powder (WP) (a.i = 25.3% cypermethrin, Zeneca Inc., Delaware) to control German cockroach infestations since 1988. All apartments were treated every 90 days, and on as-need basis.

The onset of resistance was manifested when large cockroach numbers were easily observable in many apartments that were regularly treated. Scharf *et al*. (1997) conducted resistance level bioassays on cockroaches collected from these apartments and reported 82.2 fold level of resistance to cypermethrin, and 5.2 fold level of resistance to chlorpyrifos, compared to a susceptible strain. Biochemical assays on these cockroaches revealed higher levels of cytochrome P450 monooxygenase and esterase activities, compared to the susceptible strain (Scharf *et al*. 1996). Though these studies revealed that a resistance problem exists at this location, there were no concurrent trap data from the apartments to document insecticide control failures. Ball (1981) and Zhai and Robinson (1996) emphasized the importance of documenting insecticide resistance problems in field populations of insects by demonstrating insecticide control failures, in addition to the usual lethal time and/or lethal dose bioassays. This study was therefore conducted to: (1) document insecticide resistance in the German cockroach populations at the Muncie Housing Authority's apartments through trap catch data and, (2) evaluate the efficacy of two commercially available bait products for managing these populations.

Since cockroach populations at this location were reported by Scharf *et al*. (1996, 1997) to be resistant to the pyrethoids and organophosphate insecticides, we selected Demon® Emulsifiable Concnetrate (EC) (a.i. = 25.3% cypermethrin, Zeneca Inc., Delaware), and Prescription Treatment® (PT®) Engage® (a.i. = 0.5% chlorpyrifos, Whitmire Micro-Gen Research Laboratories, St. Louis, Missouri) as the residual sprays. Avert® Prescription Treatment® (PT®) 310 dust bait (a.i. = 0.05% Abamectin B1, Whitmire Research Laboratories, St. Louis, Missouri) and Maxforce® FC gel bait (a.i. = 0.05% fipronil, Maxforce Insect Control Systems, Oakland, California) were the bait products selected. Demon® EC was applied into cracks and crevices with a 3.79-l (= 1 gal) B & G compressed air sprayer (B & G Equipment Company, Plumbsteadville, Pennsylvania), fitted with a crack-n-crevice injector tip. Each apartment received about 200 ml of formulated material. Prescription Treatment® Engage® was applied from Crack and Crevice® pressurized residual spray cans at the rate of 5 oz/apartment. Avert® dust was applied according to labeled rate while 2 'child resistant' bait stations of Maxforce® FC gel were placed in each cockroach sampling area.

A completely randomized experimental design was used to assign treatments to test apartments. A minimum catch of 12 cockroaches per apartment before treatment (n = 6 traps) was required for inclusion of an apartment in the test. A range of 8 to 10 apartments was established for each treatment, and each test apartment served as both the experimental and replicating unit. Within apartments, cockroach numbers were sampled in the kitchen and bathroom areas: under and above the kitchen sink, behind the refrigerator, behind the stove, in the utility/pantry area, and on the floor beneath the toilet seat. Populations were sampled before the initial insecticide application (0 wk) and at intervals thereafter (2, 4 and 8 wks) with Lo-Line® cockroach sticky traps (Agrisense™ Inc., Fresno, California). At each sampling interval, traps were left in place overnight, collected the next morning and counted. Trap catches were recorded as number of small nymphs (1st-3rd instar), large nymphs (4th-6th instar), adult males, females and gravid females (females carrying the egg case). Trap catch data was used to determine efficacy of treatments on population reduction. Population reduction was determined using the formula:

$\%\ \text{reduction} = (T_i - T_o)/T_i * 100$

where Ti and To are pre- and post-treatment counts, respectively. Trap catch reduction for each experimental unit was transformed by arcsine $\sqrt{p}$, where p is percentage expressed as proportion. Transformed data were analyzed according to SAS GLM procedures (SAS Institute 1996). Means were separated by Tukeys studentized range test at $\alpha = 0.05$ (SAS Institute 1996).

A trap catch reduction of ≥70% reduction was set *a-priori* as a satisfactory level of control for the treatments.

RESULTS AND DISCUSSION

The pre-treatment counts and percentage reductions in trap catches are shown in Table 1. There were no significant differences in pre-treatment counts across treatments, and this enabled us to statistically compare the efficacy of treatments at all post-treatment sampling periods. For the Demon® EC and PT® Engage® treated apartments, the mean percent population reductions was below 70%, on the average, at all post-treatment sampling periods. On the other hand, the group of apartments treated with Avert® dust and Maxforce® FC gel baits each had satisfactory level of control, because population reduction was higher than 70% at the 8-week post-treatment census. In addition, there were significant differences between the lev-

els of cockroach control recorded for Maxforce® FC, compared to the residual sprays at all the post-treatment sampling periods. These results demonstrate control failure for the Demon® EC and PT® Engage treatments. While the control failure recorded for Demon® EC can be described as pyrethroid resistance, resistance to the PT® Engage® can be described as cross-resistance since there was never a history of the use of this product or any other organophosphate to control cockroach populations at this location.

The satisfactory performance of the baits to control these resistant cockroach populations can be attributed to the fact that being stomach poisons, they have different mode of actions and act on different target sites, compared to Demon® EC and PT® Engage®. This satisfactory performance implies that these products can be employed in managing the perennial cockroach infestations at the Muncie Housing Authority's apartments. In addition to the baits, conventional insecticides mixed with juvenoids could also be integrated into the resistance management program. Kaakeh *et al*. (1997) recorded satisfactory control of cockroach populations at this location with treatment combinations that included: (1) Empire 20® + Gentrol® (i.e. chlorpyrifos and hydroprene); (2) Commodore® + Nylar® + PBO-8® (i.e. lambdacyhalothrin, pyriproxifen and piperonyl butoxide); and (3) Diacap 300CS® + Torus® (i.e. diazinon and fenoxycarb). Furthermore, some types of residual sprays as stand-alone treatments could also be included in the resistance management program. These insecticides however must have a different target site other than the ones being hit by the pyrethroid and organophosphate insecticides. Chlorfenapyr, a new chemistry discovered by the American Cyanamid Company, has been reported to disrupt insects' respiratory functions by interfering with energy production in the mitochondria but has minimal effects on the nervous system (Kuhn *et al*. 1993, Black *et al*. 1994). In a recent study at the Muncie Housing Authority's apartments, Ameen *et al*. (*In press*) reported that chlorfenapyr gave effective control of the cockroach populations.

SUMMARY

Trap catch data were used to demonstrate resistance to an organophosphate, PT® Engage®, and a pyrethroid insecticide, Demon® EC, in the cockroach populations of a low-income housing project. Based on empirical data, we arrived at the following suggestions for managing the resistance problems:

1. Discontinue the spraying of Demon® WP and any pyrethroid or organophosphate-based insecticide on a fixed spray schedule.

2. Infested apartments should be treated with the bait products: Avert® PT® 310 dust and/or Maxforce® FC gel bait, as needed.

3. Spraying conventional insecticides mixed with juvenoids as needed. In addition, residual insecticides with a target site other than the nervous system, e.g. chlorfenapyr, could also be used.

4. Educating residents about the importance of sanitation in order to reduce the perennial high levels of cockroach infestations.

Table 1. Efficacy of a pyrethroid and an organophosphate based residual control products compared to two commercial bait products for managing resistant German cockroach population in a low-income housing project

Treatment	Pre-treatment Counts (Mean ± SE)	Post-treatment % population reduction at week (Mean ± SE)		
		2	4	8
Demon® EC	46.8 ± 15.1a	19.1 ± 10.1bc	19.1 ± 11.6b	43.8 ± 12.7bc
PT® Engage®	55.3 ± 20.9a	29.1 ± 10.4bc	31.5 ± 11.2ab	32.2 ± 12.1c
Avert® Pt® 310	18.5 ± 12.5a	52.1 ± 28.9ab	62.3 ± 14.4ab	95.8 ± 2.9ab
Maxforce® FC	27.5 ± 11.2a	92.3 ± 2.1a	89.8 ± 5.2a	96.9 ± 1.9a
F	0.61	4.68	4.9	5.08
p	0.6165	0.0138	0.0116	0.0101

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Response of a Low Level Gene Amplification to Organophosphate Selection in *Culex* pipiens

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A common mechanism of organophosphate resistance in *Culex* mosquitoes is an increase in the amount of detoxifying esterase enzyme. The molecular basis of this increase is usually amplification of one or more esterase genes. One key to understanding the dynamics of gene amplification based resistance is how genetic processes interact with insecticidal selection to produce individuals with high gene copy number, high esterase activity and resistance. opulations of *C. quinquefasciatus* and *C. pipiens* in many parts of the world exhibit high frequencies of the active EST-B1 allozyme. This is the end result of years of insecticide use. Unfortunately, we know little about the process by which EST-B1 phenotypes increase in esterase activity, or the rate of this process following an initial amplification event.

Using a series of crosses between individuals from a field population and a susceptible laboratory strain, a strain was produced which had a moderate frequency (about 0.25) of a *single* chromosome isolated from a field population, S-Y. This chromosome resulted in a 3-fold increase in esterase enzyme activity in the heterozygous condition, compared to homozygous susceptible individuals. This increase in activity was associated with an increase in staining intensity of the EST-B1 band on electrophoretic gels. Since regulatory effects on esterase B1 gene expression have not been reported, this elevated activity was presumably the result of a low level esterase B1 gene amplification. The population was subjected to selection with the organophsphate insecticide temephos at a concentration sufficient to kill 75-95 percent of the treated individuals. Approximately 10,000 - 15,000 early fourth instar larvae were treated for each selection. Over the 30 generations of the study, the population was subjected to 17 selections, and esterase activity changes were monitored over time.

The strain carrying the single S-Y chromosome exhibited a significant response to temephos selection (Table 1). After Selection 5, no individuals with susceptible strain levels of esterase activity were evident, and the strain probably consisted predominantly of individuals homozygous for the S-Y chromosome conferring elevated esterase activity. Between Selection 5, and Selection 17, mean esterase activity increased about 8.5-fold. The increases in esterase activity through time were gradual, but not continuous. Significant increases in mean esterase activity occurred between Selections 5 and 7 and between Selections 12 and 17. Both of these response periods resulted in approximately 3-fold increases of mean esterase activity compared to that observed at the beginning of the period. The rapid increase in esterase activity from Selection 5 to Selection 7 suggests that there were higher esterase activity variants present at low frequency among the Selection 5 survivors. In contrast to these significant responses, no significant change in mean esterase activity was observed between Selections 7 and 12. Activity variants appear to arise randomly, and at relatively low frequency, in this system.

At the conclusion of the experiment individuals exhibited about 45 times the esterase activity characteristic of susceptible individuals, on average. The stepwise changes in esterase activity over time suggests an underlying increase in esterase B1 gene copy number on the S-Y chromosome, however changes in gene expression are also a possibility. Experiments are presently under way to determine which of these mechanisms was responsible for the changes in esterase activity. Regardless of the underlying molecular basis, our study indicates that esterase activity variants can arise and be brought to high frequency quite rapidly, even when effective population size is relatively low. This process would probably be more effective in large natural populations. Given the proper selective environment, if a low level esterase B1 gene amplification was introduced into an area by immigration, it could give rise to higher esterase activity variants, and resistance, relatively quickly.

Table 1. Esterase activites of samples of untreated offspring of the survivors of different selections. Esterase activity units are nmoles alpha-naphthyl acetate hydrolyzed/min/mg mosquito protein.

Selection	N	Mean	SE	Min.	Max
1	96	61.0	3.4	5.8	145.8
5	96	136.2	3.8	48.8	229.5
7	48	370.0	17.6	105.4	664.2
12	48	342.0	9.8	227.2	519.4
17	47	1159.1	57.8	481.2	2582.0

Sympossium Summaries

IRAC WHITEFLY WORKSHOP, ALMERIA, SPAIN, APRIL 19, 2000

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INTRODUCTION

The International Group of the Insecticide Resistance Action Committee (IRAC) held its 34th Annual Meeting on the 18-19 April, 2000, in Almeria, Spain. As part of the meeting a full day workshop was organised addressing the issues of whitefly, *Bemisia tabaci*, resistance in intensive agriculture in Almeria. A large number of local experts from institutes, universities and biological/chemical control companies attended. Invited speakers included Maria Rodriguez, Centro Investigacion y Formacion Agraria de Almeria (CIFA), Dr. Dirk Janssen, CIFH, Dr. Ian Bedford, Coordinator, European Whitefly Studies Network (EWSN), John Innes Institute and Ian Denholm, IACR Rothamsted. The meeting was divided into two sessions with the morning spent on presentations covering different aspects of the whitefly resistance problem, followed by break-out syndicate groups in the afternoon when strategies for improving the situation in the future were reviewed.

PRESENTATIONS

Gary Thompson, Chairman of IRAC International, opened the meeting by welcoming attendees and explaining the meeting objectives. This was primarily to facilitate communications on the serious problem of whitefly resistance in Almeria and to listen and make recommendations to suite local needs. The importance of encouraging actions to maintain the sustainability of products and production systems and to identify unfulfilled Insecticide Resistance Management (IRM) needs was noted. It was not anticipated or expected that a solution to the whitefly problem would be found during the meeting but rather it was hoped to increase the awareness of the issues, develop a strategy and to encourage all parties to work together to achieve the objective.

Ian Bedford provided an overview of whiteflies as agricultural pests and their importance as virus vectors. Of the species discussed *Bemisia* is by far the most damaging transmitting more than 60 different viruses. In some regions of Spain it has become impossible to grow tomatoes because of the damaging effects of the virus on production. One extreme of the situation in intensive agriculture is that with each adult having a 60% chance of spreading the virus, there is zero tolerance for *Bemisia*. This means that the normal thresholds for chemical and biological control are insufficient for *Bemisia* control. This puts enormous strains on the conventional methods of controlling insects. In addition, virus transmission often occurs before the adult, exposed to the insecticide, is affected. Also, viruses usually become visible 2 weeks after infection with the result that growers may continue to spray even after the whitefly have disappeared. Activities which can help include total removal of affected plants, use of a non-cropping period with no host plants available, use of trap plants to attract adults as they enter the plastic house, and the use of physical methods (e.g. mesh) to keep the adults out of plastic houses. Some work has started on developing resistant varieties but acceptance of the fruit by the public is a barrier.

Dirk Jansen explained that in Almeria the Q biotype of *Bemisia* was the most common. A diagnostic kit has been developed which allows identification of the virus at an early stage and a model was in construction to assess the influence on yield of the virus.

Alfred Elbert, Bayer, described the monitoring methods used to assess the sensitivity of *Bemisia* from Almeria to imidacloprid and other neonicotinoid insecticides. Imidacloprid was launched in 1992 and a monitoring method was developed based on uptake of imidachloprid solution by a cotton leaf. The LC_{50} data generated showed a steady decline in sensitivity to Imidacloprid from 1994 to 1999. A strain raised in the laboratory started with a mix of B and Q biotypes but after one year in the lab (with no selection) susceptibility had returned and there was only the B biotype detectable. However, other strains from Almeria showed no decrease in susceptibility after 4 years in culture. Work with synergists (pre treating the whitefly 2 days before imidacloprid application) indicated an MFO mechanism (synergised by PBO and PPP). The decrease in susceptibility does not generally correlate with field activity (with maintained efficacy of c85%) however some strains in Almeria show a decrease in efficacy in the field as well. There seems to be no cross resistance with pyriproxifen, pyridaben, chlofenapyr, buprofezine, while the strains show a decrease in susceptibility to

cyfluthrin, endosulfan, monocrotophos, methamidophos and pymetrozine.

Peter Wyss, Novartis described the work done to find a baseline susceptibility to thiamethoxam and pymetrozine, using a leaf disc and leaf dip assay. The results demonstrated no cross resistance between OP's and pyrethroids. Strains from Spain continued to show over the years a steady shift in tolerance to neonicotinoid insecticides. The data demonstrated some cross resistance between Imidacloprid and thiamethoxam but no cross resistance to pymetrozine. Strains of whitefly and aphids from different regions shows differences in resistance profiles but never any cross resistance between neonicatinamides and pymetrozine. The tolerance increased in Southern Spain at end of the autumn (following pressuring during the growing season). The data indicates that adults readily move from one plastic house to another emphasising the importance of regional strategy (not just house by house). The data also indicates that pymetrozine does not trigger neonicatinamide resistance but neonicatinamide resistance can generate pymetrozine resistance. Thus the recommendation is to use pymetrozine early season and not rotate imidacloprid or thiamethoxam.

Maria Delores Rodriguez explained how the predominant whitefly species has now changed from *Trialeurodes vaporariorum* to *Bemisia tabaci* including the associated viruses on the different crops. Measures needed to minimise the problem include sealing of houses to reduce migration, use of mesh that will also exclude thrips, and removal of weeds. Yellow traps to catch adults and the use of beneficial insects eg *Eretmocerus* sp are also useful techniques. These various strategies have been recommended but they need to be adapted and properly applied to the situation in Almeria.

Volker Harries, BASF, gave a presentation outlining some of the factors to consider when making IRM recommendations. Key to this was emphasis on the fact that all parties eg growers, distributors, buyers and officials are partners in IRM strategy. Methods are widespread but include economic, sanitary, education, biological and regulatory means. It was noted that key to solving the problem was to decrease total dependency on chemical control measures.

Ian Denholm finished off the presentations by giving an overview of the pests involved, the associated problems and an update on the status of resistance to different chemistries. Case histories from different parts of the world were used to show strategies adopted with varying levels of success. The importance of avoiding mixtures as a strategy was emphasised along with the need for education and monitoring.

SYNDICATE/BREAK-OUT DISCUSSIONS

Five break-out groups were formed and asked to identify the main issues and needs to help resolve the whitefly problem in Almeria. The suggestions could be grouped together under General and Research needs. Under the General category points such as physical improvements, sanitary methods, good husbandry, sensible use of insecticides, co-operation between interested parties, education and communication were identified. Under Research needs ideas such as improved knowledge on whitefly basic biology/ behaviour etc, better understanding of the consequences of spray technique, information on selectivity of different insecticides to predators, a better understanding of virus transmission and a diagnostic test for growers were all identified. Several good ideas came out from the discussions. These included the use of demonstration areas as part of an education/communication programme, use of clear messages eg "a hole lets the whitefly in and the money out the same hole", and co-operation with the plastic manufacturers to develop a better polythene house. It was also suggested that the needs of the large and smaller grower should be recognised as these are often quite different.

CONCLUSIONS

Overall the meeting was very successful and provided a good opportunity to bring together interested parties to review the current situation and to propose suggestion for improvements. It was not expected to arrive at an overall solution to the problem but rather to lay the foundation and to facilitate an overall strategy for improved IRM appropriate to the whitefly situation in Almeria . Key to this is clearly better communication, education and co-operation between all interested parties.

For more information on IRAC and the whitefly workshop in Almeria please look at our website: www.plantprotection.org/irac

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