Resistant Pest Management

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News and Reviews

Can herbicide resistant oilseed rapes from commodity shipments potentially introgress with local *Brassica w*eeds, endangering agriculture in importing countries?

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Much misinformation, disinformation and widely inaccurately-interpreted information has been promulgated about genetically engineered herbicide-resistant crops (*i.e.* Rissler & Mellon 1995), especially by those with an anti-genetic engineering and/or anti-pesticide bias. We are warned that such crops will lead to "superweeds" that will inherit the earth (Kling 1996). Not all environmentalists share these radical views (Lewis 1992). Nevertheless, severe political pressures preclude much public-sector research in this area, leading to our inability to obtain accurate information about any possible risks from these crops. This subject is discussed almost exclusively by those with a political agenda and rarely meets scientific scrutiny. The situation is further complicated by well-meaning scientists who lack the knowledge to balance the issues and make scientifically untenable extrapolations from the data. Only recently has the role of these herbicide-resistant crops been evaluated by considering the control advantages and disadvantages, avoidance or precipitation of new resistance problems, the role of these crops as volunteer weeds and the introgression of resistance genes in local weeds (Gressel 1997). This article explores the implications for shipping biotechnologically-derived, herbicide-resistant crop seed as a trade commodity.

I hope that this article invokes responses from producers, herbicide manufacturers, exporters, weed control experts and scientists from the seed importing countries in future issues of this Newsletter.

The issue

Genetically-engineered herbicide-resistant crops pose no health dangers to consumers from the engineered genes. Thus, there is no need to label international shipments of these crops or products from these crops as "transgenic" or state what genes they contain. Indeed, transgenic and non-transgenic seed may be mixed in commerce. Unfortunately this has engendered a certain level of consumer hysteria in some parts of Europe. Tons of Swiss chocolates were recently recalled, because they contained lecithin possibly extracted from transgenic U.S. soybeans. The same Europeans chose to eat cheeses that may contain rennet, a product that is often produced transgenically.

Frequently international commerce skirts the issue of whether transgenic crops pose a risk to agriculture in the importing countries. Quarantine and pesticide registration authorities can prevent advertent importation and agronomic use of such herbicide-resistant crop seeds. Should these authorities have the responsibility to prohibit inadvertent release of this seed material (*i.e.* escaping from commodity shipments)? Should exporters share these responsibilities?

Agronomists, biologists and weed scientists in importing countries often monitor the roadsides leading from ports to grain elevators, feed-meals and oil-crushing plants for volunteer weeds (escaping crops) and new weed species. Commodity seeds, unlike seeds for planting, do not have the same restrictions for contaminant weed seeds. Thus, many new weed infestations may occur in transit from port to user. Importers have been dealing with non-transgenic alien invasions for many decades, so the novel situation is when the volunteer weed carries genes detrimental to the agriculture of the importing country. Engineering resistance into crops that interbreed with related weeds can sometimes be unwise. So far authorities seem to have focused on gene introgression to weeds in the areas where transgenic crops are grown, but have not dealt with the issue where the seed is exported.

I want to further focus this discussion. The science behind how herbicide resistance was introduced into the crop is irrelevant (see Duke *et al*. 1996, Galun & Breiman 1997 for discussion). Similarly, geo-political boundaries will be ignored and commodity import/export is defined as transgenic seed from the production site to any site where it might pose danger. Both sites may be within one large country. Shipments of processed products from the crops (oil, flour, meal, *etc*.) pose no introgressional risk, and do not warrant further discussion here .

Why pick on oilseed rapes?

Herbicide-resistant maize, soybean and cotton are on the international trade market or soon will be. There are no reports of naturally-occurring herbicide resistances moving from these crops to weeds, and there is no reason to believe this will change for transgenes. However, oil-seed rapes have numerous weedy relatives throughout the world. Thus, oilseed rapes are most prone to gene introgression into weedy species found in commercial fields. The rates of movement for these genes from crop to weeds are unclear.

Herbicide resistances introduced into oilseed rapes

ALS-resistant Argentine or Canola rape (*Brassica napus*) resistant to sulfonyl urea and imidazolinone herbicides was derived from tissue culture selection (Swanson *et al.* 1989). Triazine-resistance was laboriously crossed from field-evolved resistant wild *Brassica rapa (= B. campestris)* into *B. napus*

(Souza-Machado *et al.* 1982). Both products were released without regulatory scrutiny because they were not transgenic. Similar genes introduced transgenically are *verboten* in many countries and under scrutiny elsewhere. The ALS-resistant *B. napus* is widely cultivated, especially in western Canada. The triazine-resistant *B. napus* has a 15-20 yield reduction (Gressel & Ben-Sinai 1988, Beversdorf *et al.* 1988), yet is gaining prominence in Australia, where the elimination of wild brassicas by inexpensive triazines is popular. This strategy should be effective until the brassica weeds evolve resistance to triazines, as they have in Ontario, Canada (Maltais & Bouchard 1978).

When a weed becomes resistant to any of the ALS herbicides, it is not known whether resistance evolved naturally or was introgressed through pollination. The highly mutable ALS gene (*ca.* 10^{-6} resistance in pristine populations) quickly appears in weeds. Engineering the same gene with either a two-base coding difference from the natural gene or with different introns would allow us to differentiate between mutation and introgression. Perhaps we would learn more about this phenomenon if a transgene had created this herbicide resistance. In the case of triazine resistance, resistance is maternally inherited so one might assume it will never transfer. Nevertheless maternal inheritance is not absolute, 0.2% pollen transfer was found with genetic markers.

Presently, transgenic glyphosate-, glufosinate-, and bromoxyinil-resistant oilseed rapes have been, or are about to be, released in western Canada. There is nothing to stop genetic engineers from introducing readily available genes to other herbicides such as 2,4-D (Streber & Willmitzer 1989) into oilseed rapes.

What is known about gene transfer to wild Brassicas

Controlled experiments have shown that herbicide resistances can be transferred from oilseed rapes to wild relatives, most often *Brassiea rapa* (*e.g.* Kerlan *et al*. 1993, Jorgensen & Andersen 1994, Bing *et al*. 1996, Mikkelsen *et al*. 1996, Lefol *et al.* 1996b). These studies usually resort to model or artificial systems such as hand pollination after emasculation of the weed, male sterility or self incompatibility in the weed, massive amounts of crop pollen, and/or embryo rescue of rare individuals, most of which are sterile (Darmency 1994). Conversely, gene transfer from a Brassica weed to oilseed rape has been reported. For example, McMullan *et al.* (1994) reported that deleterious weed genes introgressed into oilseed rape, lowering yield and oil quality.

When the herbicide is applied to a transgenic crop, susceptible weeds growing in the crop will not introgress the resistant genes - dead weeds don't have sex. However, resistance genes could introgress into nearby unsprayed weeds. Roadsides and nearby fields may have the wild mustards growing in them. Thus, oilseed rape may escape and show up as a volunteer weed on unsprayed roadsides. Seed set on emasculated *B. napus* was measured 1.5 km from a pollen source (Timmons *et al.* 1996).

Can these progeny compete, or survive in feral populations without the selector? Without emasculation, resistant pollen fertilized 24% of plants in the immediate vicinity, but fertilized less than 0.017% just 10 meters away.

Can the herbicide resistant transgene provide traits that increase fitness when the herbicide is not used? An unequivocal "no" is hard to provide. An alien gene metabolizing the herbicide might metabolize other substrates, supplying other traits. Typically, herbicide resistance supplies less fitness advantage than disease or insect resistance to feral pest populations (Karieva *et al.* 1996). As long as the weed can be controlled by other means, this is unlikely to lead to Frankensteinish superweeds. Modified target sites of the herbicide must be less fit than the susceptible wild type or they would be expressed in the wild type. Therefore, continual monitoring for newly-resistant weeds is required; and not just for those that may evolve by introgression with resistant crops. It is easier to eliminate nascent resistant foci, than huge areas after spread (Moody & Mack 1988, Darmency & Gasquez 1990).

Do the related indigenous weeds and the escaped volunteer oilseed rape have overlapping flowering times? If flowering does not overlap, mating is complicated beyond risk.

Are the related indigenous weeds self incompatible or do they accept foreign pollen thus enhancing chance meetings? If self pollinated, then genes transfer more slowly.

Is the herbicide-resistant pollen more or less competitive than con-specific pollen? Pollen competition can be exceedingly strong. A lack of fitness in pollen from a resistant crop and competition between conspecific pollen certainly could delay gene transfer (Charlesworth 1988, Stephenson *et al.* 1988, Mulcahy & Mulcahy 1987).

How easily can interspecific barriers be overcome where they exist? In western Canada both resistant Canola (*B. napus*) and Polish rape (*B. rapa*) are grown. The former is morphologically different, but genetically conspecific with the latter – a major Brassica weed.

Most studies rating risks of gene movement do not differentiate between reports of field transfer and field studies documenting transfer. They do not estimate how long it will take for resistance to introgress and predominate in wild populations, how long it would take resistance to evolve by natural selection, or the expected commercial lifetime of the herbicide. One study showed that *B. napus* can transfer genes to hand-pollinated *Sinapis arvensis*, at a rate of one hybrid seed per 100 flowers. However under natural field pollination, no hybrid seed was found in three million seeds (Lefol *et al.* 1996a). The lack of field studies abrogates scientific evaluation to politicians who are most influenced by those bearing an agenda, particularly those who produce the new varieties and herbicides or those who are anti-technology. One cannot make easy generalizations about the risks of resistance transfer. To predict the risk of introgression, each case must be evaluated on its merits, often after basic biological, ge-

netic and epidemiological studies. If we assume that there is an introgressional risk from oilseed rapes in many areas of the world, we must try to ascertain the levels of such risk and then ask what can be done about it scientifically and politically. There is hardly a biologist who does not expect such introgressions from proximal growth to eventually occur, although it may take many years. It is a political decision of an importing country to demand infinitesimally low or no risk of gene transfer. This could be justified by stating that few, if any, benefits of this crop accrue to the importers.

The decision-making process in western Canada

Many were surprised when the Canadian government allowed the field use of glyphosate- and glufosinate- (and soon bromoxynil) engineered transgenic oilseed rapes despite the known introgression of herbicide-resistant genes into weeds. A major use of herbicide-resistant oilseed rapes is to control wild relatives. The authorities perpetuated a double standard; ALS-R rape from mutagenesis had been on the market. Their decisions followed in-depth biological risk analyses of the local introgressions from the oilseed rapes (Anonymous 1994, 1996). Their decision allowed unrestricted field cultivation of the crops, despite the likelihood of gene introgression. They felt that the introgression would not increase weediness of crop or related weeds (Crawley *et al.* 1993). The benefits for a safer and more cost-effective weed control far outweighed the risks. The worst case scenario would be the loss of glyphosate to control such weeds (Anonymous 1995).

The Canadian authorities did not require two safeguards that might have lowered the risk of gene transfer to *B. rapa*, the weediest of the related species (Holm *et al.* 1997). This species has already demonstrated field transfer of genes (Mikkelson *et al.* 1996). First, no monitoring system was required to safeguard against this transfer. Second, they did not take prescribed genetic means to lower the risk. Canola (*B. napus)* is an ancient allopolyploid between *B. oleracea* (CC genome) and *B. rapa* (AA). Thus, if only these transgenic plants bearing resistance on the C genome were used, resistance would transfer to *rapa* by rare homologous pairing only. Glyphosate resistance is coded on two genes in a tandem construct. One gene for modifying target site and the other for degrading the herbicide. Resistance transfer could be delayed if each gene were inserted on separate C chromosomes, thus requiring two independent transfers. Whether fortuitously or by intent, the glufosinate (R. K. Downey, pers. comm.) and bromoxynil resistances for Canada are on the safer C genome, whereas the glufosinate resistance was on the A genome, a more easily introgressed genome (Mikkelson *et al.* 1986).

The question of how quickly resistance will move from *B. napus* to weedy *B. rapa* may be moot. The con-specific Polish rape (*B. rapa*) with these herbicide resistance genes will soon be released (R. K. Downey, pers. comm.). In this locale, they do not consider transfer to the con-specific weed a problem, as it is relatively rare, and where present, easily controlled by other herbicides in rotational crops. Oilseed rapes are invariably grown in rotation in this area. Whether the decision of the authorities in this area allowing this transgenic crop is wise is moot - it is their problem. What happens when commodity shipments drop seeds where related wild mustards are a problem in eastern Canada and much of the world.

What are the criteria for the importers?

Approval has been obtained from Japan, U.S.A. and Mexico for import of glyphosate resistant Canola from Canada, without a requirement for labeling. These countries account for about 85-90% of Canada's export. *B. rapa* and less related weedy species are indigenous and problematic in these countries (Holm *et al.* 1997). It would be interesting to know how these issues were considered in these countries. What were the criteria used for the allowance? Did they include the possibility of introgression from escaped volunteers? What evidence was used to negate such risks? Is it an exporter's "GATT given right" to ship unlabelled herbicide-resistant crops to countries where it might be an agronomic, just because they present no health hazard. Is a country within its rights to protect against alien weeds and exclude all shipments of oilseed rape because there may be some transgenic material included that could be potentially passed on to related indigenous weeds? Can an importing country require a monitoring program and at whose expense? If a nascent focus is not immediately eradicated, spread can be rapid (Moody & Mack 1988, Thill & Mallory-Smith 1998). Crawford *et al.* 1997 have modeled just how rapid this gene flow can be. In many importing countries, commodity seeds are transported in leaky tarpaulin-covered multi-use trucks. Clearly well-sealed bulk transport, hopper-type trucks would leak less and substantially decreasing risk. This would add expense to transport; at whose expense? I have not been able to find a resistance management specialist who knows how these decisions were made or has been asked to contribute to the decision-making process. Presumably, someone can supply this information to specialists dealing with pesticide resistance and its management through discussion in this Newsletter. The future may bring many useful herbicide-resistant crops for one area, that pose problems in other areas. We need herbicide-resistant crops and need to avoid any trade wars, but each country needs to protect themselves from generating herbicide-resistant weed problems. The problems discussed are not because the crops are biotechnologically derived. This is a new issue that we did not have to deal with a decade ago. This issue is frequently overshadowed by a political agenda that is concerned with how these new biotechnological products were created.

There are ways of preventing introgression

Care must be taken not to pass irrevocable laws banning herbicide-resistant oilseed rapes. These crops need herbicide resistances to control both interbreeding and non-related weeds. There are solutions to prevent pollen transfer such as pollen specific excision systems, obligatory apomictic systems without pollen (Koltunow *et al.* 1995). Requirements for more than one unlinked gene for resistance, tight or tandem linkage of the resistance gene in a crop with a gene deleterious to a weed, infection of the crop with disarmed RNA viruses bearing resistance genes that are not pollen carried are futuristic possibilities.

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Industry and individual company's perspective on resistance management

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This article is a summary of an oral presentation made to a formal conference on arthropod resistance at the 1997 Annual Meeting of the Entomological Society of America. The conference was organized by J. L. Flexner, DuPont Agricultural products. This presentation was my attempt to relay the industry views on resistance based on seven years experience in IRAC U.S. and from the perspective of a product development manager from DowElanco. Resistance management issues and tactics are highly debated within industry, as well as the scientific community, and these views do not necessarily represent those of the entire industry.

Background Information

Mark Whalon, Michigan State University, recently provided the following classical definition of resistance: "When a product or control tactic fails to reduce a pest population due to changes in pest susceptibility." Dr. Whalon made the point that even with common definition there are different points of views based on where an individual's interests are focused. Although most scientists may agree to a version of this definition, a biologist would view it in terms of "X-fold increase in lethal doses or % survival at a discriminating dose", while a geneticist would view it as a change in gene frequencies and a biochemist would view it as a modification at the pesticide's target site. An industry representative would have yet another perspective and modify the definition further.

Industry's definition of resistance

Industry defines resistance as when a target pest's response to a commercial product develops to a level where the product no longer performs as intended. The measurable results can range from increased operational cost and lower customer value to complete loss of the product. The economic costs of resistance to industry can be staggering. The cost of a single non-performance complaint (resistance related or not) can negate the value for 10 to 1000 individual sales. Costs can include sales representative and technical service time, replacement products, legal costs and possible crop yield replacements. Most importantly, future sales depend on the reputation of the product and the company. In addition, product value to the customer is proportional to its performance. Performance is diminished if resistance develops. All these costs apply to existing products, but significant costs can influence future products as well. The crop protection industry has encountered increased regulations and product requirements in recent years resulting in higher costs and fewer product introductions. Today, it typically takes 7 to 10 years and 40 to 100 million dollars to bring a new crop protection product to market. An ideal product would pay off the accumulated debt during the first ten years of commercialization and the income from the 2nd 10 years would fund research and development of new products. The net result is that a long financial market life for each product is a must. The current costs for developing products and the loss of a product prematurely due to resistance development would be disastrous to a company.

Resistance creates opportunities for industry?

It would have been difficult to improve on the cost efficacy of older chemistries such as DDT and the chlorinated hydrocarbons if a combination of insect resistance and environmental awareness had not derailed them. There is a myth that industry has a backlog of more profitable products once they exploit the current technology. Although numerous products have been brought forward, very few have the combination of value (cost & efficacy) and selectivity (man, environment and crop) to meet Environmental Protection Agency (EPA) registration and be profitable. While it is true that the failure of existing technology due to resistance or other reasons can increase research efforts and allow more selective products to compete, it is also true that selective products have smaller markets, need longer market life and consequently, protection from resistance development to be financially viable. There is no guarantee that new products can be found. The loss of a product for one pest situation could eliminate a cropping system and customer base for other products. It is quite clear that all stake holders in agriculture (which eventually includes everyone) have something at risk; but in many scenarios, industry and the individual company certainly have a great deal at risk, in not the most of all stake holders.

The pesticide treadmill

The perceived pesticide treadmill illustration (Figure 1) is cited all too frequently. In my opinion, this treadmill represents an unrealistic viewpoint of what often occurs in most production systems. If we had numerous and inexpensive crop protection products or if crop prices/returns would justify indiscriminate treatments or low thresholds, the perceived pesticide treadmill might apply. However since today's global trade environment keeps return margins for all crops low and few tools are available, a crop advisor's treadmill (Figure 2) is what really occurs. The key difference is that the treadmill starts when control options are lost or unavailable, not because another sprayable product is available. Practitioners of resistance management need to point out this key difference to demonstrate the value of implementing resistance management plans.

Industry Wide Efforts

Collectively, industry works on common global issues through the Global Crop Protection Association (GCPA, formerly GIFAP) and through national organizations such as the American Crop

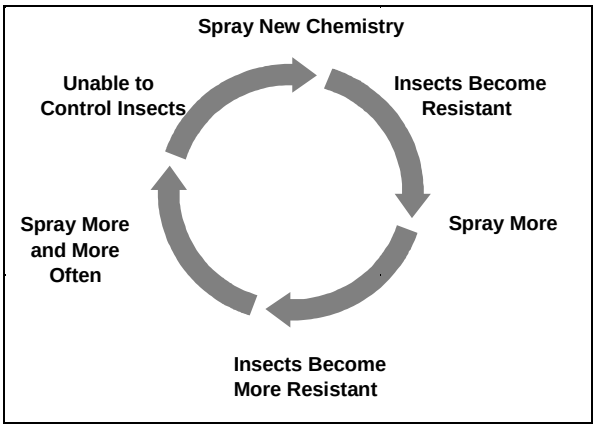

Figure 1. Perceived pesticide treadmill.

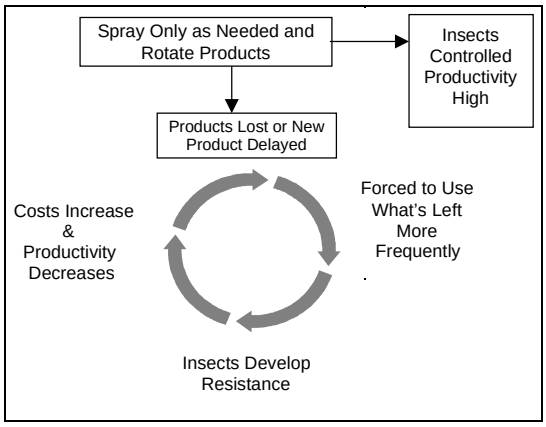

Figure 2. Crop advisor resistance treadmill.

Protection Association (ACPA formerly NACA). These organizations have formed technical committees to deal with a range of issues including resistance to pesticide products. Resistance Action Committees (RACs) have been formed to deal with Herbicides (HRAC), Fungicides (FRAC), Rodenticides (RRAC) and Insecticides (IRAC). I am most familiar with IRAC. Since it was formed in 1988, IRAC has been consistently active in the science and practice of resistance management. IRAC's accomplishments include sponsorship of pyrethroid monitoring programs, resistance surveys over multiple years, labeling efforts to address common modes of action, support for bioassay development and demonstration projects of good practices, numerous educational efforts (including support of this Pesticide Resistance Newsletter) and providing seed money for research programs that focus on resistance management.

DowElanco's approach to resistance management

DowElanco has been active in resistance management on several fronts for many years. Like most major companies, they have supported industry-wide efforts through IRAC, FRAC, and HRAC. Within business management teams, DowElanco has addressed and discussed resistance issues for individual products. In 1995, the company sponsored the formation of an advisory team, the Insecticide Resistance Management Team (IRMT), to provide advice to business units on resistance issues for all sprayable and transgenic products. The mission, vision and guiding principles of this team are outlined below together with the resistance management approach for an existing and an emerging product. These examples illustrate the importance of insecticide resistance management to DowElanco.

IRMT Mission – To provide technical/commercial counsel and support to the business in the development and implementation of arthropod resistance management strategies.

IRMT Vision - The commercial life of DowElanco's insect/mite management products is not unknowingly and/or unwillingly reduced due to the development of arthropod resistance.

IRMT Guiding Principles

Assume that the genetic basis for resistance to all current and future products occurs naturally in all arthropod populations and resistance management plans must be developed and implemented to ensure long term and consistent prosperity of DowElanco customers.

Resistance management is an integral part of product stewardship and responsible care is the responsibility of all technical and commercial functions.

DowElanco will support industry wide efforts and IRAC to manage resistance in arthropods.

DowElanco will not hesitate to consider and recommend competitive products as components of its own resistance management plans.

DowElanco believes that resistance management plans are most effective when implemented concurrently with the first commercialization of a new product.

DowElanco recognizes that resistance management plans for existing products may be both proactive and reactive and require urgent and extreme adjustments.

Lorsban™ examples

This product has a long history (30 years) of broad uses but has encountered very few resistance problems. IRMT attributes the continued success of this product with few resistance problems to the fact that it competes in most markets with several products and many target pests occupy large, untreated refugia. The few agroecosystems with resistance concerns (California red scale and wheat aphids) occurred when the alternate control options were severely limited. In each situation to date, reactive programs based on monitoring coupled with internal and external education programs have maintained the viability of Lorsban™ in all markets.

Spinosad examples

This new product with a unique mode of action is receiving its first registrations. The team that developed the product capitalized on the opportunity to develop a proactive program with assistance from DowElanco's IRMT. The goal is to maintain the effectiveness of spinosad for at least 20 years by delaying or preventing resistance development in target pests. The tactics selected by the team were practical labeling and distribution of educational materials that dis-

courage intense selection of target pests. Educational material will be designed for local needs. The effectiveness of the tactics will be assessed through periodic monitoring every three years, or every year if a 40% marketshare is obtained, and compared to the baseline susceptibility survey for each major target pest. Continual performance feedback will indicate any situations that need adjustment. Tracer Naturalyte™ is the spinosad product applied on cotton for insect control in the US. The usage information on the label is as follows:

Do not use Tracer Naturalyte or any insect control product from the same class on consecutive generations of tobacco budworm or cotton bollworm. If uncertain of the generation cycle, do not make more than 3 consecutive applications of an insect control product from the same class; then use one of the following IRM options for at least the next 30 days: No treatment or rotation to a different class of products. Consult with your local agricultural specialist or DowElanco representative for guidance and information on resistance management in your area. Always include multiple tactics (*e.g.* cultural or biological controls) within an IPM program. Do not use less than labeled rates alone or in tank mixtures and target applications against small larvae and eggs.

These labeling efforts and educational materials document this company's voluntary efforts to steward their technology with a resistance management strategy. During the label review process, EPA provided comments on the wording, but did not require or substantially change the initial submission by DowElanco. As additional crop labels are developed, it is clear that resistance management guidelines for pests, crops, and geographies differ greatly and therefore, information on labels require supplemental educational material to meet local needs.

Currently, one of the more controversial topics is regulatory requirements for resistance management tactics. My company and IRAC US have debated this topic at length. The following is an excerpt from a white paper drafted by the IRAC US committee that summarizes that committee's current logic. "Resistance management is a dynamic evolving science that should be widely debated at all levels within academia, industry, and the actual product use community. However, industry has as much to gain or lose from actions or inactions and can manage and react to resistance issues more effectively without specific regulatory requirements." Current regulatory requirements are too cumbersome and the review process is too long. These requirements only aggravate resistance development by limiting or delaying the tools that producers may use to manage resistance. The other concern is that all proposed regulations to date have been either so general as to be of little value or so specific that numerous technical exceptions are needed to be customize the product for individual geographies. Regional management plans designed by local university experts, industry, consultants and producers have proven the most effective to date. These regional plans can be modified each year as new information is obtained. They are the most practical way to implement resistance management rather than nationally-mandated programs enforced through product labeling.

SUMMARY

Pesticide resistance issues impact all stake holders in agriculture. Each stake holder is likely to form different views based on their background. Individual companies and the industry-at-large approach resistance from an economic view that considers the short and long term value to their customers. Industry's tremendous economic investment ensures that they will continue to steward their products on all issues including resistance management. Industry welcomes debate and assistance on all resistance issues, but regulatory requirement proposals would be a burden and provide minimal impact on resistance development. Regional management plans developed by local stake holders are the most effective plans and provide the flexibility needed to cope with dynamic biological processes that drive resistance development.

Concerns about managing organophosphate-resistant populations of leafrollers in New Zealand with tebufenozide

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Over recent months, there has been speculation that leafrollers resistant to organophosphates (OP) in New Zealand may have greater tolerance or resistance to other insecticides. This may have been stimulated by overseas reports of codling moth resistance to a wide range of insecticide (*i.e.* Sauphanor *et al*. 1994). The resistance of greenheaded leafroller, *Planotortrix octo*, to organophosphates and carbaryl has been reported from a restricted area in Central Otago (Wearing 1995a) and recently from Hawkes Bay (Lo *et al.* 1997). Research on OP-resistant greenheaded leafrollers from Otago shows that selection in the laboratory with either azinphos-methyl or Mimic® (tebufenozide) confers resistance to both chemicals. When colonies were established by mating females of a susceptible strain with wild males from the OP resistance area, the resulting progeny (larvae) had a low, but significant, resistance to both azinphos-methyl and tebufenozide. This research will be published.

Table 1. Assessments of leafroller damage on four apple cultivars at harvest in fruit regions with and without OP-resistant populations.

Zone	Cox	Royal Gala	Braeburn	Fuji
Resistance	0.4-1.6%	0.2-2.0%	0-0.8%	0-0.8%
No known resistance	0-0.4%	0-0.2%	0-0.4%	0-0.2%

Is this resistance reflected in field control failure? Laboratory selection is different from field selection, and leads to increase levels of resistance much faster. In the laboratory, resistant moths mate with other resistant moths; whereas in the field, resistant moths can mate with wild susceptible moths. In the case of OP insecticides, field control failure preceded the discovery of resistance. However, there is no prior history of Mimic® use in the Otago or Hawkes Bay areas. During the implementation of the NZIFP-P pilot program in 1996-97, the insecticide Mimic® was used extensively in Otago for the first time. Within the area inhabited by resistant leafrollers, mating disruption supplemented insecticide treatments, but fewer dispensers were used (250/ha) (Wearing 1995b) in an attempt to reduce costs.

Assessments of damage at harvest on four cultivars within and outside the resistance areas are shown in the Table 1. As with the earlier OP programs, damage was generally greater in orchards within the resistance area despite reliance on Mimi ® and mating disruption. However, it is hard to interpret the significance of the higher damage assessments due to possible differences in the size of leafroller populations between the areas. Nevertheless, this is the first comparison of field performance for Mimic® in areas with and without OP-resistant populations of leafrollers. When the laboratory and field data are taken together, there is reason for concern in the management of OP-resistant greenheaded leafrollers in Otago and Hawkes Bay. Although each discrete resistance episode requires separate investigation, it is possible that OP-resistant leafrollers in Otago and Hawkes Bay may have cross resistance to other insecticides such as Mimic®.

A resistance management program based on mating disruption with pheromones (1000 dispensers/ha) has provided excellent control of the OP-resistant leafrollers in Otago for the past five years (Wearing 1995b). Currently, this is our best strategy for managing leafroller resistance, because mating disruption works well on any OP resistant moths residing within the orchard. It is vital that growers within the resistance zones in Otago and Hawkes Bay continue this program to ensure maximum effectiveness in the future with either OP or Mimic® programs.

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Resistance Around the Globe

The contribution and inheritance of *Kdr* to fenvalerate and cyhalothrin resistance in *Helicoverpa armigera*

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The cotton bollworm (CBW), *Helicoverpa armigera* is a major pest of cotton in China. Pesticide resistance is a major factor contributing to the ineffective management of CBW. Previous physiological and biochemical studies with pyrethroid-resistant and susceptible populations of CBW proved that the resistance mechanisms involved are reduced cuticular penetration, enhanced metabolism and nerve insensitivity (Gunning *et al.* 1991, Wei *et al.* 1996, Wu *et al.* 1994, Zhao *et al.* 1996). Previously, a field strain of CBW was selected for resistance with cyhalothrin. The resulting resistance ratio (RR) was 30.6-fold to cyhalothrin and 337.0-fold to fenvalerate (cross-resistance). Our objectives were to investigate the mechanisms responsible for this resistance to the two pyrethroids and the inheritance of knockdown resistance *(kdr)* to fenvalerate in CBW.

The insect rearing and bioassay methods for this study are similar to Wei *et al.* (1992). We used the electrophysiological methods for the neurophysiological assay of *kdr* resistance as described by Zhao *et al.* (1996) and McCaffery *et al.* (1995). Ventral longitudinal muscle cells from 20-30 mg third instars were prepared for the assay. Spontaneous miniature excitatory junctional potentials (mEJP) were recorded with 3M KCl-filled glass microelectrodes of 15-25MΩ resistance connected to a microelectrode amplifier and photographed from the screen of a storage oscilloscope (HITACHI VC-6020). Each mEJP was identified by amplitude discrimination. A burst discharge EC_{50} was defined as the concentration of insecticide that elicited

midpoint responses from 50% of the individuals tested in each sample. A transmission block EC_{50} was defined as the concentration of an insecticide that elicited endpoint responses from 50% of the individuals tested. Data were analyzed with probit analysis. The lack of overlap between the 95%FL of EC_{50} was the criterion for a significant difference ($P<0.05$) in response.

The cyhalothrin-selected resistant (R) and nonselected susceptible (S) strain were reciprocally crossed ($R_{female}xS$, $S_{female}xR$) to produce the F1 generation for inheritance studies of *kdr* and fenvalerate resistance. The degree of dominance (D) for the F1 progeny was calculated (Stone 1968). The F1 were pooled and backcrossed to the parental R strain. Mortality for the backcrossed progeny was determined (Georghiou 1969). The hypothesis that the *kdr* resistance was controlled by a single major gene was tested with χ^2 test (Preisler *et al.* 1990).

Synergism tests on CBW pretreated with DEF (S,S,S-tributylphophorotrithiate) did not increase the susceptibility of R strain to either pyrethroid (Table 1). PBO (Piperonyl butoxide) decreased the RR of fenvalerate from 337.0- to 38.0-fold, and that of cyhalothrin from 30.6- to 5.0-fold. The synergism ratios of PBO with fenvalerate and cyhalothrin were 8.9- and 6.1-fold, respectively. This indicates that mixed-function oxidases (MFO) were important factors for resistance to both pyrethroids, but we found other mechanisms contributed to CBW resistance to pyrethroids.

Electrophysiological studies showed that the RRs were 10.7-fold to cyhalothrin and 95.8-fold to fenvalerate based on the burst discharge EC_{50} (Table 2). The RRs were 15.0-fold to cyhalothrin and 507.2-fold to fenvalerate based on transmission block EC_{50}. These results indicated that the difference in *kdr* response to fenvalerate and cyhalothrin was associated with CBW resistance to the two pyrethroids. The degree of dominance for *kdr* resistance to fenvalerate in the F_1 generation was –0.74 and –0.78 based on burst discharge and transmission block responses, respectively (Table 2). Backcrosses of the F1 to R strain (data not shown) suggested that *kdr* resistance to fenvalerate was likely monofactorial since the c?^{2} test for a monogenic model showed no significant deviation between observed and expected *kdr* ratio for the burst discharge (c?^{2}=3.72, a=0.05, df=6) or the transmission block (c?^{2}=3.10, a=0.05, df=6). Our results showed that the *kdr* resistance to fenvalerate was due to a single major autosomal gene that was incompletely recessive.

Table 1. Effect of metabolic synergists (PBO and DEF) on the response of resistant and susceptible *H. armigera* to the pyrethroids cyhalothrin and fenvalerate.

Treatment	S strain		R strain		
	Slope	LD_{50}(μg/larva) (95% FL)	Slope	LD_{50}(μg/larva) (95% FL)	RR
Cyhalothrin	2.32	0.010 (0.007-0.014)	2.31	0.306(0.212-0.442)	30.6
+PBO	2.29	0.006 (0.003-0.008)	2.60	0.030(0.021-0.042)	5.0
+DEF	2.46	0.013 (0.009-0.019)	1.68	0.289(0.180-0.464)	22.2
Fenvalerate	1.52	0.109 (0.063-0.205)	1.44	36.73(20.96-64.68)	337.0
+PBO	1.88	0.003 (0.002-0.006)	1.41	0.114(0.068-0.191)	38.0
+DEF	1.55	0.131 (0.078-0.221)	1.63	33.25(22.16-49.91)	253.8

Table 2. A comparison of neurophysiological activity in susceptible, pyrethroid-resistant, and reciprocally crossed F_1 strains of *H. armigera*.

Insecticide	Strain	mEJP repetitive burst discharge			Neuromuscular transmission block		
		Slope	EC_{50}(95% FL) ($x10^{-15}$M)	RR	Slope	EC_{50}(95% FL) ($x10^{-15}$M)	RR
Cyhalothrin	S	1.57	3.73(2.24-6.23)	1.0	1.48	36.3(21.47-61.51)	1.0
	R	1.53	39.8(23.5-67.4)	10.7	1.58	544.1(328-903)	15.0
Fenvalerate	S	1.41	0.32(0.15-0.65)	1.0	1.07	4.97(2.44-10.10)	1.0
	R	1.02	30.7(14.6-64.6)	95.8	1.36	2521(1353-4697)	507.2
	R♀xS	1.49	0.37(0.13-1.00)	1.2	1.26	13.6(5.33-34.65)	2.7
	S♀xR	1.64	0.53(0.23-1.20)	1.7	1.06	6.02(1.96-18.53)	1.2
	Pooled F_1	1.57	0.57(0.31-1.04)	1.8	1.18	9.75(4.81-19.77)	2.0

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Cross-resistance of *Bacillus thuringiensis* resistant population of diamondback moth *Plutella xylostella* (Lepidoptera: Yponomeutidae)

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ABSTRACT

Ten commercial formulations of insecticides were investigated for toxicity towards a population of diamondback moth resistant to *Bacillus thuringiensis* (B.t.). Laboratory bioassays with larvae showed no cross-resistance to fenthoate (organophosphate), benfuracarb (carbamate), fenvalerate (pyrethroid), chlorfluazuron (insect growth regulator), cartap (tertiary amine), and abamectin in a population of diamondback moth resistant to Delfin®. This population exhibited high cross-resistance to Thuricide® (B.t. formulation), but no cross-resistance to Dipel® 2x or Centari® (B.t. formulations). These results suggest that other groups of insecticides may suppress diamondback moths resistant to B.t. Increased toxicity as a result of synergism between B.t. formulations and other conventional insecticides was found for diamondback moth.

Key words : *Bacillus thuringiensis*, Delfinâ, cross-resistance, synergism.

INTRODUCTION

The diamondback moth (DBM), *Plutella xylostella* is a serious insect pests of cruciferous crops throughout the world. It has adapted to a wide range of climatic conditions. It has a short life cycle and high fecundity. The development of insecticide resistance in this Yponomeutid has made it increasingly difficult to control, especially in the tropics. In addition, frequent and excessive insecticides applications have only fueled further resistance development in this key insect pest.

Tabashnik *et al.* (1990) found the first field population of DBM resistant to *Bacillus thuringiensis* (B.t.). Since then, another strain from the Philippines was found to possess over 200-fold resistance to B.t. (Ferre 1991). High levels of B.t. resistance were detected in Japan (Homa *et al*. 1986).

Tabashnik *et al.* (1991) concluded that DBM resistance to B.t. declined slowly in the absence of selection. Conversely, McGaughey & Beeman (1988) demonstrated *Plodia interpunctella* (Indian meal moth) resistance to B.t. did not decline after two generations without selection. However, Hama (1992) documented that DBM resistance to B.t. significantly decreased within few generations. Thus, B.t. resistance in DBM seems to be unstable.

Available data suggests that resistance to B.t. resulted from selection by B.t. rather than selection by, or cross-resistance to, other insecticides. The mode of action for B.t. differs from that for conventional insecticides (Horvey *et al.* 1986), thus minimizes the possibility of cross-resistance. However, there are reports of cross-resistance between different isolates of B.t. subspecies *kurstaki*. In this study, we investigated the cross-resistance spectra of B.t.-resistant DBM to different groups of insecticides and different commercial formulations of B.t.

Noppun *et al.* (1984) found that DBM larval mortality from combinations of carbaryl/B.t. and or methomyl/B.t. were higher than that from either carbaryl or methomyl alone. However, the insect mortality from the mixture of orthene/B.t. was lower than B.t. or orthene alone. The insecticides carbaryl, methomyl and orthene combined with B.t. showed no inhibitory effect on DBM growth (Yen & Hsiao 1977). Chlordimeform and fentin hydroxide were synergistic with B.t., however demeton-s-methyl and dimethoate were highly antagonistic with B.t. (Hamilton & Attia 1977).

MATERIALS & METHODS

Insect Strains

Delfin®-resistant and non-selected (Bangkhae Strain, BKO) strains of DBM were supplied by the toxicology laboratory at the Department of Entomology, Faculty of Kasetsart University. The resistance ratio of the Delfin®-selected to the non-selected DBM strain was greater than 90 (based on LC_{50}).

DBM were reared at room temperature (25-27°C with 75% RH) and at a 16:8 (light:dark) photoperiod. For each strain, 50 pairs of pupae were place in a cage (25 x 31 x 27 cm^3) and cotton wool impregnated with a 5% honey solution was provided as a food source. After adult emergence, Chinese kale seedlings were provided for an oviposition substrate. Hatched larvae fed on the kale seedlings. As second instars, they were transferred to a paper-padded plastic box (25 x 31 x 7 cm^3) and fed on fresh cabbage leaves (*Brassica oleracea* L. var capitata). Third instars were used for the bioassays.

Insecticides formulations

The insecticides tested included: (1) fenthoate 50% EC (Nissan chemical Co., LTD), (2) fenvalerate 20% EC (Sumitomo chemical Co., LTD), (3) befuracarb 20% EC (Ostuka chemical Co., LTD), (4) chlorfluazuron 5% EC (Ishinhara Sangya Kaisha Co., LTD), (5) Cartap 50% SP (Taketa Pharmaculical, LTD), (6) Abamectin 1.8% EC (Hoechst Co.), (7) Delfin® (Sandoz, LTD), (8) Thuricide® WP (Sandoz, LTD), (9) Centari® WP (Abbott Laboratories) and (10) Dipel® 2x WP (Abbott Laboratories).

Delfin® was mixed at 1:1 ratio with the following insecticides: (1) phenthoate, (2) fenvalerate, (3) benfuracarb, (4) chlorfluazuron, (5) cartap, (6) Thuricide®, (7) Centari®, (8) Dipel® 2x and (9) abamectin.

Bioassay Procedure

Each insecticide or insecticide mixture was diluted into 5 concentrations with distilled water. A spreader/sticker (Linch to Nihon Noyaku Co., LTD, Osaka) was added at 200 ppm to each concentration. Cabbage leaves (5 x 5 cm^2) were dipped in a prepared concentration for 10 seconds. Treated cabbage leaves

Table 1. Cross resistance spectrum of a Delfin®-resistant strain of DBM to different conventional and *Bacillus thuringiensis* insecticides.

Insecticide	Selected strain		Nonselected strain		RR	
	LC_{50} [1]	LC_{95}	LC_{50}	LC_{95}	LC_{50}	LC_{95}
fenvalerate	70.5	835	630	257	2.7	3.3
benfuracarb	71.4	1140	55.3	659	1.3	1.7
phenthoate	36.7	476	16.3	139	2.2	3.4
cartap	20.4	256	28.7	347	0.71	0.74
chlorfluazuron	0.715	6.48	0.486	7.22	1.5	0.90
Abamectin®	0.305	0.190	0.0110	0.347	0.27	0.55
Delfin®	18300	1950	1290000	29700	70.4	113
Bt (A)	638000	104000	604000	36500	0.95	0.35
Thuricide®	23300	2980	869000	238000	37.3	46.3
Dipel®	398000	55000	609000	64400	1.5	1.2

[1] Values are ppm except Bt formulations where values represent dilution rates.

were placed on a paper pad and left to air-dry at room temperature. Ten 3rd instars were placed with treated leaves into a paper-padded plastic cup (10 cm in diameter and height) and held at room temperature. Mortality was recorded at 48 and 72 hours after treatment for conventional insecticides and at one week (168 hours) for IGR insecticides. Larvae that failed to respond to prodding with a pencil point were recorded as dead. The data were analyzed with probit analysis (Finney 1971). Co-toxicity coefficients were calculated as in Suo & Johnson (1960).

RESULTS

Cross-resistance spectrum for Delfinâ-resistant DBM

Delfin® resistant DBM strain showed no cross-resistance to other groups of insecticides (Table 1). The resistance ratio (RRs) for this strain was 2.2 for fenthoate (organophosphate), 2.0 for fenvalerate (pyrethroid), 1.8 for chlorfluazuron (IGR), 1.3 for benfuracarb (carbamate), 0.7 for cartap (tertiary amine) and 0.3 for abamictin. There was no cross-resistance to the B.t. formulations Centariâ® (RR = 1.1) and Dipel® 2x (RR = 1.5). However, high cross-resistance was detected for Thuricide® (RR = 37.3).

Synergism between Delfin® and other insecticides on selected Delfin®-resistant DBM

The relative efficacies and co-toxicity coefficients (CC) for the Delfin® insecticide mixtures on Delfin®-resistant and susceptible DBM are compared in Table 2 and Figure 1. For both Delfin®-resistant and susceptible DBM, there was a synergistic relationship between Delfin® and Centari®(CC = 203 for resistant and 136 for susceptible) and Delfin® and phenthoate (CC = 284 for resistant and 118 for susceptible). Synergism between Delfin®/benfuracarb (CC = 139), Delfin®/Dipel® (CC = 118) and Delfin®/Cartap (CC = 110) was detected in the Delfin®-resistant DBM only. The mixture of Delfin®/ chlorfluazuron showed no synergism in either the resistant (CC = 71) and susceptible (CC = 70) DBM strain. However, the Delfin®/abamectin mixture demonstrated antagonism toward both Delfin® resistant and susceptible DBM.

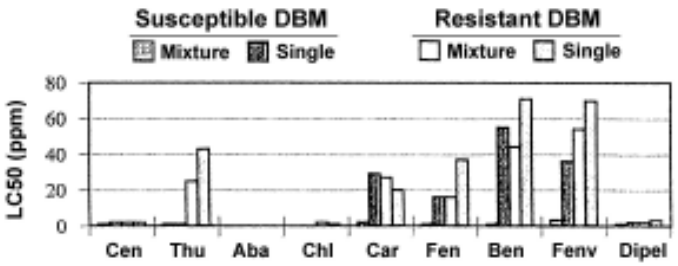

Figure 1. Toxicitiy of insecticides and mixtures to a *B. thuringiensis* susceptible and resistant strain of diamondback moth (Cen = centari; Thu = Thuricide; Aba = abamectin; Chl = Chlorfluazuron; Car = Cartap; Fen = Phenthoate; Ben = Benfuracarb; Fenv = Fenvalerate).

DISCUSSION

Cross-resistance

Our DBM strain with resistance to the B.t. formulation, Delfinâ, possessed no cross-resistance to the conventional insecticides or the other B.t. formulations except Thuricide® R. This reflects the similar mode of action for the active ingredient in these B.t. insecticides derived from B.t. subspecies *kurstaki*. The Delfin®-resistance in the DBM strain showed no cross-resistance to Centari® R. This B.t. formulation was derived from the B.t. subspecies *aizawai* not *kurstaki*. There was no cross-resistance to Dipel®another B.t. isolated from B.t. subspecies *kurstaki*. Delfin® and Dipel® gain their toxicity from different insecticidal crystal proteins that target different receptors in the insect midgut. Ferre *et al*. (1991) reported that the toxicity of three crystal proteins (CrylA (b), CrylB and CrylC) found in B.t. formulations varied between field-collected populations of DBM. They also reported that the loss of DBM susceptibility to the crystal proteins of Dipel® [CrylA (b)] did not provide resistance to the crystal

Table 2. Relative susceptibility of Delfin®-resistant and susceptible DBM to insecticide mixtures.

Insecticide mixute	Susceptible strain			Resistant strain		
	LC_{50} (ppm)	Slope	CC[2]	LC_{50} (ppm)	Slope	CC [1]
Delfin						
+ Centari	0.76	0.25	136	1.58	1.62	203
+ Thuricide	1.33	1.2	69	24.5	1.2	89
+ Abamectin	0.0292	2.29	74	0.129	1.97	47
+ Chlorfluazuron	0.352	1.44	70	2	1.29	71
+ Cartap	1.83	2.67	83	27	1.86	110
+ Phenthoate	1.26	1.26	118	15.5	1.97	284
+ Benfuracarb	1.27	2.06	85	44.3	1.82	139
+ Fenvalerate	2.73	1.42	56	53.7	2.03	81
+ Dipel	1.43	1.41	82	2.29	1.46	118

[1] Co-toxicity coefficient of the mixture (Sun & Johnson 1960)

protein, CrylB and CrylC, not present in the Dipel® formulation.

Tabashnik *et al.* (1991) suggested that insecticide rotations would not be effective in suppressing and reversing the development of B.t. resistance because this resistance decreases quite slowly. However, Hama (1992) concluded that DBM resistance to B.t. significantly declined within a few generations. Thus, we recommend that conventional insecticides and other B.t. formulations that contain different types of crystal proteins be rotated to retard the development of Delfin® resistance in DBM.

Synergism between Delfin® and other insecticides

Our data show synergistic relationships between Delfin® and conventional insecticides. This could be attributed to the slow toxic effect of B.t. formulations compared to conventional insecticides. The mixture of Delfin® with conventional insecticides also killed DBM faster than Delfin® alone. Since there was no cross-resistance between Delfin® and these conventional insecticides, mixtures could be applied to prevent multiple insecticide resistances (Yen & Hsiao 1977).

Delfinâ mixed with other B.t. formulations showed synergistic toxicity. A mixture of Delfin® and Centari® showed the highest value for synergism (CC = 203) toward Delfin®-resistant DBM. Delfin® and Centari® are formulated from different subspecies of B.t. The synergistic toxicity of this B.t. mixture was also reported by McGaughey & Johnson (1992) for the Indian meal moth. McGaughey & Johnson (1992) concluded that the application of multiple B.t. toxins may prolong the efficacy of B.t. products against B.t. resistant pests. Further work on this resistance management strategy is needed to maintain acceptable crop protection and environmental conservation.

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Decrease in the susceptibility of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) to pyrethroid insecticides in Côte d'Ivoire

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INTRODUCTION

The American bollworm *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) is a major insect pest of vegetable and cotton in West Africa. In the Côte d'Ivoire, pyrethroid insecticides have been applied for fifteen years to control *H. armigera* and other cotton bollworms [Spiny bollworm, *Earias insulana*, and pink bollworm, *Pectinophora gossypiella* (Saunders), and the false codling moth *Cryptophlebia leucotreta* (Meyrick)]. Pyrethroid insecticides are always mixed or rotated with organophosphate insecticides to control leaf pests (mites, leafrollers and sucking insects) and to prevent or delay pest resistance in bollworms.

Since 1985, CIRAD in conjunction with the cotton research program at IDESSA surveyed field populations of *H. armigera* from Cote d'Ivoire for susceptibility to insecticides. A topical application bioassay determined the response of *H. armigera* larvae to several pyrethroids and organophosphates and monitored any change in pest susceptibility (Alaux 1994, Alaux *et al.* 1997). These changes may indicate an increasing heterogeneity in the pest population as the number of resistant individuals increases. Meanwhile, bioassays carried out in France on a susceptible strain of *H. armigera* reared for many years on an artificial medium.

MATERIALS & METHODS

Our bioassay followed a protocol advised by the Entomological Society of America (Anon. 1970). Technical grades of cypermethrin and deltamethrin were mixed in analytical grade acetone and stored at 4°C. For each active ingredient, we used five serial (geometrical) concentrations. Third and fourth instar (40 to 80 mg weight) *H. armigera* were used for the bioassays. Larvae were selected immediately after molting and weighed.

We applied insecticide concentrations to the dorsum of larvae (1 ml/ 100 mg larvae) with an Arnold microapplicator. Thirty larvae were exposed to each concentration per replicate (three replicates). Mortality was assessed 48 h after application. Data was corrected with Abott's formula (Abott 1925) then subjected to probit analysis (Finney 1971). LD_{50} values for each insecticide were calculated with a PC-software developed at CIRAD (LD_{50} version 4.6) (Giner 1993). Resistance ratios were determined by dividing the LD_{50} value of the selected *H. armigera* strain by that of the susceptible strain held in France.

RESULTS

Before 1992 our surveys detected no change in pest resistance to deltamethrin or cypermethrin and the values for strains from Côte d'Ivoire were equivalent to those for susceptible strain reared at CIRAD (Figure 1). In 1994, the slope of the concentration/ response curve changed (higher slope, flatter curve) for both insecticides (Figure 2 & 3). This change was not steady, but indicated a potential increase in the proportion of resistant individuals in the *H. armigera* population.

In 1995 and 1996, an increase in LD_{50} values was recorded for *H. armigera* to both cypermethrin and deltamethrin (Figures 1) and the slopes returned to values similar to those reported in 1992 (Figures 2 & 3). When compared to either the strains collected in 1985-1992 or the susceptible strain at CIRAD, resistance ratios of 12:1 and 22:1 were recorded for these *H. armigera* for cypermethrin and deltamethrin, respectively (Figure 4).

Field efficacy trials show that 88% of first instar *H. armigera* were still controlled by the field rate of cypermethrin (40 g (AI)/ ha). However, control efficacy decreased to 30 % for middle-sized larvae, and to 0 % for late instars (up to 3 cm long) (Figure 5).

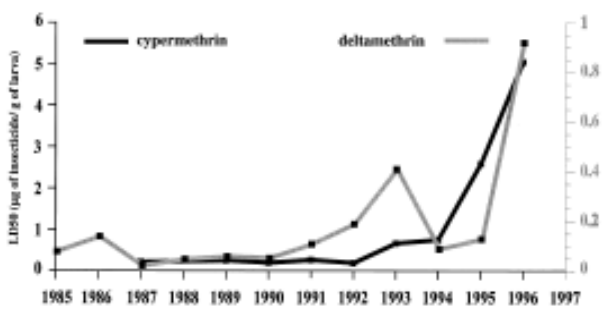

Figure 1. The increase in LD_{50} values in *H. armigera* response to cypermethrin and deltamethrin in Côte d'Ivoire between 1995 and 1996.

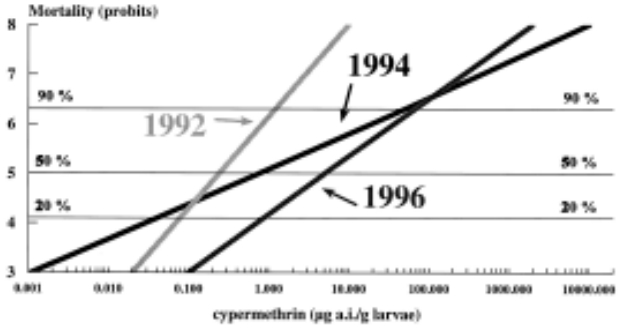

Figure 2. Cypermethrin concentration-response curves documenting a change in *H. armigera* mortality between 1992 and 1996 in Bouaké (Côte d'Ivoire).

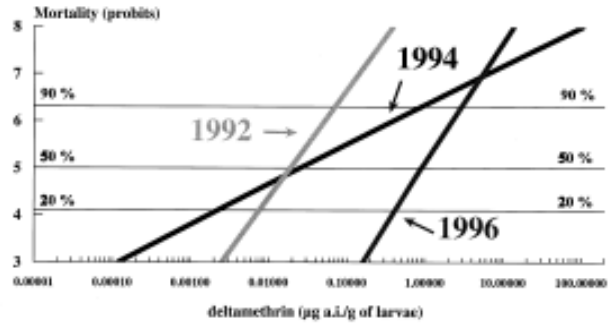

Figure 3. Deltamethrin concentration-response curves documenting a change in *H. armigera* mortality between 1992 and 1996 in Bouaké (Côte d'Ivoire).

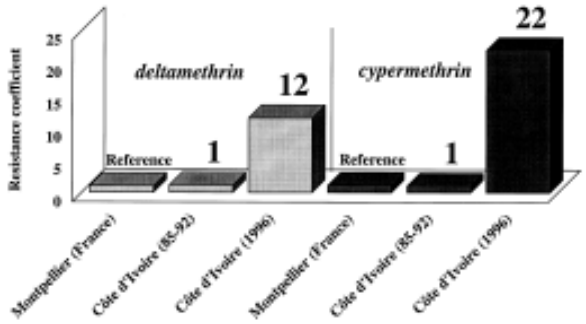

Figure 4. An increase in resistance ratios to deltamethrin and cypermethrin among *H. armigera* populations collected in Côte d'Ivoire between 1985 and 1996.

DISCUSSION

Although many countries report pyrethroid resistance problems with *H. armigera*, this is the first time a decrease in pyrethroid susceptibility and reduction in efficacy has been documented for *H. armigera* in West Africa.

Sawicki defines "resistance" as a pest control failure under field conditions (Sawicki 1987). Fortunately, no control failure has been reported in farmer fields yet. However, the field efficacy study shows that an *H. armigera* outbreak could be controlled only if the pyrethroid is applied in the first days of the pest infestation (targeting first and second instars). With respect to Sawicki's definition, resistance has not yet occurred in Côte d'Ivoire. However, laboratory and field tests together show that we are not far from control failure. The cotton growing area in West Africa covers many countries, from Senegal to Nigeria and with the migration habits of *H. armigera* (Nibouche 1994) we believe that this emerging problem is not limited to Côte d'Ivoire.

This topical bioassay procedure was a rather poor indicator of resistance, but in absence of diagnostic biochemical tests and when associated with field efficacy tests, it provides an early warning of resistance emergence. We have an extensive baseline of data on *H. armigera* susceptibility to pyrethroids and plan to select discriminating concentrations that will reduce our resistance screening and monitoring efforts.

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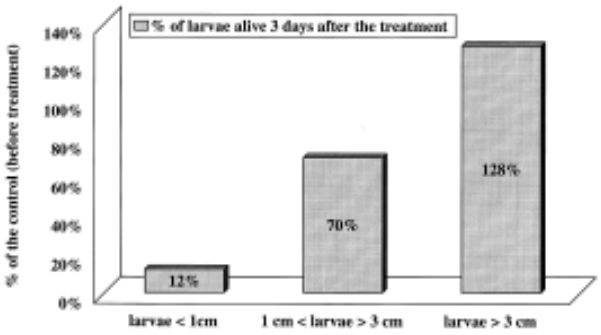

Figure 5. Relative efficacy of cypermethrin (40 g a.i./ha) among different sizes of *H. armigera* larvae collected in Côte d'Ivoire. Percent control determined three days after cypermethrin application.

Dynamics of fungicide resistance and impact on control of cucurbit powdery mildew

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INTRODUCTION

Presently, fungicides are the important tool for managing powdery mildew, a disease that affects cucurbit crops every year. Systemic fungicides are needed to obtain adequate protection on lower leaf surfaces, where conditions are more favorable for development of the pathogen, *Sphaerotheca fuliginea*, rather than on upper surfaces (McGrath 1996b). Unfortunately throughout the U.S., pathogen strains exist with resistance to the sterol-biosynthesis inhibiting (SBI) fungicide triadimefon (Bayleton) and/or to the benzimidazole fungicides benomyl (Benlate) and thiophanate-methyl (Topsin M) (McGrath *et al.* 1996). These are the only systemic fungicides currently registered in the U.S. for powdery mildew management. Despite widespread occurrence of strains highly resistant to triadimefon, this fungicide effectively suppressed powdery mildew in 1991 and 1992, because the resistant strains were rare at the start of disease development (McGrath 1996a). Following early-season treatment, the pathogen population shifted to predominantly resistant strains and consequently triadimefon did not provide full-season control (McGrath 1996a). The objectives of our study were (i) to examine the yearly dynamics of fungicide resistance, (ii) to determine how many applications of triadimefon contribute to powdery mildew control when used with chlorothalonil, (iii) to examine the impact of fungicide use on resistance, and (iv) to examine the impact of resistance on efficacy.

MATERIALS & METHODS

Field experiments were conducted in Long Island, NY, on pumpkin from 1993 to 1996. The main fungicide program was triadimefon applied on a 14-day schedule with the broad-spectrum contact fungicide chlorothalonil applied on a 7-day schedule. Treatments included triadimefon applied 1 to 4 times within this program to assess the efficacy of successive applications. Benomyl was added to some programs. Additional treatments included the newer, more effective SBI fungicides propiconazole (Tilt), myclobutanil (Nova), and triflumizole (Procure) to determine when triadimefon efficacy was compromised by resistance. Treatments were started after detecting powdery mildew by field scouting. The decision criteria used to initiate fungicide applications was 1 infected leaf out of 50 mature leaves. Fungicide programs initiated when this threshold is exceeded have been as effective in mildew management as preventive fungicide spray programs (McGrath 1996b). Applications were made with a tractor-mounted boom sprayer. Disease assessments were

Table 1. The shift in the occurrence of fungicide resistance and efficacy of fungicides on powdery mildew from pumpkin before and during an epidemic.

			Start of epidemic [a]			Middle of epidemic [b]		
			Resistant Isolates (%) [c]		Mildew severity (%) after 1st spray period [e]	Resistant Isolates (%) [c]		Mildew severity (%) after 2nd-3rd spray periods [f]
Year	Treatment	Date of Triadimefon applications [d]	Triadimefon	Benomyl		Triadimefon	Benomyl	
1993	None		3	0	14.3*			56*
	Triadimefon	August 16			0.7	71		15
1994	None		39	0	4.3*			14
	Triadimefon	2,17,30 Aug			0.8	90		19
1995	None		80	0	6.4			85
	Triadimefon	31 July; 15,29 Aug			4.2	73		77
1996	None		52	48	4.6*	56	31	4
	Triadimefon	16, 30 Aug			0.3	66	66	3

[a] Isolates collected on 11 Aug 93, 6 Aug 94, 1 Aug 95, and 20 Aug 96.
[b] Isolates collected on 1 Sept 93, 19 Aug 94, 18 Aug 95, and 19 Sept 96.
[c] Isolates able to grow on leaf disks treated with triadimefon 100 μg/ml or benomyl 200 μg/ml.
[d] Triadimefon applied at 14-day intervals with chlorothalonil applied on 7-day intervals.
[e] Severity on abaxial leaf surfaces assessed on 26 Aug 93, 16 Aug 94, 16 Aug 95, and 29 Aug 96.
[f] Severity on abaxial leaf surfaces assessed on 2 Sept 93, 6 Sept 94, 31 Aug 95, and 11 Sept 96.
* Mildew severity was significantly different (P = 0.05) between treatments.

made on lower leaf surfaces where chlorothalonil is not particularly effective (McGrath 1996b).

Fungicide sensitivity was determined with a leaf disk bioassay described by McGrath *et al*. (1996). Isolates were collected at the start of powdery mildew development and again after fungicide application.

RESULTS & DISCUSSION

Triadimefon-resistant strains were more prevalent at the start of powdery mildew development in 1994-1996 (39-80%) than in 1993 (3%) (Table 1) and in 1991-1992 (0%) (McGrath 1996a). Benomyl-resistant strains were detected in 1991 (30%), 1992 (10%), and 1996 (48%), but were at undetectable levels in 1993-1995 (Table 1).

One application of triadimefon, made after powdery mildew detection within a fungicide program with chlorothalonil, was effective when the initial frequency of triadimefon-resistant strains was less than 55% (Table 1). Powdery mildew was significantly less severe on the lower surfaces of triadimefon-treated than non-treated leaves 10-14 days after the first application in 1993, 1994, and 1996 (Table 1). There were no significant differences in disease severity in 1995 when the initial frequency of triadimefon-resistant strains was 80%. A second application of triadimefon was not effective; there were no significant differences in disease severity on plants sprayed once or twice with triadimefon in 1993 and 1994. Loss of efficacy was due to resistance as the frequency of triadimefon-resistant strains had increased to 71-90% 16-17 days after the first fungicide application. Furthermore, other systemic fungicides were more effective than triadimefon, especially during the second half of the epidemic.

Benomyl incorporated in the triadimefon plus chlorothalonil fungicide program contributed to powdery mildew control in 1995, when benomyl resistance was not detected before treatment. However, in 1996 benomyl did not contribute to mildew control when 48% of the pathogen population was resistant to both triadimefon and benomyl before treatment (Table 1).

Although cross resistance occurs among the SBI fungicides (McGrath *et al*. 1996), triadimefon-resistant strains were controlled by propiconazole, myclobutanil, and triflumizole (data not shown). After 2 or 3 SBI fungicide applications at 14-day intervals, powdery mildew on lower surfaces of leaves treated with these newer SBIs was quite low (<1%) and was significantly less than on leaves treated with triadimefon (3-17%). Propiconazole did not control powdery mildew on lower leaf surfaces in 1995 as well as in 1993 and 1994 perhaps because the rate was halved to 2 oz/A. The strobilurine fungicide kresoxim-methyl (Sovran), with a different mode of action from SBIs, was as effective as triflumizole when tested in 1996. These results confirm that the decline in triadimefon efficacy during the growing season was due to selection of resistant strains rather than another cause.

In conclusion, successful management of cucurbit powdery mildew with currently-registered fungicides is challenged by the occurrence and yearly dynamics of resistance to triadimefon and to benomyl. It is not possible to predict the efficacy of these fungicides based on the frequency of resistant strains the previous year. After one application of these fungicides, the pathogen population rapidly shifts within two weeks to predominantly resistant strains. Consequently, more than one application does not provide additional disease control. Proper timing is critical to ensure that one application is made only after the threshold is reached for greatest impact. However, the initial frequency of resistant strains can become so high that control failure may still occur. Triadimefon and benomyl are still recommended for managing powdery mildew; however, they should always combined with protectant fungicides that are not at-risk for resistance development.

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Evidence for genetic heterogeneity in a malathion-resistant population of *Drosophila simulans*

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In the analysis of the genetic basis of pesticide resistance, a number of issues have been debated. First, is field resistance typically a monogenic or polygenic phenomenon? While both theoretical considerations and experimental results suggest that monogenic resistance is the most likely to evolve, there are numerous examples of resistance that appear to involve multiple genes and/or mechanisms. This may be critical to the success of resistance management programs targeted against agricultural pest species. Second, does pest resistance result directly from pesticide-mediated selection, or from natural variation in susceptibility that exists in a population (Robertson *et al.* 1995)? Worded differently, does natural variation reflect the genetic variation on which pesticide selection acts? If so, then certain predictions can be made: A) resistance is more apt to be polygenic, since susceptibility is inherently a quantitative trait, determined by contribution and interactions between multiple loci, and B) the rate at which resistance evolves in a particular popu-

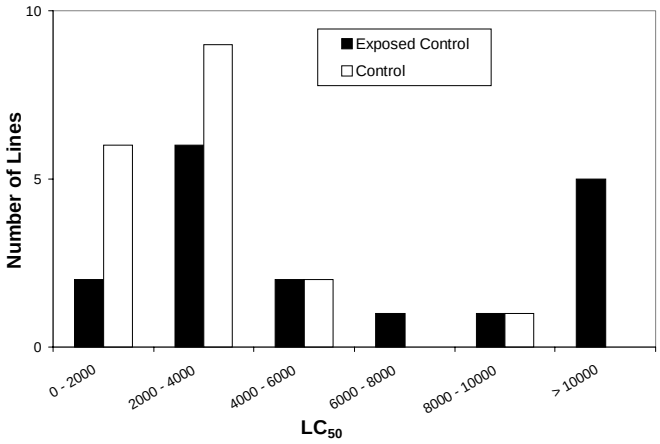

Figure 1. Distribution of LC_{50} values from *Drosophilia* lines collected at sites exposed to and free from malathion.

lation is influenced by the amount of variation in susceptibility that exists prior to pesticide usage. Finally if resistance does not involve the spread to fixation of a single major allele, then it is quite likely that significant genetic variation in resistance will remain in the population even after extensive selection.

We recently reported high levels of resistance to malathion in a population of *Drosophila simulans* where malathion had been used exclusively and intensively for the control of salt marsh mosquitoes (Windelspecht *et al.* 1995). In that report, we surveyed 17 isofemale lines of *D. simulans* for malathion resistance and observed LC_{50} values ranging from 14,00 ppm to 81,500 ppm. In contrast, four lines collected from one control site displayed LC_{50}s from 1,800 to 2,800 ppm. We collected an additional 18 lines from various nonexposed sites in the Tampa Bay vicinity and assayed them for malathion resistance (Windelspecht *et al.* 1995). Figure 1 compares the distribution of LC_{50}s in several lines from control versus exposed sites. The distribution of LC_{50}s for lines from control sites approaches a normal distribution. This seems to be the case for lines from the exposed site except for the five lines with LC_{50}s greater than 10,000. If we exclude those five lines, the mean LC_{50}s for the two site groups (2,900 for control and 4,000 for exposed) are not significantly different (t=0.16, df = 28, n.s.). The five lines from the exposed sites demonstrate resistance levels that are outside of the phenotypic range found in control populations. The LC_{50}s from the control populations do substantially overlap with those from the exposed populations.

Is this resistance at the exposed sites polygenic and does it result from selection on natural variation present in the existing base population? We crossed two of the five resistant lines, CF11 (LC_{50} = 18,000 ppm) and CF34(LC_{50} = 66,000 ppm) to a susceptible line (Guatemala 3) and backcrossed the F_1 progeny to the resistant parental line. The slopes of the dose-response curves for the resistant parental line (CF34) were distinctly lower than those for the susceptible parental line (Figure 2). This suggests that significant heterogeneity remains in the resistant CF34 line compared to the susceptible parental line. The slope of the dose-response curve for the backcross population was similarly flat. If resistance was due to a single gene, we would expect the backcross progeny to consist of two genotypes, RR and RS, with different resistance levels. The continuous nature of the dose-response curves shown in figure 2 argues against this hypothesis. Results with the CF11 resistant line were comparable. Thus, we conclude that resistance was due to multiple genes, and that these resistance alleles were not fixed even in our most resistant lines.

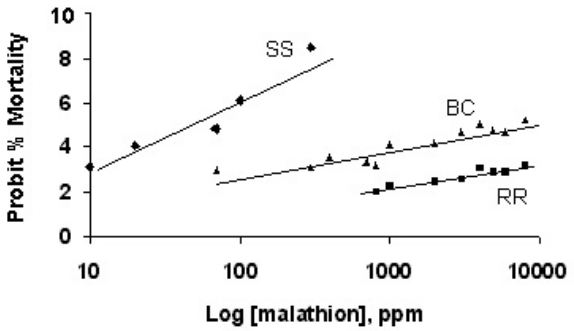

Figure 2. Dose-response kinetics of malathion-sensitive *Drosophilia* line (SS), malathion-resistant line (RR) and the backcross F_1 progeny (BC) to malathion.

Did pesticide-mediated selection act on variation that was preexisting in *D. simulans* prior to malathion use? If so, then we would expect that the differences in mortality between control and exposed populations would reflect differences in frequencies of resistance alleles rather than presence or absence of resistant alleles. Malathion resistance in *Drosophila* and other species is attributed to point mutations in the gene encoding acetylcholinesterase (*Ace*), the target site of malaoxon (Mutero *et al.* 1994). We have identified this mutation (Leu to Met substitution, position 299) in the *Ace* allele carried by the CF11 and CF34 line. This mutation dramatically lowers the affinity of the acetylcholinesterase for malaoxon. The gene allele cosegregates with malathion resistance in isogenic lines derived from the F_1 progeny (Cochrane *et al.* in prep.). By comparing the frequency of this allele in control and exposed populations, we should be able to determine the degree that populations differ in their response to malathion.

Flies were collected at two control sites (Lutz and South Tampa) and at one site with a malathion-resistant line (Riverview). We determined the genotype and allele frequencies at the *Ace*

locus, as well as at a randomly selected control locus (*md20*, a microsatellite locus on chromosome II) (Table 1). A heteroduplex analysis detected three alleles in all three populations. In the Riverview population, the C allele, detected in malathion-resistant lines, was at a higher frequency than in the other two control populations. This difference in frequency was significantly different from zero based on Wright=s F_{st} (c^2 = 12.76, 4 d. f., $p < 0.05$). No similar evidence for differentiation was observed at the control locus although average heterozygosity at this locus was comparable to that at *Ace* (data not shown). These observations suggest that the malathion selection was associated with the change in allele frequencies at a particular locus and the resistance-associated allele exists at high frequencies in populations not subject to malathion selection.

Until recently, malathion has been used widely in Florida for mosquito control, especially in the urban areas that serve as prime habitats for *Drosophila simulans*. Thus, it is likely that all populations of this *Drosophilia* species in Florida have been subject to malathion selection pressure. In our case, pesticide selection acted on a preexisting genetic variation. Malathion resistance appears to result from contributions of multiple genetic loci. Since *Drosophila* species have not been targets for malathion control, selection may have been less intense than it would have been for a pest species. Furthermore, based on a report by Aquadro *et al.* (1988) and the climate of region, the population size of this cosmopolitan species of *Drosophila* is quite large. Even in our most resistant *Drosophilia* lines, the potential for further malathion selection exists. These conditions are ideal for the evolution of polygenic resistance and the distinction between resistance-mediated selection or selection on natural variation in susceptibility is far from obvious.

Table 1. *Ace* allele frequencies in three *Drosophilia* populations collected in the Tampa Bay area.

Location	Sample Size	Allele		
		A	B	C
Lutz	104	.082	.386	.563
South Tampa	73	.048	.384	.568
Riverview	82	.061	.237	.701

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Gene pyramiding: An effective strategy of resistance management for *Helicoverpa armigera* and *Bacillus thuringiensis*

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Research on transgenic plants expressing insecticidal crystal protein (ICP) from *Bacillus thuringiensis* (Bt) has progressed rapidly in recent years. The commercialization of Bt cotton in USA and Australia in 1996 indicates the importance of transgenic Bt crops in integrated pest management (IPM) for the near future. However, the importance of these crops could be seriously diminished by widespread development of resistance to these ICPs by the target pests. One tactic to prevent insects from adapting to transgenic Bt crops is to deploy multiple insecticidal genes (pyramiding) in transgenic plants (McGaughey & Whalon 1992). Transgenic tobacco expressing modified insecticidal proteins from Bt (Cry1A) and cowpea trypsin inhibitor (CpTI) was developed by the Biotechnology Research Center at the Chinese Academy of Agricultural Sciences (Zhao *et al.* 1995). The objectives of this study were to: 1) evaluate of transgenic tobacco with Bt and CpTI genes for insecticidal activity on the cotton bollworm (CBW), *Helicoverpa armigera*; and, 2) determine CBW's ability to adopt to transgenic tobacco expressing both the Bt and CpTI proteins versus tobacco with the Bt protein alone.

Over 200 CBW adults were collected from six counties in three provinces of North China to initiate a laboratory strain (Zhao *et al.* 1996). Larvae were initially reared on artificial diet at 27±1°C and a 14:10 (L:D) photoperiod (Wei *et al.* 1992). Each instar was exposed to one of four treatments: 1) a transgenic tobacco line 'Kongchong 931' expressing both Bt and CpTI proteins (Zhao *et al.* 1995), 2) a transgenic tobacco line ex-

Table 1 *H. armigera* mortality when reared on transgenic tobacco expressing Bt or Bt + CpTI genes, nontransgenic tobacco, and artificial diet.

Instar Exposed	Treatment duration	% Mortality ± SEM[1] Transgenic Bt and CpTI genes	Transgenic Bt gene alone	Nontransgenic control	Artificial diet control
1	3 Day	99.3±0.7 a	88.2±0.7 b	67.4±0.7 c	4.2±4.2 d
	To pupation	100 a	100 a	100 a	30.5±2.8 b
2	3 Day	83.3±4.8 a	55.6±5.6 b	27.8±11.1c	2.8±0.8 d
	To pupation	100 a	100 a	99.9±0.03 a	22.2±2.8 b
3	3 Day	80.5±2.8 a	50.0±8.3 b	22.2±2.8 c	16.7±4.8 c
	To pupation	100 a	100 a	77.8±2.7 b	19.4±7.3 c
4	3 Day	14.7±5.6 a	15.7±4.1 a	15.0±6.4 a	0 b
	To pupation	100 a	92.5±2.5 a	67.5±2.5 b	10.0±5.7 c
5	3 Day	6.0±4.0 a	4.0±4.0 a	5.0±4.0 a	4.0±4.0 a
	To pupation	72.5±7.5 a	67.0±9.7 a	42.5±2.5 b	6.7±6.7 c

[1] Means (SEM) within rows followed by different letters are significantly different (α=0.05, HSD).

pressing the Bt protein alone, 3) a nontransgenic tobacco line 'NC89', and 4) an artificial diet control. The three lines of tobacco plants were grown in the greenhouse and the tobacco leaves were used for this study. Each treatment exposed 48 first, 24 second, 20 third, 20 fourth and 20 fifth instar CBW to one of four treatments. Larvae were placed separately in one cell of a plastic plate and the plate was held at the above laboratory rearing conditions. CBW survivorship was recorded after larvae were exposed to a treatments for 3 days and repeated again after pupation.

Mortality of first, second and third instar CBW after three days of exposure was significantly lower on transgenic tobacco expressing the Bt protein compared to tobacco expressing both the Bt and the CpTI protein (Table 1). Both fourth and fifth instars were able to pupate when reared on tobacco with the Bt protein, but only fifth instars could pupate on transgenic tobacco expressing both Bt and CpTI proteins.

For eleven generations, second instar CBW were placed and reared on both lines of transgenic tobacco, nontransgenic tobacco and artificial diet. From the 2,000 larvae/generation placed on transgenic tobacco 61.5% (50.0 - 84.4%) survived on tobacco expressing Bt and CpTI proteins and 59.2% (31.5 - 78.0) survived on transgenic tobacco with Bt protein alone. After the selection period, second instar (2-4 mg) CBW from each treatment were screened for susceptibility to a microbial BT insecticide with the CryIAc ICP (MVP, Mycogen). The bioassay procedure reported by Zhao *et al.* (1996) was used. Larval mortality was recorded 14 days after exposure. The resulting concentration/response curves were calculated and LC_{50}s considered significantly different if their 95% FL did not overlap.

After eleven generations of selection, CBW reared on transgenic tobacco expressing Bt and ICP proteins demonstrated resistance ratios to formulated CryIAc ICP of 2.4 (LC_{50}) and 1.9 (LC_{90})(Table 2). In contrast, CBW reared on transgenic tobacco expressing the Bt gene only demonstrated higher resistance ratios of 5.5 (LC_{50}) and 13.6 (LC_{90}). The slope of concentration/response curve for the CBW reared on transgenic tobacco expressing the Bt protein alone is much lower than other treatments, suggesting greater variability and higher potential for resistance development.

Table 2. Resistance ratios (RR) of *H. armigera* larvae to Cry1Ac Bt ICP after 11 generations of selection on transgenic tobacco.

Selection treatment	b (SE)	LC_{50} (mg/l)	95%FL	RR1[1]	LC_{90}	RR2[2]
Bt and CpTI genes	2.48(0.25)	31.2	23.9-40.8	2.4 b	102.9	1.9
Bt gene	1.27(0.11)	70.2	52.4-94.0	5.5 a	723.8	13.6
Nontransgenic control	2.10(0.24)	13.1	9.4-18.1	1.0 c	53.5	1.0
Artificial diet control	2.10(0.19)	12.8	10.1-16.3	1.0 c	53.2	1.0

[1] RR1=LC_{50} treatment/LC_{50} of artificial diet control, RRs within a column followed by different letters are significantly different.

[2] RR2=LC_{90} treatment/ LC_{90} of artificial diet control.

This study suggests that transgenic tobacco with both Bt and CpTI proteins will delay resistance development in *H. armigera* to Bt ICP, relative to transgenic tobacco with the Bt protein only. Gene pyramiding could be a valuable strategy for resistance management and the sustainable use of Bt transgenic crops.

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Genetic basis of diamondback moth resistance to *Bacillus thuringiensis* toxin Cry1C

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Field-evolved resistance to *Bacillus thuringiensis* (Bt) by diamondback moth has been reported from many locations around the world (Tabashnik 1994, Perez & Shelton 1997). Almost all these reports involve resistance to *B. thuringiensis* subsp. *kurstaki* (Btk) (Tabashnik 1994, Liu *et al*. 1996). We recently reported field-evolved resistance to toxin Cry1C of *B. thuringiensis* subsp. *aizawai* (Bta), as well as low level resistance to a spore-crystal formulation of Bta (Liu *et al*. 1996, Liu & Tabashnik 1996). From the resistant field population, we established a Cry1C resistant laboratory colony. This article summarizes our efforts 1) to further increase resistance to Cry1C in this laboratory strain through selection, and 2) to assess the genetic basis of resistance to Cry1C (Liu & Tabashnik 1997a).

MATERIALS & METHODS

A laboratory strain (lab-p) of diamondback moth served as our susceptible strain in this study. In the field, our resistant strain (NO-95) had evolved about 22-fold and 4-fold resistance to Cry1C and Bta, respectively (Liu *et al*. 1996). The NO-95 was selected with Cry1C six times in the laboratory to give rise to the selected strain NO-95C. Leaf-residue bioassays (Liu *et al*. 1995, Tabashnik *et al*. 1993) were used for the selection process and toxicity comparisons. We used a liquid formulation of Cry1C for the selections and liquid formulations of Cry1C and Cry1Ab (Mycogen, San Diego, CA, USA) for toxicity comparisons. All bioassays were conducted at 28ºC and 14:10 (L:D) photoperiod.

After six selections, we did reciprocal mass crosses between NO-95C and LAB-P. Males were distinguished from females by the lighter coloration on the fifth abdominal segment of mature larvae (Liu and Tabashnik 1997b). We crossed thirty NO-95C females with thirty LAB-P males and crossed thirty LAB-P females with thirty NO-95C males. Third instars from the F_1 progeny and the parental strains, LAB-P and NO-95C, were bioassayed with six concentrations of the Cry1C toxin.

The F_1 progeny were then backcrossed with LAB-P. The F_2 offspring were reared and mated as 16 single-pair families. The F_3 larvae from each family were bioassayed with single concentrations of Cry1C and Cry1Ab.

We used probit analysis (SAS Institute 1985) to estimate LC_{50} values and 95% fiducial limits for the F_1 and parental colonies exposed to Cry1C. Resistance ratios were calculated by dividing the LC_{50} for a strain by the LC_{50} for the susceptible strain LAB-P. We also calculated the realized heritability of resistance from the selection with Cry1C (Liu & Tabashnik 1997a, Tabashnik 1992). Dominance of resistance was estimated by combining Stone's (1968) method based on LC_{50}s and Hartl's (1992) method based on single-concentrations. The degree of dominance (D) from the Stone's (1968) was converted to the dominance (h) from Hartl's (1992) definition as $h=(D+1)/2$. Values of h range from 0 (completely recessive resistance) to 1 (completely dominant resistance). Resistance is described as codominant or additive if $h = 0.5$, partially recessive if $0 < h < 0.5$, and partially dominant if $0.5 < h < 1$ (Liu & Tabashnik 1997a).

To test for genetic linkage between resistance to Cry1C and resistance to Cry1A, we used correlation analysis (SAS Institute 1985) to compare mortality to Cry1C and Cry1Ab among the F_3 split broods of single-pair families.

RESULTS & DISCUSSION

After six selections with Cry1C, the resistance level to Cry1C for the NO-95C had increased from 22-fold to 62-fold. The realized heritability of resistance to Cry1C was 0.10 (Liu and Tabashnik 1997a). Resistance to Cry1C was autosomally inherited as no significant difference in LC_{50} occurred in either reciprocal crosses between the NO-95C and LAB-P strains.

The dominance of Cry1C resistance depended on the formulation concentration. At the LC_{50} of Cry1C, F_1 progeny from the mass crosses (reciprocal crosses pooled) were significantly less susceptible to Cry1C than LAB-P, but did not differ significantly from NO-95C. Resistance to Cry1C was partially dominant where $h = 0.63$ (converted from $D = 0.26$). Dominance of Cry1C resistance increased as the concentration decreased. Resistance was recessive at 10 ml Cry1C formulation per liter ($h = 0$), partially recessive at 2 ml per liter ($h = 0.44$), and almost completely dominant at either 0.08 or 0.4 ml per liter ($h = 0.88$ and 0.98, respectively).

Resistance to Cry1C was not linked with resistance to Cry1Ab. Mortality caused by Cry1C was not correlated with mortality caused by Cry1Ab in the split-brood bioassays of F_3 progeny from single-pair families of F_2 backcross progeny. This indicates that the gene or genes that confer resistance to Cry1C segre-

gate independently of the gene(s) that confers resistance to Cry1Ab.

The value of 0.10 for the realized heritability of resistance to Cry1C was intermediate between the low values (0.022 and 0.069) for heritability of resistance to Bta (Liu & Tabashnik 1997c) and the high values (0.14 - 0.18) for heritability of resistance to Btk (Tabashnik 1992). Autosomal inheritance of resistance to Cry1C is consistent with all other reported Bt resistance. Bioassays from split broods of single-pair families confirm that the genes for Cry1C and Cry1Ab resistance segregate independently in diamondback moth (Liu et al. 1996).

This field population of diamondback moth from Hawaii harbors at least one recessive mutation conferring resistance to Cry1A toxins (Tabashnik *et al.* 1997a), as well as gene(s) that confer partially dominant resistance to Cry1C. Thus, the dominance of resistance can vary among Bt toxins for a single insect population. Also, for a given toxin, the dominance of resistance can vary among diamondback moth populations from different locations (Tabashnik *et al.* 1997b). These results cast some doubt on the effectiveness of resistance management tactics, *i.e.*, the refuge/high dose strategy expected to works best when resistance is recessive (Liu & Tabashnik 1997c, Tabashnik 1994b). The independence of Cry1C resistance from Cry1A resistance suggests that Cry1C and Cry1A toxins might be useful in rotations or mixtures for delaying resistance.

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Honey bee hygienic behavior as a defense against *Varroa jacobsoni* mites

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The article is an update of research reported in this newsletter in 1996, vol. 8, no. 1, pp. 42-44. The reader is referred to Spivak (1996) and Spivak and Reuter (1997) for more detailed descriptions of the methods.

The parasitic mite, *Varroa jacobsoni*, is the most destructive pest of honey bees, *Apis mellifera*, in the U.S. and Europe. Since its introduction into the U.S. in 1987, this mite has reduced the quality and quantity of bee colonies available for honey production and pollination. Currently, the only approved treatment for the mite is the pesticide Apistan® (fluvalinate) which is applied in strips within the bee hive. The risks of contaminating honey with pesticides and the development of mites resistant to the pesticide are formidable. It is important, therefore, to determine if honey bees have any natural, heritable defense mechanisms against the mite which may be readily incorporated into breeding programs.

Hygienic behavior is one mode of resistance to at least two diseases of larval and pupal honey bees and is a defense against *Varroa* mites. Hygienic bees detect and remove diseased brood from the nest before the pathogen becomes infectious, and remove mite-infested pupae interrupting the reproductive cycle of the mite.

A two-way selection program for hygienic behavior was initiated at the University of Minnesota in 1992. Colonies are selected for hygienic behavior using a freeze-killed brood assay in which the time taken to remove a 5 cm x 6 cm section of frozen pupae is recorded. Hygienic queens are reared from colonies that remove all of the freeze-killed brood within 48 hours over two trials. The queens are instrumentally inseminated with semen from drones of different hygienic colonies.

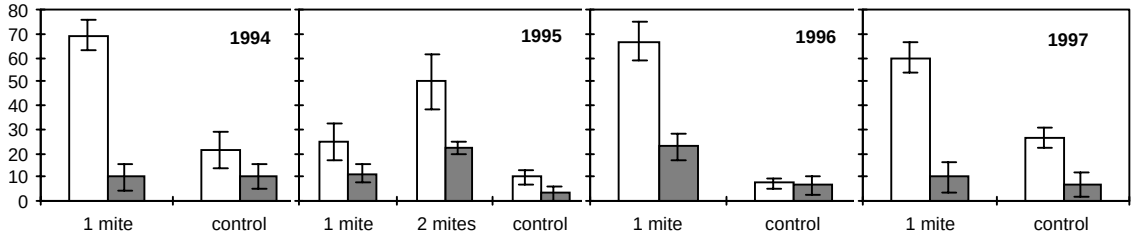

Figure 1. The mean percent removal of pupae experimentally infested with *Varroa* mites by the hygienic (open bars) and non-hygienic (solid bars) colonies from 1994 through 1997. The numbers of infested pupae removed were counted on the tenth day after the mites were introduced (or one day before the pupae eclosed). One mite per cell was introduced into 10 cells containing a recently sealed 5th instar larvae. The mites were introduced through the bases of specially constructed cells. The control cells were opened and closed with no mite introduction.

Lines of hygienic and non-hygienic colonies were bred and tested for their ability to remove pupae experimentally infested with *Varroa* mites (Spivak 1996). The hygienic and non-hygienic lines used in the experiment were bred from stock derived from Italian *A. mellifera ligustica*.

The results of four years are shown in Figure 1. Results of 2-way ANOVA for each year indicated that the hygienic colonies removed significantly more infested pupae than the non-hygienic colonies in all years except 1995. In addition, the hygienic colonies removed significantly more pupae infested with one mite per cell than control pupae, except in 1995 when they removed more pupae only when two mites per cell were introduced. There were no differences between the amount of infested and control pupae removed by the non-hygienic colonies. Some of the hygienic colonies (with the same queens) that removed low numbers of infested pupae in 1995 removed large numbers in 1996, indicating that the low removal rates in 1995 were due to environmental rather than genetic factors; however, the nature of those factors remains unclear.

Inseminated queens are used as breeder stock within the beekeeping community, but naturally mated queens are preferred in colonies used for honey production and pollination. Early genetic studies on hygienic behavior suggested that the hygienic trait is recessive (Rothenbuhler 1964). However, experiments in 1995 and 1996 indicated that queens raised from inseminated stock retained the hygienic trait when they were outcrossed with unselected males (Spivak & Reuter 1997).

If hygienic queens are to be utilized by the beekeeping industry, it is important to determine whether colonies with naturally mated queens from hygienic stock produce as much honey, have lower incidences of diseases and have lower levels of *Varroa* mites than colonies bred from commercial stock.

In March 1996, hygienic queens were reared and naturally mated in an apiary of a commercial beekeeper in Texas. For comparison, unselected "commercial" queens were reared and mated in the same location. The colonies were transported to Wisconsin in May, and those with marked hygienic queens (n = 49) and marked commercial queens (n = 46) were distributed in four apiaries. In June, the colonies were evaluated for population size, incidence of diseases, and temperament. In September, the colonies were evaluated for honey production, and mite loads. The results indicated that the

Table 1. Comparison of hygienic (n=49) and commercial (n=46) colonies headed by open mated queens. Values shown for hygienic and commercial colonies are means ± standard deviations. Evaluations of frames of bees, frames of brood, temperament, and chalkbrood were made in June 1996. Remaining measures were made in September 1996. All colonies were scored independently by 2 people and scores were averaged. Last column indicates whether values are statistically different: ns = not significant, $P > 0.05$; * = significant, $P \geq 0.05$ (2-way ANOVA comparing bee line and apiary site).

Criteria	Measurement	Hygienic	Commercial	*P*
Frames Bees	range 1-20 frames	17.4 ± 1.38	17.3 ± 1.74	ns
Frames Brood	range 1-20 frames	10.1 ± 1.85	10.0 ± 1.52	ns

Temperament	# stings received:
	0 = none; 1= one or more
		0.14 ± 0.32	0.02 ± 0.15	ns

Chalkbrood Disease	# mummies on 2 frames:
	0= none; 1=<5; 2= 5-20; 3=>20
		0.67 ± 0.85	1.78 ± 1.07	*

Honey Production	pounds harvested	90.0 ± 36.56	66.8 ± 32.20	*
Varroa mite load	# mites / 100 bees	0.6 ± 0.86	1.04 ± 1.09	*

hygienic colonies had significantly lower levels of chalkbrood disease, no Amercian foulbrood disease (compared to 6 commercial colonies with the disease) and produced significantly more honey. Importantly, the hygienic colonies had significantly lower levels of mites in three of the four apiaries. All other measures were the same between the hygienic and commercial colonies.

The same experiment was repeated in 1997 in collaboration with a different commercial beekeeper. The aims were to compare the hygienic colonies to "Starline" colonies, a commercial line renown for high honey production, and to compare the mite loads among the colonies over a longer period of time. Although the data is being tabulated currently, preliminary results indicate the hygienic colonies again have lower infestation levels. They also had significantly lower incidence of disease and produced as much honey as the Starline colonies (113 lbs and 101 lbs average, respectively).

In sum, the inclusion of hygienic behavior as a selection criterion in breeding programs is highly desirable because it provides a natural defense against American foulbrood disease, chalkbrood disease, and *Varroa* mites. In addition, it is possible to select for hygienic behavior without compromising honey production. Extension efforts are underway to encourage bee breeders to select for the behavior to so that hygienic queens from various lines of bees may be available to beekeepers.

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Inheritance of resistance to chlorpyrifos in the German cockroach

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Resistance to chlorpyrifos in isolated populations of the German cockroach, *Blattella germanica* (L.), has been known for many years (Milio *et al.* 1987, Schal 1988, Cochran 1989, Rust & Reierson 1991). Often the level of resistance detected is quite low (Cochran 1995), but varies among populations and is significantly influenced by the bioassay method (Milio *et al.* 1987, Cochran 1989, Siegfried *et al.* 1990, Rust & Reierson 1991). A LT_{50} (lethal time to kill 50% of a population) resistance ratio of 5.0 has been shown as the equivalent to a LD_{50} resistance ratio of 10-20 or even higher (Milio *et al.* 1987, Siegfried *et al.* 1990, Scharf 1994). However, in a tarsal-contact bioassay (lethal time or jar test), the results may differ greatly depending on the insecticide concentration (Cochran 1997). If too high of a concentration chlorpyrifos is used, resistance may be essentially masked.

Despite these difficulties, my studies conducted with the tarsal-contact bioassay indicate that instances of high-level resistance to chlorpyrifos in the German cockroach have increased sharply since 1990 (Figure 1). The ability to reliably detect this high-level resistance (Cochran 1997), allowed me to use a discriminating time to separate phenotypes. This simplified this study of inheritance mechanism of high-level chlorpyrifos resistance in the German cockroach.

In an earlier study, Siegfried *et al.* (1990) reported that chlorpyrifos resistance in the German cockroach was controlled by more than one factor. They also reported that this resistant strain showed an enhanced ability to hydrolyze chlorpyrifos and an increased level of cytochrome P450-dependent monooxygenase activity in comparison with a susceptible strain. This strain had an LT_{50} resistance ratio of 4.5. In spite of an LD_{50} resistance ratio of 22, this strain may not have had the high-level resistance (LT_{50} > 20) to chlorpyrifos described here. In addition, the biochemical differences between the resistant and susceptible strains were not large (Siegfried *et al.* 1990, Siegfried & Scott 1992). It is not clear what, if any, relationship those biochemical mechanisms have to the high-level resistance mechanism now present in many populations of German cockroaches.

In this study, I crossed the highly-resistant Las Palms strain with the VPI-susceptible strain. The results indicated that one major gene was responsible for most, if not all, of the chlorpyrifos resistance in this strain.

MATERIALS & METHODS

Insects

This study was performed with a VPI-susceptible strain and a Las Palms resistant strain (Las Palms) collected in Miami, FL in 1990. The Las Palms strain was maintained in the laboratory without selection pressure.

Genetic Crosses

Reciprocal crosses between these two strains were conducted. The resulting F_1 progeny were examined for their response to chlorpyrifos. Backcrosses were also made between the F_1 progeny and the Las Palms strain. The χ^2 goodness-of-fit test determined if observed results differed significantly from expected ratios based on an hypothesis of autosomal, monofactorial, Mendelian inheritance.

Toxicity Bioassays

Large nymphs (5-6th instars) were tested for their response to

chlorpyrifos by exposure to glass surfaces treated with 0.5 µg (AI)/cm^2 chlorpyrifos (Cochran 1989). Technical grade chlorpyrifos (94%) was supplied by DowElanco (Indianapolis, IN). Five replicates of ten insects from both F_1 progeny groups were tested with chlorpyrifos and mortality was recorded over time. The results were pooled and analyzed by probit analysis (SAS 1985). Similar analyses were conducted on the VPI and Las Palms strains, and a discriminating time was established from responses of susceptible, heterozygous, and resistant individuals. All tests were done at 21-23°C.

Synergist Studies

The synergists studied were piperonyl butoxide (PBO) and S,S,S-tributylphosphorotrithioate (DEF). Both were applied at a concentration of 3.0 nl (AI) /cm^2 and a synergist:insecticide ratio of 6:1. The results are reported as differences in mortality resulting from exposure to the insecticide-synergist combination in comparison with insecticide alone.

Gene Frequency Estimate

The gene frequency of the chlorpyrifos-resistance gene in the Las Palms strain was estimated with the Hardy-Weinberg equilibrium expression (Falconer 1981) as described by Cochran (1994a, 1994b). The essential features of this approach were that the trait in question be inherited monofactorially and that at least one phenotype be distinguishable from the others present by toxicological testing. These conditions were met.

RESULTS & DISCUSSION

The Las Palms strain was highly resistant to chlorpyrifos. Originally, no mortality among these roaches occurred during a 48 h exposure period. Scharf (1994) reported an LD_{50} resistance ratio of about 50 for this strain, but an LT_{50} of 5. He did not state the concentration of chlorpyrifos used in the tarsal-contact method, but it appears that it was high enough to mask resistance. The data shown in Table 1 for this strain are based on a 72 h exposure to chlorpyrifos at 0.5 mg (AI)/cm^2. Even longer exposure would result in a higher resistance ratio since little or no increase in mortality occurred.

Results from the genetic analysis of chlorpyrifos resistance are shown in Tables 1 and 2. F_1 progeny resulting from reciprocal crosses between the VPI-susceptible strain and the Las Palms chlorpyrifos-resistant strain were susceptible to chlorpyrifos (Table 1). LT_{50} resistance ratios for the F_1 progeny were 2.2 and 2.7 from reciprocal crosses. These data indicate that chlorpyrifos resistance is not sex linked and that the inheritance mechanism is incompletely recessive.

Table 2 confirms that all individuals from VPI-susceptible strain were killed by the 48-h exposure to 0.5 mg (AI)/cm^2 chlorpyrifos. In comparison, the Las Palms strain had a mortality of 6.6% based on more than 200 individuals. We estimate that the frequency of the resistance gene was >0.95 (Cochran 1994a). Bioassays on the F_1 reciprocal-cross progeny produced mortalities above 90%. Theoretically, values nearer to 100% were expected. Heterozygotes were slightly more tolerant of exposure to chlorpyrifos than susceptible individuals (Table 1) and 100% mortality was not always achieved. Apparently, a small percentage of heterozygotes can survive exposure to 0.5 mg (AI)/cm^2 chlorpyrifos for 48 h. A longer exposure period would have resulted in higher mortality. Backcrosses to the Las Palms strain produced progeny that showed approximately 50% mortality when exposed to chlorpyrifos. In both (Table 2) backcrosses, the data fit a 1:1 ratio ($c^2 = 0.64$, df = 1, P >0.3 and $c^2 = 1.44$, df = 1, P >0.2). These results are consistent with a hypothesis of a simple, autosomal, incompletely recessive inheritance mechanism for chlorpyrifos resistance in the Las Palms strain. It is likely that this inheritance pattern is common among populations of German cockroaches showing high-level resistance to chlorpyrifos as demonstrated by the tarsal-contact bioassay.

Neither piperonyl butoxide or DEF had a detectable effect on the mortality of VPI-susceptible strain when exposed to chlorpyrifos (Table 3). Similarly, piperonyl butoxide had no effect on mortality in the Las Palms strain when exposed to chlorpyrifos. In contrast, DEF increased mortality to >70%. While not conclusive proof, these data suggest that enhanced hydrolytic-enzyme activity is the principal biochemical mechanism involved in the Las Palms strain's resistance to chlorpyrifos.

This conclusion does not agree with Siegfried *et al.* (1990). Several factors may contribute to this discrepancy. First, the mortality data presented by Siegfried *et al.* (1990) revealed an overlap between the regression lines representing the phenotypes tested. Without a clear separation of at least one phenotype, it would be difficult to show monofactorial

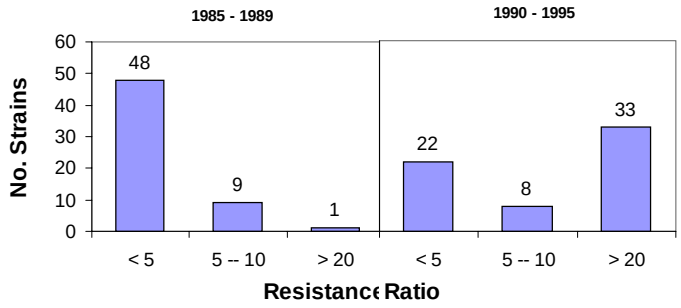

Figure 1. Changes in the level of resistance to chlorpyrifos over the past decade in field-collected populations of the German cockroach. Resistance Ratio (LT_{50} resistance ratio/ LT_{50} susceptible strain) determined with tarsal-bioassay.

inheritance (Cochran 1994a). Also since the line separation was not great, the dominance relationship would be difficult to establish. Our data shows that the resistant phenotype can be separated with a high degree of resolution by the tarsal-contact bioassay (Tables 1 and 2). Second, it appears that a new mechanism of resistance to chlorpyrifos may have developed in the German cockroach since 1990 as suggested by the synergism between the hydrolytic-enzyme inhibitor DEF and chlorpyrifos on roach mortality (Table 3). This factor by itself could explain the apparent discrepancy mentioned above. Third, it is possible that the resistance mechanism employed by the insect may be influenced by the bioassay used to detect resistance. Differences in uptake and distribution of the insecticide achieved by the two bioassay methods (Siegfried *et al*. 1990) could lead to differences in how synergists influence the level of resistance detected.

Table 1. German cockroach response to chlorpyrifos for progeny from reciprocal crosses between the VPI susceptible and Las Palms resistant.

Strain/cross	LT_{50} (min.)	Slope ± SE	RR
VPI susceptible	128	4.0 ± 0.3	---
Las Palms M x VPI F	277	3.9 ± 0.3	2.2
VPI M x Las Palms F	345	2.7 ± 0.3	2.7
Las Palms resistant	>4320[a]	---	>30

[a]Based on 72 h of exposure to chlorpyrifos-treated surfaces. Even longer exposures did not increase mortality significantly, but produced higher resistance ratios.

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Table 2. German cockroach mortality after exposure to chlorpyrifos for the VPI susceptible strain, the Las Palms resistant strain, their reciprocal crosses and F_1 backcrosses to the resistant strain.

Strain/cross	Percent mortality[a]
VPI susceptible	100
Las Palms resistant	6.6
VPI M x Las Palms F (F_1^1)	94.6
Las Palms M x VPI F (F_1^2)	92.7
F_1^1 M x Las Palms F	46.0
Las Palms M x F_1^1 F	56.0

[a]Mortality was based on a 48 h exposure to glass surfaces treated with 0.5 μg (AI)/cm^2 chlorpyrifos. Approximately 100 individuals were tested from each category, except >200 from the Las Palms strain.

Table 3. Effects of two synergists on mortality among the VPI susceptible and Las Palms resistant strains of German cockroaches when exposed to chlorpyrifos.

Strain	Treatment[a]	% Mortality[b]
VPI susceptible	Chlorpyrifos	100
	Chlorpyrifos + PBO[c]	100
	Chlorpyrifos + DEF[c]	100
Las Palms	Chlorpyrifos	0
	Chlorpyrifos + PBO	0
	Chlorpyrifos + DEF	71.4

[a]Insects were exposed to glass surfaces treated with 0.5μg (AI)/cm^2 chlorpyrifos alone or plus the synergist. Concentration of the synergists was 3.0 nl (AI)/cm^2. The synergist to insecticide ratio was 6:1.

[b]Based on 48 h of exposure to the treated surfaces.

[c]PBO is piperonyl butoxide. DEF is S,S,S-tributylphosphorotrithioate.

Insecticide resistance in turnip aphids, *Lipaphis erysimi* (Kaltenbach), from Beijing suburbs

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INTRODUCTION

Turnip aphid, *Lipaphis erysimi*, is an important pest of vegetable crops in Beijing, China. In the early 1980s, pyrethroids replaced the organophosphate insecticdes for aphid control. By 1986, the aphids evolved resistance to the pyrethroids with levels reported as high as 126.5-fold for deltamethrin and 412.4 for fenvalerate (Wei *et al.* 1988). Four years later, we monitored resistance to pyrethroid and organophosphate insecticides in aphids collected in Beijing suburbs.

MATERIALS & METHODS

Turnip aphids were collected in Langfang County, Hebei Province and in Beijing suburbs. The four populations of aphids from Beijing were sampled from vegetables at the following locations: Malianwa (MA), Shauangqing (SH), Evergreen (EV) and the Horticultural Department of China Agricultural University greenhouse (HO). Langfang County aphids were collected from Chinese cabbage that had not been treated with insecticides since 1990. These aphids exhibited high sensitivity to insecticides and were the susceptible colony in this study. Technical grade deltamethrin (98%; Roussel-Uclaf, France), fenvalerate (95.6%; Sumitomo Chemical Co.) dimethoate (97.4%; Shanghai Pesticide Factory) and omethoate (72.08%; Zhang Dian Pesticide Factory) were used for toxicity tests. A bioassay that exposed aphids to topical applications of insecticides was performed. This bioassay, recommended by the Division of Entomology, Nanjing Agricultural University (1983), was modified from earlier protocols (Kung *et al.* 1964, Zang 1982). Aphid mortality was scored 5 h after treatment.

RESULTS

Turnip aphid populations from Beijing suburbs expressed high levels of resistance to all pesticides tested (Table 1). The resistance levels were higher for the pyrethroids than organophosphates and appeared closely related to the insecticide history of the region.

Aphids exposed to the synergist piperonyl butoxide (PB) exhibited a high synergism ratio (SR) with fenvalerate and omethoate, 4.9 – 7.2-fold and 5.1-fold, respectively (Table 2). Aphids exposed to the synergist triphenyl phosphate (TPP) exhibited a synergism ratio of 3.4-fold with omethoate and 4.3-fold with dimethoate, but not with fenvalerate. These bioassay results suggest that mixed-function oxidases (MFO) play an important role in aphid resistance to pyrethroids and organophosphates.

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Table 1. The response and resistance to pyrethroid insecticides among turnip aphid populations collected from Beijing suburbs.

Insecticide	Population	Slope (SE)	LD_{50} (ug/aphid)	(95% CL)	RR*
Deltamethrin	LA	2.02 (0.49)	0.00014	(0.000086-0.00022)	1
	EV	1.55 (0.16)	0.00070	(0.00068-0.00093)	5
	SH	1.53 (0.19)	0.0051	(0.0031-0.0086)	36.4
	HO	1.02 (0.06)	0.012	(0.008-0.018)	85
	MA	1.97 (0.03)	0.20	(0.15-0.28)	1429.6
Fenvalerate	LA	2.35 (0.12)	0.0015	(0.0012-0.0018)	1
	SH	1.64 (0.16)	1.2	(0.8-1.3)	800
	EV	1.73 (0.39)	1.4	(1.0-1.9)	933.3
	MA	2.25 (0.22)	1.6	(1.3-1.9)	1066.7
	HO	0.94 (0.07)	1.7	(1.1-2.5)	1133.3
Dimethoate	LA	3.65 (0.24)	0.0092	(0.0082-0.003)	1
	HO	1.17 (0.09)	1.4	(0.8-2.3)	152.2
	EV	2.23 (0.29)	2.1	(0.6-2.6)	228.3
	MA	1.97 (0.26)	2.5	(2.0-3.0)	271.7
Omethoate	LA	3.67 (0.29)	0.0057	(0.0052-0.0063)	1
	EV	1.26 (0.20)	0.66	(0.41-0.0108)	115.8
	HO	1.07 (0.13)	0.82	(0.34-1.29)	143.9
	MA	1.84 (0.29)	1.0	(0.8-1.3)	175.4

*RR = LD_{50} of each populations/ LD_{50} of the susceptible LA colony

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Table 2. Response of turnip aphids to pyrethroid insecticides determined with and without the synergists piperonyl butoxide (PB) and triphenyl phosphate (TPP).

Population	Insecticide + Synergist	Slope (SE)	LD_{50} (95% CL)[a] (ug/aphid)	SR*
EV	Fenvalerate	1.73 (0.39)	1.37 (1.00-1.89)	
	+ PB (1:1.7)	1.31 (0.15)	0.19 (0.12-0.32)	7.2
	+ TPP (1:1.7)	1.86 (0.29)	2.96 (2.30-3.81)	0.5
SH	Fenvalerate	1.64 (0.16)	1.17 (0.81-1.32)	
	+ PB (1:1.7)	1.62 (0.39)	0.24 (0.17-0.35)	4.9
	+ TPP (1:1.7)	1.66 (0.27)	1.06 (0.73-1.53)	1.1
HO	Dimethoate	1.07 (0.07)	0.82 (0.34-1.29)	
	+ PB (1:3.2)	1.17 (0.25)	0.16 (0.10-0.26)	5.1
	+ TPP (1:3.2)	1.36 (0.07)	0.19 (0.11-0.34)	4.3
MA	Fenvalerate	2.25 (0.22)	1.59 (1.29-1.95)	
	+ PB (1:1.7)	1.60 (0.27)	0.27 (0.19-0.38)	5.9
	Omethoate	1.84 (0.29)	1.02 (0.80-1.30)	
	+ TPP (1:3.2)	1.55 (0.12)	0.30 (0.20-0.47)	3.4

*SR = population LD_{50} without synergist/ LD_{50} with synergist

The phorid fly, *Megaselia scalaris* (Loew), as a candidate for managing molluscicide-resistant round snail, *Bradybaena similaris* (Ferussas)

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The round snail, *Bradybaena similaris* Ferussas (Helicidae), is a terrestrial species widely distributed in the Tropics and prevalent at altitudes over 1,000 meters above sea level (Nor'Aini *et al.* 1995). This snail has potential to become an important agricultural pest, especially on Brassica crops such as *B. chinensis* L. and *B. juncea* Cosson (Murali 1991, Ahmad & Ho 1980). We observed damage on cabbage by *B. similaris* that was comparable to damage by *Plutella xylostella* L.(diamondback moth larvae) - the major insect pest of cabbage.

Currently, the snail pests are controlled with SiputoxÒ, a bait with 3% and 5% metaldehyde. Increasing resistance of the snails to this molluscicide (Salmijah *et al.* 1996) is supported by decreasing field efficacy of the baited Siputox at 3% and increasing use of the 5% metaldehyde bait by the farmers in the Cameron Highland. Laboratory studies by Noran *et al.* (1992) showed that 1.5% of *B. similaris* were killed by metaldehyde. Furthermore, Noran *et al.* (1995) found that *B. similaris* was unaffected by the bioinsecticide from extracts of *Azadirachta indica* leaves, shown to be toxic to the aquatic snail, *Indoplanorbis exustus*. Currently, there are no reports of effective biocontrol methods against this pest. However, we found that a laboratory culture of round snails taken from the wild were heavily parasitized by the phorid fly, *Megaselia scalaris* (Diptera: Phoridae). In Malaysia, only the giant African snail, *Achatina fulica* Fer (Achatinidae) was reported as parasitized by phorid flies (*Aphiochaeta scallaris* Loew and *Spiniphora genetalis* Schmitt) (Ahmad & Ho 1980). Our objectives were to determine the percentage of *B. similaris* parasitized by the phorid fly, *M. scalaris* and the life cycle of this parasitoid.

The host snail, *B. similaris* was collected from cabbage fields of the Kea Farms, Cameron Highlands, Pahang, Malaysia and reared in the laboratory at 25 +/- 2°C. Five snails and one female phorid fly were put in each plastic observation arena. We recorded the number of snails with fly eggs and the number of larvae produced per snail with each area. Larvae were transferred to Petri dish (10 cm diameter with a nutri-

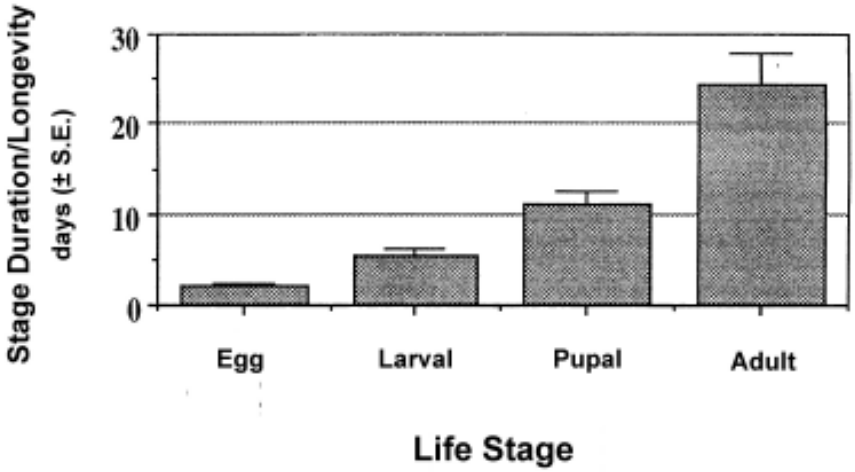

Figure 1. Duration/ longevity for each life stage of *M. scalaris* reared on the round snail, *B. similaris*.

ent agar diet 3% Gelose nutritive + distilled water, w/v) and held until pupation. The pupae were removed and adults reared on diet in plastic observation arenas maintained at high humidity by placing moist cotton wool inside the container and sealing the container. We recorded the time taken for each larva to reach the pupal stage, the numbers of pupae per snail, the number of emerged adults and adult longevity.

Roughly 55% (± 4.3) of *B. similaris* were parasitized by the phorid fly. On average, 28.8 larvae were produced per parastized snail, 85.9% (± 9.5) of these larvae pupated, and 76.7% (± 8.6) of the pupae produced healthy adults. It took 2 days for the eggs to hatch, 5.5 days for the larvae to reach pupation, 11.2 days for adults to emerge from pupae and these adults survived for 24.3 days (Figure 1).

This phorid fly, *M. scalaris*, has the potential to manage metaldehyde-resistant populations of the round snail as indicated by the parasitism rate (>50%) and relatively high fecundity of the parasitoid in our studies. Both pest and parasite species are easy to culture in the laboratory; and therefore, field release of the parasite may be considered as a practical control measure. However in the field, parasitism may be affected by exposure to pesticides and the availability of alternate hosts. Therefore, field studies should be initiated to assess the affect of pesticides on the dynamics of the parasite/snail interaction and determine relative preference and availability of alternative snail hosts. This and other ecological information will better estimate the potential of native and released populations of *M. scalaris* in managing the round snail, a potential threat to Malaysian agriculture as a result of resistance development to metaldehyde.

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The potential role of *Cleome rutidosperma* D.C. and *Brassica juncea* L. (Indian mustard) in resistance management of diamondback moth, *Plutella xylostella* (L.), in Malaysia

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INTRODUCTION

The diamondback moth (DBM), *Plutella xylostella* (L.), is a major pest of *Brassica* crops worldwide and has the potential to develop resistance to all pesticides including *Bacillus thuringiensis* (Talekar & Shelton 1993). DBM can be found on many Brassicaceae plants and some non-Brassicaceae plants that contain mustard oils (Marsh 1917, Thorsteinson 1953, Gupta & Thorsteinson 1960, Harcourt 1986). The abundance of host plants and natural enemies are key factors that impact DBM population dynamics (Marsh 1917, Harcourt 1986, Fox *et al*. 1990, Ooi 1992). Idris & Grafius (1994, 1996a, 1996b) reported the possible impact of the weed host, *Brassica vulgaris* R. BR., on resistance management of DBM in Michigan, USA. Indian mustard, *Brassica juncea* (L.)(Czen.) was used successfully as a trap crop for DBM in India (Srinivasan & Krishna Moorthy 1992). However in Malaysia and Indonesia, this approach was not practical (Sivapragasam & Loke 1995, Omoy *et al*. 1995). The weed, *Cleome rutidosperma* DC. (Capparidaceae), is reported as a suitable host plant for the cabbage webworm, *Hellula undalis* (F.), in Malaysia (Sivapragasam *et al.* 1994). Larval and pupal development for cabbage webworm on *C. rutidosperma* was significantly shorter than on cabbage and the eggs oviposited per females was more on *C. rutidosperma* than cabbage. The objective of our study was to assess the effect of *C. rutidosperma* on larval DBM behavior and feeding development, and on adult DBM egg production.

MATERIALS & METHODS

The following host plants were raised in clay plots under greenhouse conditions: Four cultivated *Brassica* (*Brassica juncea* Cosson, *B. alba* Rebenh, *B. juncea* Coss.var. *Rugose* Bally, *B. aboglabra* Bally), one wild *Brassica* (*B. juncea* L. = Indian Mustard), and one Capparidaceae, *Cleome rutidosperma*. A pesticide-resistant colony of DBM was donated by the Malaysian Research Development Institute (MARDI).

DBM development on each host plant was evaluated by placing 1st instars in 15 cm diameter petri dishes with leaf cuttings (2 cm^2). The host leaf was re-

Table 1. Developmental times for diamondback moth larvae and pupae on different host plants.

Host Plants [1]	Local Name	Developmental time (days ± S.E.) [2]				
		1st Instar	2nd Instar	3rd Instar	4th Instar	Pupae
Cultivated						
Brassica juncea Cosson	Sawi	2.51 ± 0.22b	2.32 ± 0.34a	1.54 ± 0.31a	3.73 ± 1.24ab	4.62 ± 0.81b
B. juncea Cosson var. *Rugose* Bally	Kai Choy	2.62 ± 0.50b	1.98 ± 0.35a	1.61 ± 0.56a	2.91 ± 0.72c	4.10 ± 0.54bc
B. alba Rebenh	Kai Lan	2.64 ± 0.26b	2.23 ± 0.35a	1.60 ± 0.43a	4.42 ± 1.12a	2.34 ± 1.22c
B. alboglabra Bally	Sayur Putih	2.55 ± 0.35b	2.25 ± 0.57a	1.65 ± 0.32a	2.93 ± 0.87c	4.01 ± 1.56bc
Wild						
B. juncea L. (Czern.)	Indian Mustard	3.01 ± 0.21a	2.46 ± 0.54a	1.45 ± 0.45a	2.97 ± 1.01c	4.86 ± 0.78a
Cleome rutidosperma D.C	Purple Maman	3.04 ± 0.34a	2.25 ± 0.46a	1.43 ± 0.54a	3.21 ± 0.67b	5.01 ± 0.85a

[1] All host plants are Brassicaceae except *C. rutidosperma* (Capparidaceae)

[2] Means followed with same letter within a column are not significantly different (P > 0.05, Fisher Protected LSD)

placed every 2 d to maintain freshness. The petri dishes were placed in the growth chamber and kept at 25 ± 2°C, a photoperiod of 12:12 h (L:D) and 50% R.H. Treatments were checked every day to record the larval and pupal developmental time. Pupae were weighed 1-2 d before adult emergence. The observation period was complete when adults emerged. These developmental treatments were replicated five times.

A 500 ml plastic container, modified with screened openings on the top (4 x 5 cm) and on the sides (3 x 3 cm), was used as an oviposition cage. Cages were placed under white inflorescence light (160 Watt, 50 cm above the top cage). A 15 ml test tube (3 cm x 6 cm) filled with 10% (v/v) diluted honey was placed inside the cage to fed the DBM adults. A single aluminum foil strip, 2.5 cm x 4 cm, coated with juice from cabbage leaves was replaced everyday, and served as an oviposition substrate (Idris 1995). One pair of DBM adults were released in these cages and after 2 d the male removed. Each treatment was replicated four times. Female oviposition (number of eggs) was recorded each day until no eggs were found.

We used choice and no-choice tests to compare feeding behavior of DBM larvae on different host plants. Petri dishes (15 cm diameter) were fitted with screen lids for proper ventilation and used as testing arenas. Host plants evaluated were cultivated *Brassica*, *B. juncea* Cosson, and *B. alba* Rabenh, and weed species, *B. juncea* L. (Indian mustard) and *C. rutidosperma*. In no-choice test, three leaf cuts (2 cm^2 cut per host species; placed 1.0 cm from perimeter, 4.5 cm from center, and 7.0 cm between cuts) were placed inside each petri dish. In the choice tests, four leaf cuts (2 cm^2) of each host species were placed inside the petri dishes (1.0 cm from perimeter, 4.5 cm from center, 5.0 cm between

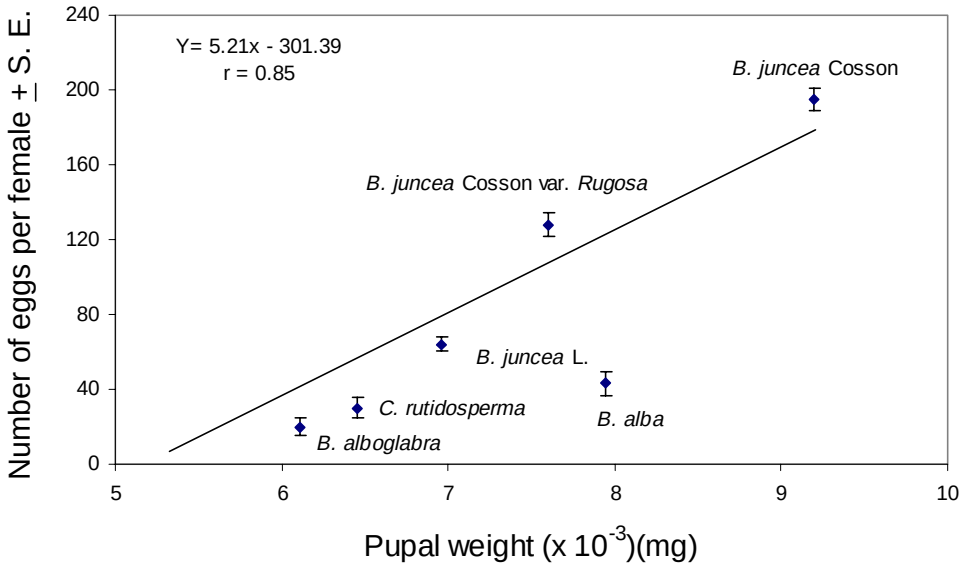

Figure 1. Correlation between pupal weight and egg-laying activity for diamondback moth reared on several host plants.

Table 2. Time spent by diamondback moth larvae to reach, remain, or feed on different host plants in no-choice and choice tests (observed for 3 hr)[1].

	No-choice test			Choice test		
Host plants	To reach food (minutes + S.E.)	On food (minutes + S.E.)	Feeding (minutes + S.E.)	To reach food (minutes + S.E.)	On food (minutes + S.E.)	Feeding (minutes + S.E.)
Brassica juncea Cosson	25.31 ± 10.32b	52.43 ± 16.72ab	30.02 ± 10.31b	4.43 ± 2.10b	8.52 ± 3.52a	35.58 ± 12.38a
B. juncea Cosson var. *Rugose* Bally	10.54 ± 3.12c	15.72 ± 7.53c	45.83 ± 8.92 a	5.21 ± 2.31b	1.26 ± 0.86c	20.65 ± 10.54b
B. juncea L (Czern.)	50.01 ± 16.67a	10.54 ± 2.43c	15.23 ± 3.50c	53.32 ± 20.53a	7.51 ± 2.52ab	10.89 ± 3.54c
Cleome rutidosperma D.C	12.43 + 4.21c	58.33 + 20.35a	16.54 + 3.21c	8.93 + 3.23b	5.12 + 2.11b	18.75 + 6.87b

[1] Means followed with same letter within a column are not significantly different (P > 0.05, Fisher Protected LSD)

cuts). In both tests, petri dishes were held as in the developmental studies. One 3rd instar, starved for 6 h, was released in the center of the dish with a fine brush and dish sealed. The times taken by larvae to reach then leave the leaf cuts were recorded with a microtape recorder for 3 h (from 1400 - 1700 h). In both no-choice and choice tests, treatments (host plants) were arranged in complete block design with four replications. Data were analyzed with a one-way ANOVA (Abacus Concepts 1991).

RESULTS & DISCUSSION

There was no significant different in developmental time for 2nd and 3rd instar DBM fed on four cultivated and two wild host plants (P > 0.005, Fisher's Protected LSD) (Table 1). However, two wild host plants, Indian mustard and *C. rutidosperma*, significantly prolonged developmental time for 1st instar DBM when compared to the four cultivated host plants. The developmental time for 4th instar was significantly shorter when larvae were fed on Indian mustard (wild), *B. juncea* var. *Rugose* and *B. alboglabra* (cultivated) than on the *B. alba* (cultivated) (P< 0.05, Fisher's Protected LSD). The two wild host plants may have antifeedants that affect the feeding and developmental time of the 1st instar DBM. The wild host plants also caused significantly longer pupal development than the cultivated host plants species (P< 0.05, Fisher's Protected LSD)(Table 1). An antifeedant found in cabbage prolonged developmental time of *P. rapae* larvae to pupation (Hough-Goldstein & Hahn 1992).

The numbers of eggs laid by adult DBM females were positively correlated with the weight of pupae that varied based on host plants (r = 0.85, P < 0.05) (Fig. 1). DBM on *B. juncea* Cosson had the highest pupal weight and number of eggs produced. DBM pupal weight and numbers of eggs produced were lowest when larvae were fed *B. alboglabra* or *C. rutidosperma*. These differences in pupal weight and number of eggs laid may be influenced by the host quality. Fox *et al.* (1990) reported that host plant quality determined DBM larval size and influenced the percent parasitism of DBM by its parasitoid, *Diadegma insulare* (Cresson).

DBM larvae took significantly less time to reach *C. rutidosperma* and *B. juncea* var. *Rugose* than on other host plants in both no-choice and choice tests (P > 0.05, Fisher's Protected LSD) (Table 2). Both host plants appear to have higher concentration of feeding attractants for DBM. DBM larvae remained significantly longer on *C. rutidosperma* than on wild mustard *B. juncea* var. *Rugose* in no-choice test; but when given a choice, DBM preferred the others. This suggests that *C. rutidosperma* has less feeding stimulants. In a no-choice test, DBM spent significantly less time feeding on the wild host plants, Indian mustard and *C. rutidosperma* than the cultivated host plants. DBM larvae spent less time feeding and more time wandering on Indian mustard than on cultivated *B. juncea* Cosson in a choice test. Again, this suggests that wild host plants have less feeding stimulants than the cultivated plants.

Glucosinolates serve as feeding attractants or stimulants and different compounds and concentrations have been identified in host plants (Brassicacaeae) and non-host plants (Cole 1976). Our results showed that the wild host plants, Indian mustard. and *C. rutidosperma*, may have higher concentrations of feeding attractants or stimulants. However, the lower pupal weight and reduction in the number of eggs laid by females reared on these weeds indicates a poor host quality.

We conclude that the poor host quality plus increased attractiveness exhibited by *C. rutidosperma* and Indian mustard can be manipulated for managing insecticide-resistant DBM population. *C. rutidosperma* is a ubiquitous weed in Malaysia while Indian mustard was introduced from India (Anderson 1974, Sivapragasam & Loke 1995). Indian mustard also possesses oviposition attractants that make it attractive as trap crop (Srinivasan & Krishna Moorthy, 1991). Although it is not practical to interplant Indian mustard within the field (Sivapargasam & Loke 1995), we can plant it around the field and apply insecticides as necessary. This can reduce DBM and other cabbage pests in field as well as prevent insecticide resistance development. The effect of *C. rutidosperma* on DBM oviposition behavior has yet to be studied. Nevertheless, we can divert the DBM oviposition activity from cultivated plants to wild host plants around or within a field, especially near or at the critical growth stages that

determine maximum yield and income per hectare. In addition, *C. rutidosperma* can be manually weeded out easily, removing the need for herbicides.

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Quantitative structure-activity relationships and computer-aided molecular modelling: A preliminary study on insecticide resistance

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INTRODUCTION

Many aspects of pesticide resistance have been studied over the years. Numerous studies have focused on detecting and monitoring resistance based on biological, genetic and biochemical studies, and more recently, through molecular biology studies on how resistance manifests itself. While much useful scientific data is gathered and major advances are made in our understanding of resistance, a major frustration is that the science is often too late to influence problems in the field.

Computational chemistry has been used for many years in the pharmaceutical and agrochemical industries to predict how chemicals can be made more toxic. Perhaps similar approaches could be used to predict how pesticides might fare when pest resistance to any compound in their class arises. In this preliminary study, molecular modelling and computational chemistry were applied to insect resistance and the organophosphorous insecticides.

Quantitative Structure-Activity Relationships

Quantitative Structure-Activity Relationships, (QSAR) are computational chemistry techniques used to relate one or more properties of a chemical group to the activity of those molecules in a biological system. Once the relationship is established, it can predict the effect of other related molecules in the same biological system.

The action that a drug or agrochemical exerts on its target must be due to some characteristic of the chemical. It has been known for many years that certain fundamental properties of bioactive molecules are important determinants of biological activity. Early studies identified three main categories of chemical properties as determinants of bioactivity - hydrophobic, electronic and steric. Hydrophobic properties describe the partitioning of chemicals between an organic solvent and water and are good indicators of biological activity in many cases. It is generally assumed that this property is closely related to a chemical's ability to cross or dissolve in the lipids of

Table 1. Molecular properties and insect resistance factors for 15 organophosphorous compounds selected as the learning series for the QSAR analysis.

Compound	DIPOLE	Energy	LogP	HOMO	LUMO	P+	ConArea	Rf C12	Rf Qd	RF MdG
chlorpyrifos	3.22	62	4.1	10.21	1.7	0.702	287			
chlorpyrifos methyl	3.61	54	2.4	10.28	1.65	0.7	238	4		
diazinon	3.27	57	3.38	10.08	1.5	0.694	299	11	10	8.2
dichlorvos	0.63	0	2.36	10.5	1.07	0.921	195	3		22
etrimphos	2.93	59	1.23	10.3	1.4	0.7	228			
fenitrothion	5.41	57	2.2	10.6	2.05	0.694	251	4	4	9.4
malathion	1.88	54	2.8	10.27	9.87	0.52	305	18	8	
methacrifos	3.96	55	2.7	9.87	2.29	0.7	207		6	4.4
pirimiphos methyl	3.13	59	0.7	9.67	1.47	0.7	287		55	30
Tetrachlorvinphos	1.36	58	4.4	10.21	1.88	0.694	277	227		3372
temephos	3.2	58	2.75	9.24	1.76	0.693	408	10		
phoxim	3.2	74	5	10.04	1.74	0.698	290	10		
Trichlorphon	2.4	0	1.99	11.96	1.28	0.92	212			24
Bromophos	2.81	9	4.28	10.32	1.87	0.694	255			58
Iodofenphos	2.45	9	5.7	10.17	1.89	0.694	262			94

Dipole=dipole moment; Energy= molecular mechanics energy difference between parent compound and the active oxon; LogP= octanol/water partition calculated from the program CLogP; HOMO= energy of the highest occupied molecular orbital from the semi-empirical QM program MOPAC; LUMO= energy of the lowest unoccupied orbital; P+=partial charge on the phosphorus atom; ConArea=surface area of molecule exposed to a 1.4 Angstrom probe (water); Rf C12, Rf Qd, Rf MDG=Resistance factors (LD_{50}) for the three insect strains.

biological membranes. Electronic properties essentially describe reactivity or the ability of molecules to donate or accept electrons. Steric properties describe the size and shape of the molecules. While a range of 'constants' for some of these properties have been published, some properties are difficult to measure in the laboratory and some are theoretical and not obtained through practical chemistry. Thus the ability to calculate or estimate such properties from fundamental principles is an important aspect of QSAR studies.

The mathematical relationship between one or more properties in a series of molecules (known as the 'training' or 'learning' series) is classically determined by multiple regression analysis. However when constructing QSARs, it is important to remember that a straight line relationship is unlikely to be fully predictive. There are optimum properties of a molecule that confer biological activity and the relationship between a given chemical property and biological activity will be hyperbolic. Thus the relationship is not described by the straight line equation $y=mx+c$, but by the square function $y=mx-nx^2+c$.

There are three factors that mitigate in favour of a good QSAR - 1) a large number of molecules in the learning series, 2) those molecules are closely related, and 3) accurate biological data. The best QSAR would rely on a learning series of greater than 20 compounds that differ by a single substitution of the parent molecule. The best biological studies have minimal biological variability, for example *in vitro* inhibition of a key enzyme.

In recent years, QSAR has been applied to less ideal situations. For example QSARs have been constructed for *in vivo* toxicity of drugs and agrochemicals and for evaluating the environmental effects of a loosely-related pollutants. The further one gets from the 'ideal' situation for QSAR, the less appropriate multiple regression analysis becomes. A series of non-parametric techniques have evolved to tease out trends in highly variable data. These techniques include cluster analysis and molecular similarity techniques. Due to the preliminary nature of this study, these methods will not be discussed.

Computer Assisted Molecular Modelling

Recent developments in technology allow desktop computers to inexpensively run molecular modelling. A simple program will model molecules on the basis of stored bond lengths, angles and torsion angles with molecular mechanic formalisms. Since even simple molecules can exist in many conformations due to rotation about single bonds, more complex programs can calculate the energy of a molecule. This allows us to make an intelligent assessment of the shape molecule will adopt since many calculated properties will vary with the shape of the molecule. Many programs include 'energy minimisers' to further aid the operator in establishing the lowest energy conformation of a molecule and the probable shape adopted in nature. The most complex (and expensive) programs include quantum mechanics that solve highly complex equations and predict the positions of the electrons on a molecule.

MODELLING OF ORGANOPHOSPHOROUS INSECTICIDES

I examined the relationship between the chemical properties of some organophosphorous insecticides (OPs) and the ability of insects to develop OP resistance. This study had some inher-

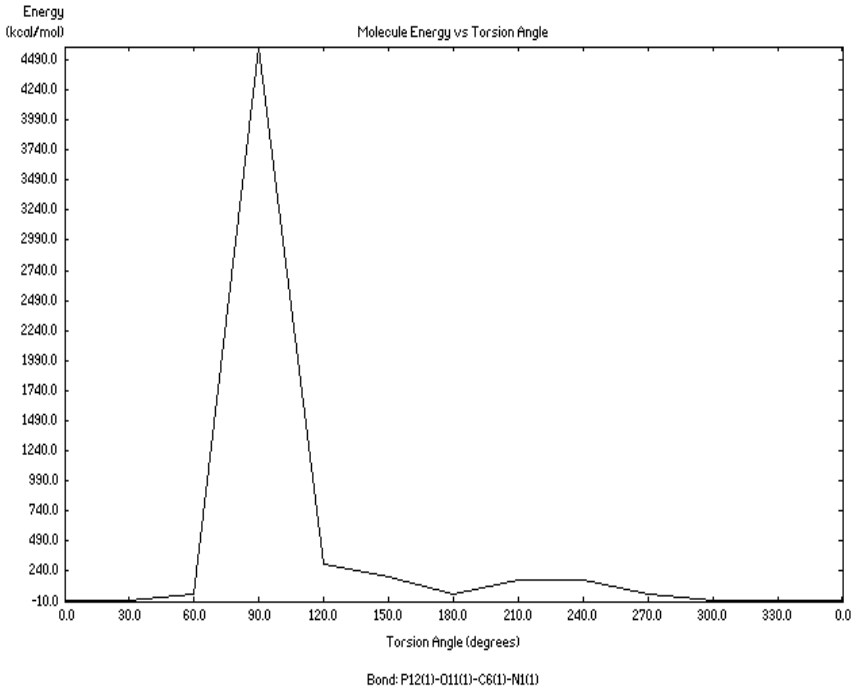

Figure 1. An energy plot of the conformers of chlorpyrifos generated by rotation of the oxygne-ring bond.

ent difficulties:

OPs contain the P=S bond that is uncommon and not parameterised in some modelling software.

OPs need to be oxidized to the P=O compound before they are toxic. This introduces an extra variable.

Some OP insecticides are P=O compounds in the parent form and may form a second 'family' of the learning series.

The biological data from *in vivo* studies is potentially highly variable.

The learning series is small.

These factors make the establishment of statistically significant QSARs difficult. This is unfortunate since there is a vast body of studies available on OP resistance. Furthermore, each study conducts resistance assessment in different ways and the comparison across different insects, insecticides and methods is not usually possible.

Building

The OP compounds used in this learning series are shown in Table 1. They were modelled CHEMX, (Chemical Design Ltd, Chipping Norton, UK). The series was based on the crystal structure of chlorpyrifos obtained from the Cambridge Crystallographic Database. In the past, crystal structures were considered to be the definitive 3D structure of molecules. However, crystals represent an unnatural molecule in a solid state and may not be the same as in the biochemical context. Molecular mechanic calculations in CHEMX indicated that although the crystal structure of chlorpyrifos was a low energy one, some bonds were strained and an energy minimisation procedure relaxed the molecule from 55 kcals to 4.2 kcals without significantly altering the shape.

Conformers

To verify that the most relaxed structure was obtained, conformational space was searched systematically through X-ray spectrometery. The chlorpyrifos molecule has a number of bonds that rotate freely. For example as the bond between the pyridine ring and the oxygen bridge atom rotate, a range of orientations between the ring and the rest of the structure are possible, each with a characteristic potential energy. The software enables this rotation to be simulated and the potential energies calculated as shown in Figure 1.

The change in energy of the molecule with the rotation of the oxygen ring bond shows that there is a local energy minimum at 180 degrees. The molecule is most 'at rest' when the ring is between 330 and 360 (or 0) degrees with respect to the P-O bond. A full conformational search on all bonds that impact on the 3D structure of chlorpyrifos was performed interactively with conformational searching tools available in CHEMX. The 'global minimum' conformation of chlorpyrifos was found to be the energy minimised crystal structure shown in Figure 2.

Once the basic OP 'shell' from chlorpyrifos was established (Figure 3) substituents were built onto this basic structure for other OPs. While a full conformational search on all structures would be valid, they are likely to exist in similar conformations since all OPs to exert the same biochemical effect on acetylcholinesterase (the target enzyme in the insect nervous system). Indeed, each model OP was oriented in a similar way to chlorpyrifos in a low energy form (Figure 4). I assumed this commonality of 3D structure among the OPs. The similarities can be seen when structures are fitted to a least squares routine.

Quantum mechanics

Single-step quantum mechanic calculations were run on the structures to allow QSAR parameters to be estimated. The calculations were performed within the CHEMX environment with the semi-empirical molecular orbital program MOPAC. This enabled estimation of the dipole moment, energies of highest occupied, (HOMO) and lowest unoccupied, (LUMO) orbitals, heats of formation, electronic energies, superdelocalisations, partial charges and other properties for each compound in the study.

QSAR PROPERTIES

The selection of chemical properties to correlate with biological activity was largely intuitive and subjective. Many studies involve a hundred or more properties for correlation because modern computational techniques can handle the large data sets relatively easily. In this

preliminary study, a small number of properties were selected as representative of gross descriptors:

1) Dipole moment. This is a measure of charge distribution and thus polarity. Energy of the P=S compound minus that of the P=O compound. An indication of the metabolic energy required to convert from the inactive to the active form of the insecticide.

2) LogP. Octanol/water partition as calculated by the program CLogP. This is the hydrophobic parameter.

3) HOMO. The energy of the highest occupied molecular orbital and a measure of nucleophilic reactivity.

4) LUMO. The energy of the lowest unoccupied molecular orbital and a measure of electrophilic reactivity.

5) P +. Partial charge on the phosphorus atom calculated both by molecular and quantum mechanics methods.

6) Connolly surface area. The surface of the molecule exposed to a 1.4 Angstrom diameter probe. This is a measure for surface area for the molecule, and is a representation of the molecular area accessible to water molecules.

BIOLOGICAL DATA.

There is a vast body of data on insect resistance to insecticides. Since many of the 'ideals' have already been compromised is the QSAR study, the biological data should be as good as possible. For example, the data should be based on dose response lines with resistance factors at the LD_{50} or the LD_{99}. Resistance tests should be performed in the same way for each data set and the resistance factors determined with similar bioassay techniques (*e.g.* topical application of insecticide to insect). Finally, the data sets should contain resistance factors to as many OP compounds as possible. The span of these should be as wide as possible to minimise the risk of chance correlations. Given these criteria, there are surprisingly few publications dealing with OP cross resistance that were suitable for this study.

Three data sets were selected for this preliminary study:

Table 2. The cross correlation matrix for the molecular properties used in QSAR analysis. The lightly shaded boxes highlight variables that were cross correlated (r+0.4 or greater).

	DIPOLE	Energy	LogP	HOMO	LUMO	P+	ConArea
Dipole		0.47	0.16	0.16	0.17	0.29	0.1
Energy			0.09	0.57	0.15	0.62	0.47
LogP				0.11	0.007	0.2	0.19
HOMO					0.04	0.56	0.61
LUMO						0.64	0.22
P+							0.51
ConArea							

Housefly, *Musca domestica*, collected in 1980 from a hog farm in the United Kingdom. This strain (MdG) was found to be resistant to OPs, pyrethroids and carbamates.

Flour beetle, *Tribolium castaneum*, collected during the Global resistance survey conducted by the FAO in 1975. Two sets of data for two strains C12, and Qd were utilized.

RESULTS.

The calculated properties and the measured resistance factors are shown in the Table 1.

The first task was to identify any combination of variables that were cross correlated. Such combinations cannot be used in the QSAR relationship since they are not independent of each other and may cause erroneously high correlations with the biological data. A cross correlation matrix was constructed to identify such combinations (Table 2) and wight variable combinations were excluded.

Table 3 shows the correlation coefficient, (r) for specific variables with the biological data for each insect strain. The results of single correlations show that only LogP (the hydrophobicity parameter) had a significant correlation with biological activity in 2 out of the 3 sets of data. For the Qd strain, the apparent improved correlation obtained when both LogP and $LogP^2$ were used with the biological data must be treated with caution since, although statistically significant, there were only to 5 data points.

A number of valid combinations of variables was examined for improved correlation with the resistance data. An excellent relationship was found for the resistance factor of MdG strain with dipole moment and the Connolly surface area. This relationship was found to be:

$$Log.Rf=-0.53Dipole+0.25ConArea-0.0005ConArea^2-28$$

The correlation coefficient (r = 0.9) indicated that 81% of the variation in the observed data was explained by this equation and the F statistic indicates that this correlation was significant at the 95% level. While the two variables individually were not well correlated to resistance, the combination of dipole moment

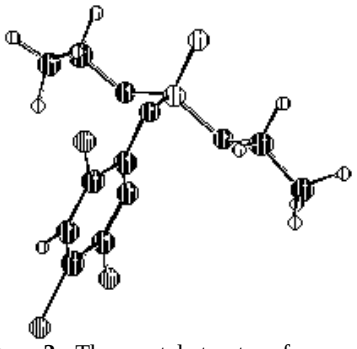

Figure 2. The crystal structure for chlorpyrifos minimized for energy.

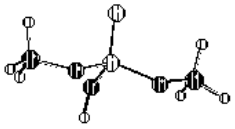

Figure 3. The basic OP 'shell' structure.

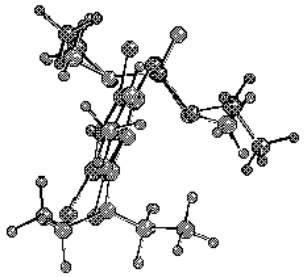

Figure 4. Chlorpyrifos and pirimiphos methyl superimposed.

and the exposed surface area of the molecule form a good descriptor of the biological activity. The equation indicates that a molecular surface area of 258 would have a maximum resistance factor of 3,216. The observed biological value of 3,372 was a good approximation.

These two variables also showed a good correlation for the data on the flour beetle strain C12. The equation was:

$$Log.Rf = -0.24Dipole + 0.049ConArea - .000074ConArea^2 - 5.9$$

This relationship was not such a good fit to the data ($r = 0.8$, $F = 2.2$, $P = 0.22$), but the data set was rather small.

A similar relationship was obtained for the Qd strain of *T. castaneum* (r=0.75), but the data set was small compared to the number of variables. It was not possible to say whether the equation was statistically significant. Nevertheless, the trend observed is the same for all three sets of data examined.

Table 3. Correlation between molecular properties and biological data for three OP-resistant strains of insects. The r value (number in brackets) obtained when the variable and its square were included in the regression analysis. The percent significance number indicates the likelihood of the relationship arising by chance. Where no significance figures are shown, the value is greater than 50%.

	Rf C12	%sig	Rf Qd	%sig	Rf MdG	%sig
Dipole	0.38(.45)	30(55)	0.43(.63)	46(59)	0.56(.56)	10(30)
Energy	0.37(.39)		0.65(.94)		0.02(.05)	
LogP	0.62(.78)	10(10)	0.72(.97)	16(5)	0.5(.54)	17(35)
HOMO	0.17(.48)		0.76(.82)		0.03(.18)	
LUMO	0.18(.4)		0.2(.76)		0.03(.46)	
P+	0.37(.4)		0.15(.54)		0.14(.4)	
ConArea	0.28(.55)	48(40)	0.42(.43)		0.32(.42)	40(54)

CONCLUSIONS.

Since this was a preliminary study, only three sets of data were modelled and analysed. Nevertheless QSAR approaches do offer potential in predicting relative levels of insecticide resistance. I established a statistically significant relationship between two physicochemical properties and insecticide resistance. Clearly the model will be affected by a number of factors including mechanism of resistance, cross or multiple resistance. It would be interesting to construct models for pest species with multiple resistance mechanisms to the same class of compounds.

Any model should be validated with more biological data and extended to increase its significance. The model then may predictively assess the likelihood of resistance emerging to other OPs not in the learning set. Larger data sets of comparable biological data would be required. I am interested in hearing from researchers who may have such data for OPs for other pesticide groups.

The relationship between carboxylesterases and peach potato aphid resistance to sumithion and to fenvalerate

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MATERIALS & METHODS

Aphids

Field-collected peach potato aphids, *Myzus persicae* (Sulzer), were divided into four colonies: FE, SU, FS and CK. The former three colonies were selected once per week for 14 weeks with fenvalerate (FE), sumithion (SU) and a mixture of the two (FS) (fenvalerate:sumithion = 3:7). The later colony, CK, was treated with water only. Carboxylesterase activity and pesticide toxicity were determined for each colony throughout the selection period.

Toxicity/Synergist bioassays

Insecticide toxicity towards these aphid colonies was determined with a topical application method recommended by FAO. Resistant ratios were calculated as LD_{50} of each generation divided by the LD_{50} of the parental generation. In the synergist bioassays, insecticides were applied to aphids one hour after the synergist was applied. For each aphid population, the synergist ratio was calculated as the toxicity (LD_{50}) of the insecticide divided by the toxicity

Table 1. Relationship between pyrethroid resistance and general carboxylesterase activity between green peach aphid colonies over 14 weeks of pesticide selection.

Week of Selection	CK			FE			SU			FS		
	S-ratio	Activity*	Km (μm)	R-ratio	Activity*	Km (μm)	R-ratio	Activity*	Km (μm)	R-ratio	Activity*	Km (μm)
0	1.00	759.92	229.48	1.00	759.92	229.48	1.00	759.92	229.48	1.00	759.94	229.48
3	1.06	728.15	220.78	1.23	748.57	212.45	1.33	797.40	222.04	1.06	741.74	223.06
6	0.72	767.94	201.59	2.18	565.13	202.85	1.42	824.49	223.20	1.29	722.04	222.98
9	1.40	756.32	219.92	18.20	547.31	190.87	3.42	926.43	241.96	1.73	713.85	214.78
11	1.87	746.43	220.49	21.82	508.91	176.98	4.35	1076.49	225.86	3.24	751.18	211.57
14	2.23	742.72	226.21	52.61	504.63	150.67	11.11	1175.26	226.53	3.51	767.19	202.08

* μm/15 min. mg protein = change in response to fenvalerate for the susceptible CK colony
S-ratio = susceptible ratio
R-ratio = resistant ratio

Table 2. Synergy between triphenyl phosphate (TPP) and pyrethroids in resistant colonies of green peach aphid.

Colony	Resistance level		Resistance modified by TPP		Synergist ratio
	Y = a + bx	LD_{50} (µg/ aphid)	Y = a + bx	LD_{50} (µg/ aphid)	
FE	Y=6.289+1.134x	0.07296	Y=6.454+1.246x	0.0817	1.07
SU	Y=5.825+0.92x	0.12784	Y=5.943+0.503x	0.01332	9.60
FS	Y=7.534+1.139x	0.00596	Y=6.581+0.693x	0.00522	1.14

(LD_{50}) of the insecticide plus the synergist.

Enzyme preparation

From each colony, three groups of ten apterous adult aphids were selected for the carboxylesterase activity assay. Each aphid group was homogenized in approximately 2 ml sodium phosphate buffer (0.04 M, pH 7.0). Then 1 ml alpha-homogenate was added into 5 ml substrate solution, automixed in a thermomax chamber for 15 minutes and held at 30° C in a water bath. Absorbence measurements were taken at a wavelength of 600 nm, ten minutes after adding the colorimetric dye.

Electrofocusing

Esterase banding patterns from aphid colony homogenates, prepared as in the carboxylesterase activity tests, were resolved on isoelectric focusing gels over a wide range of pH (5.0 - 9.5). The resolved esterase isozymes were visualized by a naphtholic ester-fast blue staining protocol. The band intensity was detected with the scanner. The intensity of the esterase band was detected with a Shimadzu Dual-Wavelength TLC-Scanner (S-900) (comparison wavelength = 700 nm, sample wavelength = 590 nm, scanning speed = 40 mm/min).

RESULTS & DISCUSSION

General carboxylesterase activity

We anticipated and saw that the activity and Km value of carboxylesterases in the CK colony remained constant during the 14 "selections" with water (Table 1). The general carboxylesterase activity in the SU colony increased gradually as did aphid resistance to sumithion; however, the Km value remained constant. This implies that the binding affinity of the carboxylesterase remained the same during the selections, and that the sumithion resistance in the aphid was attributed to a quantitative increase in general carboxylesterase activity. There was a decline in carboxylesterase activity in the FE but not the FS colony (Table 1), making it difficult to extrapolate the relationship between enzyme activity and aphid resistance to fenvalerate. Meanwhile, the Km value in the FE and FS colony steadily declined as resistance evolved. This suggests that the binding affinity of carboxylesterase in the two colonies has changed.

Synergist bioassay

At the conclusion of the selection process (14 weeks), synergist bioassays were performed with triphenyl phosphate (TPP), an inhibitor of general carboxylesterase activity. Table 2 shows that TPP did significantly reduce the resistance level expressed by the SU colony. Now we infer that aphid resistance to sumithion was due to the increase in general carboxylesterase activity.

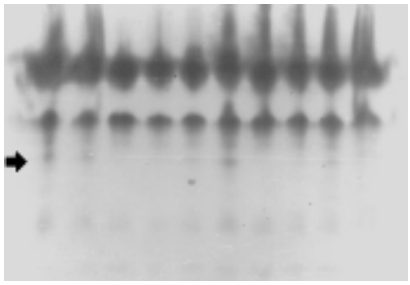

Figure 1. Esterase banding patterns from homogenates of individuals from four colonies of green peach aphid. Note the 3rd esterase band (arrow) was highly expressed in the SU colony resistant to sumithion.

Electrofocusing

Figures 1 and 2 show that third band of esterase from the SU colony was darker than that from CK colony. This implies that the increase in general carboxylesterase activity was associated with an increase in expression of the enzyme in this band. It is interesting that neither the FE or the FS colony displayed the third band. Furthermore, there was

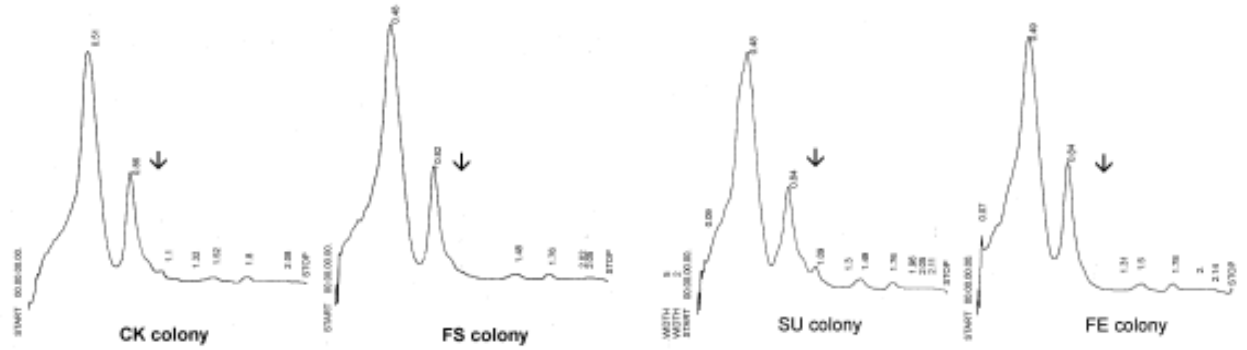

Figure 2. Intensity of esterase banding patterns for individuals representing four colonies of green peach aphids. Note the 3rd esterase band (arrow) highly expressed in the SU colony resistant to sumithion.

no unique esterase band detected in aphids from the FE or FS colony.

CONCLUSIONS

We conclude that the resistance of peach potato aphids to sumithion was caused by an increase in carboxylesterase activity. The activity was linked to the increase in expression of an enzyme captured in the 3rd esterase band in an electrofocused gel of aphid homogenate. Aphid resistance to fenvalerate was not associated with an increase in carboxylesterase activity and must have another resistance mechanism.

Susceptibility of the Argentine cotton stainer, *Dysdercus chaquensis* (Heteroptera : Pyrrhocoridae), to selected insecticides

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INTRODUCTION

The genus *Dysdercus* includes several species that severely damage to cotton plants in different regions of Asia, Africa and South America. Six *Dysdercus* species have been identified in Argentina and three of them (*D. chaquensis, D. albofasciatus* and *D. ruficollis*) occur in the different cotton growing areas of this country (Stadler & Cappozzo 1988). These species feed and reproduce on their natural hosts (Malvales), while *D. chaquensis* is the only species able to switch from its natural host to commercial cotton. *D. chaquensis* populations develop in the forest surrounding the cropping areas. The species feeds and breeds on two natural host plant-species, *Sphaeralcea bonaerensis* and *Wissadula densiflora* (Malvaceae) (Stadler & Diz, unpublished). During the cotton flowering period, the cotton stainer moves from the forest to the cotton fields. Two or three generations of *D. chaquensis* can be a pest of cotton depending on environmental conditions.

Early in the cropping season, the cotton stainer feeds on cotton squares. Later when feeding on cotton bolls, the bug introduces bacteria and fungi into the developing fruit resulting in a rotten inner boll. Finally, after boll opening, *Dysdercus* feeds on seeds killing the seed embryo by puncturing and sucking out contents. The bug also deposits its feces on the developing lint where bacteria and fungi develop. The microbial activity results in loss of commercial value of the fiber because it becomes stained and weakened.

In the field, an entomopathogenic fungus (*Sporothrix* sp.) and a tachinid parasitoid (*Acaulona brasiliana*) are two main natural enemies of *D. chaquensis*. The former belongs to the Deuteromycetes group and was found associated with field populations of the host in northern Argentina (Zerba 1996). The parasitoid, *A. brasiliana*, was found infesting *D. chaquensis* nymphs. A field survey assessed parasitism by this tachinid as geographically variable and rarely above 50%. Thus, the parasitoid is not viewed as an effective natural control agent for cotton stainer due to the frequent and unpredictable fluctuations of parasite/pest densities and also due to the host's patchy distribution (Stadler & Schang 1994).

Historically, the cotton stainer was considered as a secondary pest in the north-eastern Argentine cotton cropping area. Occasionally outbreaks surpassed the economic threshold. However in the last five years, *D. chaquensis* has become a main pest in the northern Argentine cotton cropping region. To minimize the severe damage caused by *Dysdercus* to the crop, up to two additional pesticide treatments were needed. This practice introduces extra management costs and ensues drawbacks to the agroecosystem such as the destruction of nontarget biological control agents and environmental pollution.

Typically, *D. chaquensis* populations can be kept under control by simple cultural methods combined with natural control by parasites and predators. However, population outbreaks must be suppressed immediately thus requiring pesticide applications. In both cases, biological and toxicological information about the pest and its natural enemies must be assessed before an efficient and sustainable management program can be realized. In order to attain baseline information for in the framework of cotton IPM programs in Argentina and to monitor pesticide resistance in the cotton stainer, pest susceptibility to the pesticides frequently applied in cotton was investigated in laboratory conditions.

MATERIALS & METHODS

The insecticides tested were technical grade carbaryl 92% (Rhone Poulenc), chlorpyrifos 95% (BASF), cypermethrin 96% (Bayer), deltamethrin 98% (HOECHST), endosulfan 95% (HOECHST), lambdacyhalothrin 92% (ICI), monocrotophos 50% (CIBA), beta-cypermethrin 98.5% (Chem. Sintyal) and beta-cyfluthrin 98% (Bayer). Each insecticide was dissolved in analytical grade acetone (Merck) to make stock solutions of 10 mg/ml. Serial dilutions for each insecticide-stock solution were prepared in acetone the day of each assay.

Our colony of cotton strainers were reared on cotton seed diet and standard rearing conditions (28ºC, 70-75% RH, 12 hours light). For the bioassays, 5th instars were immobilized with CO_2. Larvae selected were 5 to 7 days old and weighed 55 ± 2.7 mg (CL 95%). Dilutions were applied topically to the ventral abdominal surface of the bug with a Hamilton repeating dispenser PB 600 equipped with a 50 µl syringe, calibrated to deliver 1µl droplets. Following pesticide application, the bugs were placed in petri dishes and held at standard rearing conditions.

Mortality was assessed 24 hours after pesticide application. Cotton stainer response (LD_{50}) to each insecticide was based on a minimum of four doses. Three replicates with 30 bugs each were assayed for a total sample of 90 bugs per dose (Robertson & Preisler 1992) . Dose-mortality data were analyzed by a probit analysis (Russell *et al.* 1977). When necessary, control mortality was adjusted with Abbot's formula (Abbot 1925).

Table 1. Toxicity of selected insecticides to the fifth instar cotton stainer, *D. chaquensis*.

Pesticide	LD_{50} µg/insect	Confidence Interval (95%)	Slope + SE
carbaryl 92 %	3.67	1.66 - 7.12	3.49±0.45
chlorpyriphos 95%	0.14	0.13 - 0.25	3.19±0.39
cypermethrin 96%	0.030	0.018 - 0.052	2.15±0.31
deltamethrin 98%	0.006	0.0045 - 0.0110	3.09±0.21
endosulfan 95%	7.97	3.23 - 18.03	1.47±0.23
lambda-cyhalothrin 92%	0.005	0.0036 - 0.0098	1.33±0.22
monocrotophos 50%	0.050	0.036 - 0.188	2.78±0.40
ß-cyfluthrin 98%	0.003	0.0019 - 0.0096	1.03±0.23
ß-cypermethrin 98.5%	0.004	0.0010 - 0.0067	1.00±0.15

RESULTS & DISCUSSION

Significant differences in LD_{50}s occurred among insecticides for LD_{50} and slope estimates (Table 1). The relative toxicity of the different insecticides to the 5th instar cotton stainers in ascending order was endosulfan < carbaryl < chlorpyrifos < monocrotophos < cypermethrin < deltamethrin < lambdacyhalothrin < b-cypermethrin < b-cyfluthrin. Overall, the pyrethroids b-cyfluthrin and b-cypermethrin were the most toxic to the 5th instars. The organophosphate, endosulfan and the carbamate, carbaryl were the least toxic. The organochlorine, monocrotophos and chlorpyrifos were of intermediate toxicity These results encourage the application of pyrethroids at lower field rates relative to organophosphates and carbamates.

There is no need yet for chemical control measures of *D. chaquensis* populations in northeastern Argentina because this species still is considered as a secondary pest or simply ignored. However in the northern part of the country where cotton production is in its infancy, *Dysdercus* is considered a primary pest. Our assessment of the susceptibility of *D. chaquensis* to these pesticides represents an accurate baseline due to the lack of previous pesticide pressure directed towards this species. These data will serve as future baseline comparisons for other *D. chaquensis* populations and life stages, as well as for assessing any pesticide resistance that may develop in this species. We will also use the results to set the foundation for developing and implementing an integrated pest management program in cotton that will manage the cotton stainer at minimal cost and low environmental impact.

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