RESISTANT PEST MANAGEMENT

A Biannual Newsletter of the Pesticide Research Center (PRC) in Cooperation with the
Western Regional Coordinating Committee (WRCC-60), the
United States Department of Agriculture/Cooperation States Research Service (USDA/CSRS) the
International Organization for Resistant Pest Management (IPRM) and the
Insecticide Resistance Action Committee (IRAC)

Volume 4, No. 1

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Call for Articles

The *RESISTANT PEST MANAGEMENT NEWSLETTER* continues to grow in subscribers and contributions. Since it functions largely by resistance workers contributing articles to update colleagues on their work, we need your contributions to fulfill our joint communication goal. Please consider submitting an article for the next *NEWSLETTER*. We can accept articles on disk from any IBM software package, or any hard copy of text or graphics. You may also FAX your articles to (517) 353-5598. The submission deadline date is August 31, 1992.

Thank you for your interest and commitment to sharing resistance information.

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Editorial

We apologize for the delay in getting this newsletter to you. We have been experiencing some budget shortfalls and had to wait to raise additional support. It is our hope that future issues will not be delayed and that we will have them on a regular January, July, schedule. If you have any suggestions for additional support or, particularly the mailing costs of this newsletter we would be very much appreciative of your input. Our next issue will be published in October

Mark E. Whalon and
Robert Hollingworth
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News/Review

The International Organization for Resistant Pest Management

The International Organization for Resistant Pest Management (IOPRM) will convene the First International Congress November 1, through November 4, 1992 in the Washington, D.C. area.

The purpose of this invitational Congress will be the presentation and discussion of resistant pest management programs developed by IOPRM working groups for: mites, insects, and the Fire Blight pathogen on apples in Mexico; diseases and mites on apples in Poland; *Heliothis* on cotton in India, Diamondback moth on crucifers in Central America; and white fly on roll crops in Mexico.

Invited participants in the Congress will include representatives of government agricultural research, extension and regulatory agencies, United Nations Development Program, the World Bank and other international development banks, agrichemical industry and industry associations, non-governmental organization, and international development agencies.

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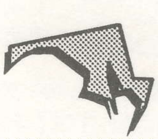

WRCC-60 1992 Annual Meeting in Conjunction with the Annual Meetin the Weed Science Society of America

WRCC-60 Administrative Report: Chair Wolfenbarger introduced the WRCC-60 a role in providing a forum for discussions of resista E. Bernays gave the administrative report. The ren for the WRCC-60 for 1991-94 was approved last summer, contingent upon adding "interdisciplinary involvement". The objectives of the WRCC-60 we discussed.

National Funding Initiative: J. Parochetti, CSRS provided a handout on the proposed CSRS budget f 1993, which includes an increase of the FRI (competitive grants program) to $150,000. Paroche discussed the overall CSRS budget, which provides 20% of Experiment Station budgets.

IRAC and PEG: C. Staetz provided a handout o these two committees anddiscussed their history.

Cost of Simulating Resistance by Tobacco Budworm in Cotton: D. Wolfenbarger gave a brief research report on the above topic, and provided a handout summarizing his findings.

Insect Resistance to *Bacillus thuringiensis*: D. Heckel gave a report on genetic linkage analysis of resistance in the tobacco budworm, and provided a handout to attendees. Discussion followed on geneti of resistance.

Overview of Resistance in Weeds: H. LeBaron discussed herbicide resistance and provided a handou on distribution of herbicide resistant weeds. Discussi followed on design on herbicides to avoid single targe site mechanisms of resistance. Discussion also dealt with herbicide resistance in general, and principles tha related to insect resistance. The criteria for what constitutes meaningful resistance were discussed, and whether such information should be used in the EPA's registration data requirements for pesticides.

B.t. Management Working Group: M. Dimock gave a report on B.t. mechanisms of action, products under development, resistance to B.t. , resistance managemen tactics, and the B.t. Management Working Group. A handout was provided.

ALS/AHAS Inhibitor Resistance Working Group (AIRWG): C. Carson discussed the history of ALS resistance, and the AIRWG goals and activities and scientists from the different companies view or perceive resistance of the target species.

WSSA Herbicide Resistant Weeds Committee (HRWC): J. Dekker reported on goals and activities of the HRWC, a standing committee of the Weed Science Society of America.

Chair Wolfenbarger asked for comments about the meeting from the administrative advisor. Dr. Bernays felt that WRCC-60 should focus on a single topic instead of trying to cover all topics. She felt there was not enough notice. A goal should be to coordinate one topic by the three disciplines.

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A Field Test Kit for Detecting Insecticide Resistance in Stored-Product Pests

Considerable damage may be caused to grain and similar commodities after harvest by insect pests, particularly in warm climates. The number of insecticides available for protecting grain has never been great, due partly to the relatively small market sector. Increasingly, however, a major factor is the cost and timescale involved for manufacturers in obtaining the data necessary for clearance and registration of grain protectants. It must now be considered essential to retain the relatively few registered insecticides at maximum levels of effectiveness. The important insecticide malathion has ceased being used as a grain protectant in many countries because of the development of resistance to it by one or more major grain pests. It is vital to prevent a recurrence of this situation with more recently introduced insecticides for as long as possible. Early detection of resistance by screening insects in the field, can help in the planning of recommendations to avoid or delay the onset of resistance to a particular chemical compound

be effectively retained for six months when sealed in aluminium foil and stored at 5°C. Pre-treated filter papers form the basis of a self-contained field test kit, which can be easily distributed. The kit also contains plastic rings for confining insects on filter papers, together with perforated covers and PTFE emulsion to prevent insect escape by flying or crawling (Taylor 1990).

The number of discriminating doses for different insecticide/insect combinations, and for which test papers are provided, is at present limited. It includes, in addition to malathion (data for which have been available for many years), pirimiphos methyl and fenitrothion for evaluating samples of *Tribolium castaneum* and *Sitophilus* spp. Discriminating doses for other insecticide/insect combinations will be introduced in the future, and NRI's present research program includes the determination of appropriate doses of pyrethroid insecticides for economically important bostrichid beetle pests of stored products.

The resistance test kit has been developed with particular reference to developing countries, where local preparation of insecticide-treated filter papers often presents problems. In association with the chemical manufacturers' organization GIFAP, NRI has recently commenced an introductory resistance screening program in four African countries which have been supplied with test kits. The program aims to validate the kit under field conditions, while at the same time gathering data on the resistance status of several major grain pests to insecticides currently used. Since publication of the FAO global survey in 1976 (Champ and Dyte 1976), there have been few studies of resistance to commonly-used insecticides in field strains (see Champ 1985) and very little information has been gathered from African countries.

References

Anonymous. 1974. Recommended methods for the detection and measurement of resistance of agricultural pests to pesticides. Tentative method for adults of some major beetle pests of stored cereals with malathion and lindane. FAO Method No. 15. FAO Plant Protection Bulletin 22: 127-137.

5. Food and Agriculture Organization of the United Nations, Rome. 297 pp.

Taylor, R.W.D. 1990. Field detection of resistance in beetle pests to contact insecticides. Proceedings of the 5th International Working Conference on Stored-Product Protection, Bordeaux, France. (In press)

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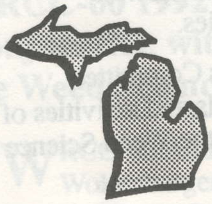
IPRM Deciduous Fruit Team Visits Mexico

IOPRM is dedicated to implementing resistance management programs worldwide. Several IPRM teams have addressed resistance problems throughout the world in 1991. Among these teams, a deciduous fruit team was formed and dispatched to Mexico.

The team of nine scientists from the U.S., United Kingdom, Argentina and Germany visited the Sierra Chihuaha apple production region in Cuouhtemoc, Mexico. The local growers, grower organizations and researchers hosted a four day on-site program. The combined Mexican and international meeting was made up of representatives from academic institutions, policy makers, government researchers, agrichemical industry, and international donor agency, local growers, local extension workers and local industry.

The meetings objectives included an on-site assessment of pesticide resistance in apple production, and to write a preliminary resistance management proposal to be submitted to donor agencies. The team together with local support, identified two severe pesticide field failures 1) fireblight and 2)white apple leafhopper. Resistance is also suspected in codling moth and phytophagous mites.



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Workshop on Integrated Pest Management and Insecticide Resistance Management in Asian Grain Legume Crops[1]

19-22 March 1991

Some 40 delegates representing the major legume growing countries of Asia, the agrochemical industry, and international research and policy organizations[2] met in Chiang Mai, Thailand to discuss the integrated management of grain legume pests in Asia and the related topic of insecticide resistance management. The meeting was sponsored by IDRC, Ciba-Geigy (Thailand) Ltd., and ICRISAT, as an activity of the Asian Grain Legumes Network (AGLN).

The workshop was divided into two two-day meetings: the first meeting dealt with IPM per se, the second with insecticide resistance management (IRM), recognizing that IRM is a facet of IPM.

The Objectives of the workshop were to:

- determine the need and the strength of support for network activity among legume entomologists in Asia, and if the need was demonstrated to:
- highlight priority areas (research topics and key insect pests),
- examine the feasibility of increasing the interaction between public sector researchers and the agrochemical industry,
- determine the extent and intensity of insecticide resistance in the farming systems that include grain legumes, and
- to discuss policies that would prevent insecticide resistance in legume crops reaching the grave levels found in other commodities.

Brief review of the proceedings

Day 1. Country delegates presented an overview of the major insect problems that beset grain legume crops in their countries. A Ciba-Geigy representative outlined the new policy of his company towards IPM, and indicated the kind of information the private sector would like to receive from public sector scientists.

Day 2. The morning was devoted firstly to reviewing the policy milieu in Asia as it might influence the implementation of IPM in farmer's fields. Discussion was free and wide and touched on such matters as open and hidden subsidies and rational behavior when discussing the economic importance of insects. Even though IPM researchers depend upon pest damage to justify their continuing employment it was agreed that they were doing their profession a disservice by overstating the losses caused by insects and other pests.

Delegates then discussed the technology transfer "loop" in their countries. The loop starts with the transmission of the message from the farmers about what they really need and want to know, to the sources or providers of new or existing information, which should then be transferred to the farmer.

In the afternoon the major problem areas were distinguished and separated into topics that could be handled by discrete working groups. A ballot was taken to determine the relative importance of the potential working groups across Asia. A set of recommendations for action by NARS and the international research sector was then drawn up (below).

Day 3. Country representatives outlined the insecticide resistance problems in their countries. The discussion centered on legume crops, but was extended to cover the problems of the relevant farming systems, especially where they contain crops that are susceptible to polyphagous insects that are likely to become resistant to insecticides. Cotton and *Helicoverpa* was the combination most frequently referred to.

The Ciba-Geigy representatives gave an account of how the industry, via the Insecticide Resistance Action Committee (IRAC) and a US Government backed international consortium of representatives of industry, academia and the public sector International

Day 4. The final day was devoted to discussing approaches to dealing with insecticide resistance problems. Guidelines drawn up in Australia following experience in managing pyrethroid resistance formed a basis for this discussion. The need to detect insecticide resistance before it manifested itself in the form of pesticide failures was stressed. A set of recommendations that indicate how the delegates perceived the need for, and direction of, future action was drawn up (below).

Recommendations Leading to the Formation of a Sub-Network Dedicated to the Integrated Control of Insect Pests of Grain Legumes in Asia.

1. It was recommended that a network should be formed, under the aegis of VAR, FAY and ICRISAT (AGLN) (Note 1) to promote:

- the exchange of information on grain legume pests (Note 2). Specific mention was made of the need to communicate information on the results of pest surveys carried out by members of national programs;
- the exchange of natural control agents, including pathogens, and germplasm and breeders material with insect resistance in its profile;
- human resource development by the interchange of trainees and organization of specialist training courses;
- the development and application of biotechnological techniques specifically orientated to the needs of IPM schemes.
- rational insecticide management; and taxonomic support for the identification of insect pests and their natural enemies, ideally through a Regional Center.

Note 1.1 A coordinating body with this structure is necessary to accommodate all the relevant grain legume crops in Asia and the needs of the relevant countries.

Note 1.2 The term "pests" normally includes all biotic constraints. The possibility of linking with other legume constraint networks or of extending the proposed network to include fungal pathogens, vertebrate pests, and weeds in the future was accepted where accessible, from international institutes in the region and from institutes on other continents.

The Working Groups highlighted in discussion, in priority order of topic, are (notes 2.1-7):

• Pesticide management	(1)
• Agromyzid flies	(2=)
• Storage pests	(2=)
• Insecticide application	(4)
• *Helicoverpa*	(5)
• Maruca	(6)
• Mirus vectors	(7)
• Soil insects	(8=)
• Pod borers	(8=)
• Defoliators	(10)
• Thrips	(11)
• Heteroptera	(12)
• Insect pathogens (see note 2)	

<u>Note 2.1.</u> The ranking was determined by ballot and indicates the importance of the areas of potential working groups in terms of constraint intensity. It was acknowledged that the priority order would be different (almost reversed) in terms of the need to gather and collate information about specific pests.

<u>Note 2.2.</u> The exploitation of insect pathogens was noted to be of highest priority by researchers but the ranking of this topic was depressed because it is currently of lesser importance to the private sector although research is ongoing.

<u>Note2.3.</u> The anticipated needs of Myanmar, Nepal and Sri Lanka were indicated by ICRISAT representatives because delegates from these countries had been unable to attend.

<u>Note 2.4</u>. Industry and the extension sector indicated that researchers should provide them with information about the life cycles, phenology, population dynamics, natural enemies and damage-yield loss relationships of key pests. This is included in the information required about specific pests or pest groups together with indications of potential IPM strategies.

<u>Note 2.5.</u> Species included under "pod borers" = *Etiella*, plume moth, blue butterflies, and *Eucosma;* "defoliators" = *Spodoptera litura*, groundnut leaf

Riptortus, Clavigralla) and mirids (e.g. *Campyloma*); "storage pests" refers specifically to bruchids.

<u>Note 2.6.</u> A "thrips network" has already been initiated by VAR and the needs of legume entomologists can be accommodate therin.

<u>Note 2.7.</u> It was agreed that studies of the natural enemies of specific insects or insect groups would be included in the activities of the relevant working groups.

3. The need to monitor the effectiveness of IPM in economic and socioeconomic terms was stressed, and specific recommendations were made to:

   - in the near future, hold a workshop to compile all available base-line data on the relationships between pest density and yield loss for grain legume crops;
   - to initiate studies on the effectiveness and farmer perceptions of IPM in grain legume crops;
   - analize the impact of the policy environment in the furtherance of IPM.

4. Technology exchange and information transfer should be facilitated by:

   - newsletter(s)
   - meetings of Working Groups
   - construction of an IPM data-base
   - investigating the possibility of organizing an International Grain Legumes Workshop to be staged in 1993/94 by ICRISAT in India
   - procuring support for inter-country study tours.

5. The widening of the membership of the network should be sought to increase the pool of experience available within the network and to attract donor support. Specific mention was made of:

   - ADB
   - AIDAB/ACIAR
   - CGIAR (CIAT, IITA, IRRI, ICARDA)
   - CP-CRSP
   - FAY (Rome, Bangkok, Manila)
   - GIFAP/IRAC
   - GTZ/BMZ
   - ICIPE
   - IDRC

- PAN
- P-CRSP
- TARC
- UNDP
- WWF
-

6. Attemps should be made to link with other networks with common interests
7. A Steering Committee based and administered by AGLN at ICRISAT Center should be formed to promote the activities of his sub-network. It should be chaired by an ICRISAT Legumes Entomologist and composed of AGLN country representatives or their nominees if the representatives is not a plant protection specialist. The private sector, AVRDC, FAO and NGOs should be represented on this steering committee.

Insecticide Resistance Management in Asian Grain Legumes

Recommendations:

1. The group recognized the importance of Insecticide Resistance Management (IRM) as a component of the integrated management of legume pests and wished to link IRM with the IPM Network proposed above through the pesticide management working group. It also recognized that many of the insect pests of legumes live on other crops and stressed the importance of the coordination of IRM activities by insect species and across farming systems (as opposed to the existing emphasis on crops and commodities).
2. The group emphasized the need for accumulating base-line data about key or high risk pests with respect to their resistance to different classes of insecticides, where possible before resistance was detected or suspected. Initial research projects should focus on:
   - Maruca
   - *Spodoptera*
   - *Helicoverpa*
   - Aphids, jassids and white flies

Monitoring techniques should be identified, standardized, and developed or refined where necessary.

5. IRM/IPM strategies should be formulated on the basis of site specific, base-line susceptibility and resistance data as well as on the results of resistance.
6. There is a need for the continuous evaluation of IRM strategies.
7. Every effort should be made to ensure the full participation of policy makers, researchers, industry and farmers to guarantee the success of IPM/IRM programs.
8. The Asian Grain Legumes IRM Network should establish linkages with the donor community, IOPERM, IRAC, FAY and other international bodies to sustain work on IRM.

Summary proceedings were available in September 1991. Delegates will automatically receive a copy.

Communicated by:

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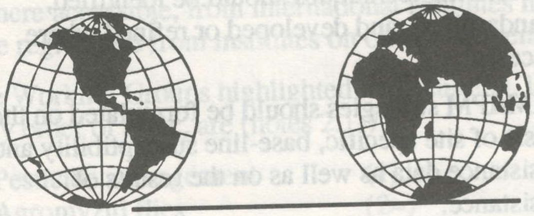


Resistance Around the Globe

Toxicokinetics of permethrin in single insects: A Method

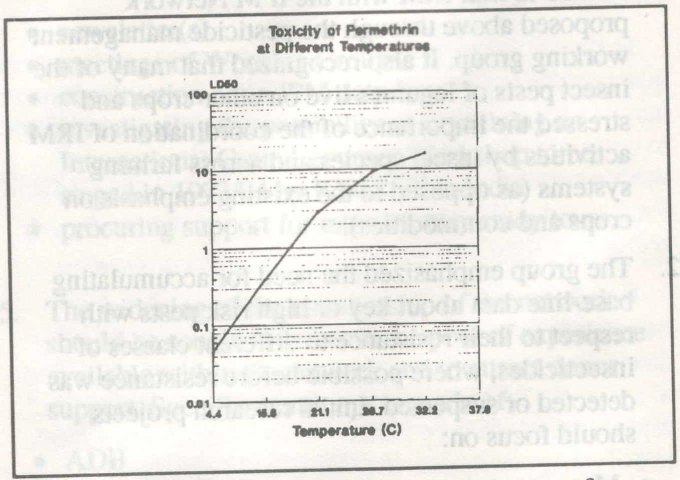
We have known for a long time that some pyrethrins and DDT become more toxic as temperatures decrease. Tom Sparks provided toxicities of a number of pyrethroids for tobacco budworm larvae and boll weevils. His data seemed to indicate that the toxicity of α-cyano pyrethroids, such as cypermethrin fenvalerate and deltamethrin, did not vary greatly with temperature, whereas permethrin, with no cyano substitution alpha to the chrysanthemic acid moiety had a pronounced negative temperature coefficient of toxicity.

The symptoms of intoxication from 4 ng of permethrin applied topically to susceptible house fly adults were reversed immediately by moving the fly from 24°C to 32°C. The symptoms of poisoning reappeared if the adult were cooled again. This process was reversible, and symptoms could be obtained at will for some hours after topical treatment. Gradually, however, the temperature at which poisoning symptoms appeared grew lower as the time after topical treatment grew longer. At some point, the symptoms did not reappear even when the adults were cooled to 12°C below which the adults entered cold "stupor," a condition in which poisoning symptoms were very difficult to discern.

I have felt for some years that observing the point at which poisoning symptoms could be seen in the manner described above was, in essence, watching the minutes of topical application. The decline in permethrin concentration in the hemolymph duplicated the decline in temperature at which poisoning symptoms were seen in parallel experiment protocols. I have been calling this decline "elimination rate kinetics," but as pointed out by Bill Plapp, that is not correct. Elimination already means something else and it would be confusing to borrow the term for what is going on here. What I mean by elimination is the decline in concentration of permethrin at the site of action, but I don't have a fancy sounding term to describe it. Plapp came up with "removal."

Last year I decided to examine these removal rates in the pink bollworm, *Pectinophora gossypiella* Saunders with the assistance of Dr. Moustafa Ali from the Pesticides Laboratory, Ministry of Agriculture, Alexandria, Egypt under the auspices of a National Agricultural Research Program exchange fellowship.

Figure 1 shows the dose-mortality curve for d-trans permethrin applied topically to adult pink bollworm. Note that toxicity covers two orders of magnitude between 12°C and 32°C. Doses are shown in ng per moth. The adults weigh on average about 8 mg, and these data are from our laboratory susceptible strain.

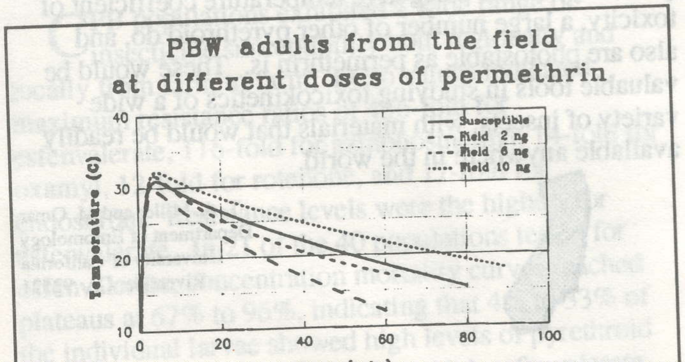


The protocol for determining removal rates of a given dose was as follows. Five adult pink bollworms were treated topically on the underside of the abdomen, and placed at one end of a thin narrow aluminum table. The table was kept at 34°C at one end and the opposite end was placed in an ice bath so that the upper surface that symptoms of poisoning were never obtained when the adults were held at the warm end of the aluminum slab (34°C).

Immediately after treatment, the adults were placed on the warm end of the table. As soon as practicable, the insects were moved towards the cool end of the table. The temperature at which poisoning symptoms started to appear was recorded for each insect and the insects were then immediately moved back to the warm in the of table and kept at 34° for about five to ten minutes.

After the first time interval, another temperature was determined at which poisoning symptoms occurred identical to the first measurement. Again, after the determination, the adults were removed to the warm end of the table. This process continued until symptoms could not be obtained above cold stupor or for about 2 hours. Afterwards, the data were plotted as the highest temperature poisoning symptoms were seen at a given time following topical treatment. Figure 2 shows typical results.

In Figure 2 the solid line shows the data from our susceptible strain treated with 2 ng of permethrin. A quick glance at figure one shows that 2 ng was the LD_{50} obtained if the adults were kept constantly at 20°C for two days; whereas, if the adults were kept at above 32°C, the lethal dose was well above 10 ng permethrin. Thus for the purposes of these experiments, we were keeping the adults under conditions which were sublethal.



after which no symptoms were obtained. This meant that the removal rates for permethrin in field animals were considerably higher than those for susceptible animals when all tests were conducted under identical conditions.

Removal rates in the field animals could be obtained that more similar to those obtained by permethrin treatment of the susceptible insects merely by increasing the dose. A glance at figure two shows that removal rates of 6 ng of permethrin on the field strain were still faster than 2 ng applied to the susceptible strain, but the field strain removed a 10 ng dose of permethrin slower than a 2 ng dose applied to the susceptible strain. These data suggested that the LD_{50} for permethrin topically applied to the field strain was around 4-fold higher than for the susceptible insects, and this is what we found from our ordinary topical toxicity studies.

What does all of this mean? I interpret the data in Figure 2 to mean that permethrin is present in the hemolymph of the insect five minutes after topical application as a bolus of dose. Our toxicokinetics measurements of permethrin entry into adult house fly shows something similar to Figure 2, with the same sort of time course.

If nervous tissues are dissected out of an adult house fly ten minutes after topical application of permethrin, and flushed with saline, the tissues will exhibit poisoning symptoms to the same extent they would if they were dissected fresh from untreated adult house flies, the perfused at a concentration of permethrin in saline equivalent to that found when hemolymph is collected and analyzed ten minutes after topical application. All of this tends to suggest that the highest concentration of permethrin in the hemolymph is obtained within minutes of treatment. Thereafter, the concentration of internal permethrin declines at a rate that depends directly on how fast the insect is able to metabolize or otherwise eliminate it.

For these and other reasons, we feel that the temperature values given on the ordinate of figure two are directly related to permethrin concentration in the hemolymph of the treated insect. For this reason these amount of permethrin that were actually in the hemolymph could be determined with great accuracy, there is virtually no way to be sure what it means in terms of poisoning concentration at the multitude of sites of action in the nervous tissues.

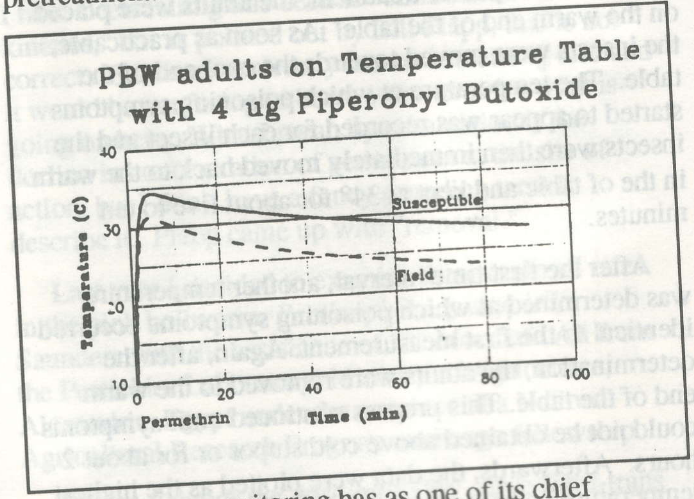
As good as radiolabelling is as a technique, in our studies of the concentration of permethrin in the adult house fly, we still found it convenient to pool samples. This was partly because the hemolymph one can obtain from the adult house fly was around 1-10 µliters and was not especially reproducible. The enormous advantage in the very simple procedure just outlined here is that the rates could be determined in individual insects unambiguously. In addition, size was not a factor. The same procedures could be applied to the smallest insects such as white fly and thrips. All that is required was for the symptoms of poisoning to be visible by whatever means.

The beauty of the determination of elimination rate kinetics by a temperature table is that it represents the first time toxicity can be determined from single insects, or the measurement of a toxicity phenotype. The fact that the data are obtained non-destructively, means that the insects can be saved for breeding studies or merely added back to a laboratory colony once the initial tests are finished.

This method also is able to check something that has confounded insect toxicology from the beginning of the field. For the first time we can check if removal rates in larvae of holometabola are different from those in the adult. We have determined that, for pink bollworm at least, removal rates of permethrin are identical for the larvae and the adult of the same animal. Larvae were tested on the temperature table as described above, then held through pupation and eclosion. The subsequent adults showed removal rates that were indistinguishable from rates that were obtained when they were larvae.

Removal rates should be susceptible to change if animals are treated with metabolic inhibitors such as piperonyl butoxide that interfere with metabolism of permethrin. We have obtained removal rate results from pink bollworms treated with piperonyl butoxide one hour before topical treatment with permethrin. To eliminate interference between the applications of different materials, p.b. was applied to the dorsal thorax

bollworms that were pretreated with 4 µpg of p.b. Comparing Figure 3 to Figure 2 shows that in both cases removal rates were slowed considerably by p.b. pretreatment.



Resistance monitoring has as one of its chief characteristics the fact that toxicity measurements are averages and are destructive. The method described above greatly extends the amount of information one can obtain from a single insect and a very simple test protocol. It allows phenotypes for toxicity to be measured non-destructively.

While the protocol described here applies to permethrin because of its negative temperature coefficient of toxicity, the information learned from the effects of synergists on removal rates might have implications for ability to oxidatively metabolize other insecticides. Although the α-cyano pyrethroids do not generally exhibit a negative temperature coefficient of toxicity, a large number of other pyrethroid do, and also are photostable as permethrin is. These would be valuable tools in studying toxicokinetics of a wide variety of insects, with materials that would be readily available anywhere in the world.

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Baseline Levels and Factors Associated with Insecticide Resistance of Colorado Potato Beetle Populations in Maryland

Since the introduction of DDT, Colorado potato beetle (CPB) has rapidly evolved resistance to all classes of chemical insecticides. Although resistant CPB populations exist in many areas of the Eastern Shore, growers in other areas of Maryland report satisfactory control. As a first step in the development of a resistance management program, this study determined the geographic extent and magnitude of the CPB resistance problem, and the cropping and insecticide use practices associated with the resistance episode in Maryland.

CPB populations were sampled in 1987 and 1988 at 56 farms statewide and assayed for insecticide susceptibility to esfenvalerate, azinphosmethyl, oxamyl, endosulfan, and rotenone. Concentration-mortality responses were determined by exposing first instar larvae for 24 hours to filter paper treated with each insecticide (Heim *et al.* 1990). Each population was subjected to a full dilution series of concentrations plus an acetone control. Ten larvae were exposed in each dish, and each test was replicated at least 6 times. To document factors associated with CPB resistance development, questionnaire information on insecticide use and cropping practices during the past 5 years (1983-87) was obtained from 235 growers.

Insecticide Susceptibility

CPB populations exhibited a wide range of insecticide susceptibility both regionally and locally from farm to farm. LC_{50} values indicated maximum resistance ratios of 456-fold for esfenvalerate, 116-fold for azinphosmethyl, 14-fold for oxamyl, 12-fold for rotenone, and 17-fold for endosulfan. Resistance levels were the highest for esfenvalerate. In 21 of the 40 populations tested for esfenvalerate, concentration mortality curves reached plateaus at 67% to 96%, indicating that 4% to 33% of the individual larvae showed high levels of pyrethroid resistance. Populations exhibiting high esfenvalerate resistance were associated with commercial growers who had extensively used this class of insecticides since its introduction in the early 1980's.

Populations exhibiting high resistance to azinphosmethyl were associated with growers who had extensively used azinphosmethyl alone; most growers routinely mix azinphosmethyl with other insecticides such as oxamyl, endosulfan or parathion to achieve effective control. Many populations from western, central, and southern Maryland were susceptible to azinphosmethyl, as evident by the RR values ranging from 0.4 to 8.6. Interestingly, many growers in these regions shifted away from azinphosmethyl use, not because of its lack of effectiveness to control CPB, but instead, because more economical and less toxic insecticides became available.

CPB populations were generally more susceptible to oxamyl than to the other insecticides. Variations in resistance ratios among populations were much lower, ranging from 0.2 to 13.8. In the tomato production areas of the Eastern Shore, moderate levels of CPB resistance to oxamyl, along with an apparent decline in effectiveness as a foliar treatment, may be attributed to the prolonged selection pressure imposed by the use of oxamyl as a systemic transplant drench.

Most populations tested were moderately to highly resistant to endosulfan. Only 27% of the growers used endosulfan alone during the past 5 years and of these only 52 percent reported satisfactory control. The range in CPB susceptibility to rotenone (RR varied from 0.4 to 12.3) was much narrower. Rotenone is not used extensively in Maryland, thus little is known about field effectiveness and potential resistance problems. The fact that rotenone effectiveness is enhanced by synergists and moderate levels of rotenone insensitivity exist suggests that resistance mechanisms are already present in CPB populations.

Spatial Distribution of Resistance

Frequencies of questionnaire responses and median RRs for each insecticide were summarized by susceptibility category and geographic region. The most resistant CPB populations were concentrated on the Eastern Shore, where the majority of commercial tomato and potato acreage is grown. Overall median RR values averaged 9.8 and 19.5 for the upper and lower Eastern Shore, respectively (Table 1). Only one location west of the Chesapeake Bay was categorized resistant. All 30 resistant populations were associated with areas of continuous and relatively intensive production of commercial host crops, where 87 percent of growers have made significant changes in management practices during the past five years to deal lower Eastern Shore because of the close proximity of host crops among neighboring farms.

Populations rated moderately resistant were about evenly distributed between the eastern and western portions of the state. Eighty-five percent of these locations also were associated with commercial growers. However, the types of farming operations represented here were much more diversified than those associated with resistant populations. Most susceptible populations were located in counties west of the Bay, as indicated by the overall RRs (Table 1). Two-thirds of the 167 susceptible populations were associated with non-commercial growers, primarily home gardeners. Susceptible populations were also scattered throughout counties with high levels of resistance development. In several areas of the Eastern Shore, susceptible populations were found in close proximity (less than 10 km apart) to resistant ones. These distinct differences among local populations suggests the presence of strong selection pressure within populations and limited gene flow among neighboring populations.

Factors Associated with Resistance

A categorical data modeling procedure was used to fit questionnaire data to a linear model of susceptibility as a function of the insecticide use and cropping practices. High levels of resistance on the Eastern Shore were related to CPB population density. Because of warmer summers and mild winters, Eastern Shore populations exhibited more generation turnover and reached higher levels that populations in other regions of the state. Eighty percent of the growers with resistant populations reported that greater than 50% yield loss would occur if insecticidal controls were not applied, whereas responses was more evenly distributed for the susceptible locations (Table 2).

CPB populations on the Eastern Shore were apparently more adapted to tomato as a host crop. Compared to the rest of the state, a greater percentage of both commercial growers and home gardeners reported yield reductions greater than 20% on tomatoes if populations were left uncontrolled. Many growers with resistant populations were restricted in their use of crop rotation because of specialized production systems. The worst cases of resistance were found on

The number of insecticide applications was the major factor that significantly contributed to the linear model of insecticide susceptibility. Highly resistant populations were associated with growers who applied more insecticide sprays during the past five years (Table 2). Resistant populations were also exposed to significantly more at-planting treatments of aldicarb on potatoes or oxamyl applied as a transplant drench on tomatoes. Seventy % of the highly resistant populations were located on farms with greater than 10 acres of host crops, whereas the majority of susceptible populations were associated with small plantings of host crops, primarily home gardens. The timing of insecticide applications during the growing season was not significantly different among susceptibility categories. About two-thirds of the sprays were applied prior to July and primarily targeted against the overwintered adults and first generation larvae.

Table 1. Questionnaire summary of management practices, expected losses, and Colorado potato beetle resistance ratios on farms of 235 growers grouped by region of the state.

	Region of State				
	Western	Central	Southern	Upper Shore	Lower Shore
Number of Respondents					
Commercial	11	30	27	29	24
Non-Commercial	10	38	13	22	31
Average host Crop Average	22.5	3.8	1.0	11.3	40.0
Average loss rating withoug CPB controls[a]					
Totato	1.9	3.8	4.6	5.1	3.8
Potato	4.9	5.1	5.8	4.7	5.0
Average Number of insecticide sprays during past 5 years					
	13.5	19.5	20.5	27.0	26.5
Median resistance ratios[b] **for:**					
Esfenvalerate	0.8	7.7	6.0	4.8	38.0
Azinphos-methyl	2.3	5.0	2.7	32.3	39.0
Oxamyl	1.9	1.5	1.2	3.3	5.3
Rotenone	0.8	3.0	2.1	4.5	4.6
Endosulfan	1.3	1.0	1.0	3.7	10.8
Overall average	1.4	3.6	2.6	9.8	19.5

[a] Expected yield loss in each host crop was rated as: 1 = <1%, 2 = 1-5%, 3 = 6-10%, 4 = 11-20%, 5 = 21-50%, and 6 = >50%.

[b] Resistance ratios were calculated as the ratio of the

Table 2. Questionnarie summary of frequencies of management practices and expected yield losses on farms of 235 growers grouped by the overall insecticide susceptibility of Colorado potato beetle populations on each farm.

	Insecticide Susceptibility Category[a]		
	Susceptible	Moderately Resistant	Highly Resistant
No. of Respondents	167	38	30
	Percentage of Respondents		
Type of Grower			
Non-Commercial	65.7	15.8	0.0
Commercial	34.5	64.2	100.0
Host Crop Average			
< 1	68.8	26.3	3.3
1 - 10	28.7	36.6	26.6
> 10	2.4	36.8	70.0
Extent of crop rotation and isolation from previous year's crop			
No rotation	55.4	31.5	16.6
Adjacent field	21.3	31.5	20.0
One field between	18.2	26.3	46.6
Isolated on new land	4.9	10.5	16.6
Percentage yield loss expected withoug CPB controls			
<1	15.6	2.6	0.0
1 - 5	11.2	2.6	0.0
6 - 10	5.0	0.0	0.0
11 - 20	16.2	5.3	0.0

After July 1	34.7	28.9	26.7
No. of insecticidal sprays during past 5 years			
0 - 10	42.7	9.7	0.0
11 - 25	40.0	48.4	22.2
25 - 50	14.7	25.8	33.3
> 50	2.7	16.1	44.4
Management practices employed during past 5 years			
Used soil insecticides	3.6	26.3	46.6
Increased sprays	16.1	39.4	63.5
Increased rates	6.6	13.1	46.6
Changed insecticides	19.7	55.2	76.6
Added synergist	1.2	26.3	90.0
Rotated insecticide	10.7	39.4	83.3

[a] Susceptibility category was arbitrarily assigned on the basis of resistance ratios (available for only 56 farms) and questionnaire data, including insecticide use patterns, frequency of control failures, and the respondent's perceived effectiveness of the insecticides used. A population was considered susceptible if any of the registered insecticide (excluding carbaryl) provided economically acceptable control, without any perceived loss of relative efficacy during the past five years. If a loss of economic efficacy was reported for endosulfan and organophosphates but not for oxamyl or unsynergized pyrethroids, then the population was categorized moderately resistant. For highly resistant populations respondents reported a loss of economic control for all groups of insecticides, and only synergized pyrethroids or combinatons of materials provided acceptable field performance.

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Baseline Monitoring of Colorado Potat Beetle Sensitivity to *Bacillus thuringiensis* and Associations with Pyrethroid Resistance

The increased use of *Bacillus thuringiensis* (Bt) based insecticide products and recent development of transgenic plants containing Bt insecticidal proteins has brought attention to the possibility of widespread resistance (Delannay *et al.* 1989). Although there is no evidence of field resistan to Bt for Colorado potato beetle (CPB), recent reports of resistance in field populations of the diamondback moth have documented the consequences of repeated Bt applications in isolated insect populations (Tabashnik *et al.* 1990). Baseline determination of susceptibility to Bt is a necessary step in the development of a resistance management program fo CPB. Previous resistance monitoring work in Maryland has revealed significant geographical variations in CPB resistance to chemical insecticides. This study determined if CPB populations differ in B susceptibility and whether this response is related to existing resistance patterns with chemical insecticide

In 1990, bioassays of both Bt and chemical insecticides were conducted on 12 Maryland populations of CPB, selected for their wide range of susceptibility to chemical insecticides. A potato leaf-dip bioassay using 3 aqueous concentrations of a spray dried powder of *B.t.* var. *san diego* was used to screen populations for relative sensitivity. Second instar larvae were exposed to the treated leaves at 27 for 72 hours at which time the tests were scored for mortality. Bioassays were repeated at least 3 times f each population. Concurrently, mortality responses discriminating concentrations of esfenvalerate, oxam azinphosmethyl, endosulfan, and rotenone were determined for each population by exposing first inst larvae to insecticide residue on filter paper in small petri dishes (Heim *et al.* 1990). Each test consisted o 20 larvae per dish, replicated 10 times for each population and chemical.

Mortality responses to chemical insecticides vari use on any of the 12 populations, differences in susceptibility were assumed to be due to natural variations. Comparisons of Bt responses revealed no significant correlations with azinphosmethyl, oxamyl, endosulfan, and rotenone susceptibility. However, LC_{50} estimates of the Bt response and percent mortalities caused by esfenvalerate were positively correlated (r= 0.704, P 0.01). Populations that were most resistant to esfenvalerate were the most sensitive to Bt (Fig. 1).

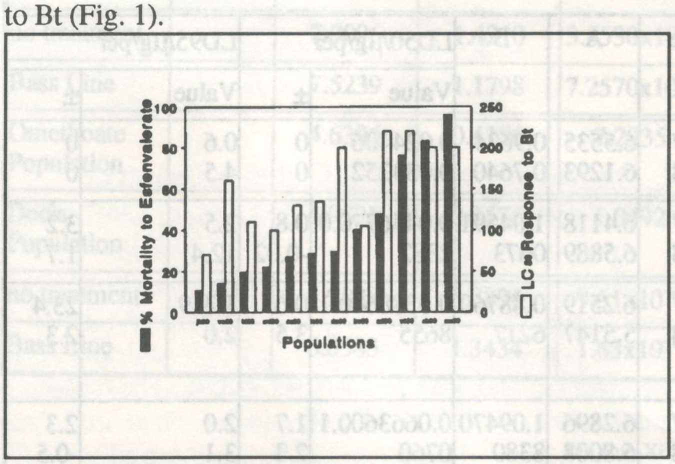

Figure 1. LC_{50} responses of 12 Colorado potato beetle populations to *B. thuringiensis* var. *san diego* ranked in ascending order according to their mortality response to a discriminating concentration (100µg/ml) of esfenvalerate. 1990.

To test this hypothesis more rigorously, two groups of CPB populations were selected in 1991, six of which had very high levels of pyrethroid resistance and six with no resistance. The following CPB populations from outside Maryland also were included: a pyrethroid resistant field population from Long Island, NY; a University of Massachusetts CPB colony characterized pyrethroid resistant; and a susceptible field population from St. Johns, ME. Leaf-dip bioassays involving a full dilution series were performed on each population using a similar but less potent Bt preparation as in 1990. Only esfenvalerate was used as an indicator of chemical resistance.

Discriminating exposure tests with esfenvalerate segregated populations into the resistant and susceptible groups, which averaged 17.1% and 88.9% mortality, respectively (Table 1). Tests based on the

pyrethroid susceptible populations with an overall LC value of 481 g/ml (Table 1). LC_{50} values among individual populations varied significantly but differe by no more than 4-fold. Correlations between esfenvalerate resistance and Bt response were again significant (r= 0.618, P 0.05 for LC_{50} values; r= 0.65 P 0.05 for slopes).

Table 1. Concentration-mortality responses of pyrethroids susceptible and resistant groups of Colorado potato beetle populations to *B. thuringiensis* var. *san diego* and esfenvalerate. 1991.

Group	% mortality (±SE) to 100 µg/ml esfenvalerate	LC50 response to B.t.	95% high-low LC50 values	Slopes (±SE)
Resistant	17.1 (2.25)	216	246-188	1.79 (0.061)
Susceptible	88.9 (2.39)	481	564-394	2.50 (0.113)

In summary, CPB populations varied significantly in response to Bt but did not differ by more than 4-fold. Populations that exhibited high levels of pyrethroid resistance were the most sensitive to Bt. There is no precedent for a negative correlation between chemical insecticide resistance and Bt sensitivity. Since there is no evidence of a related biochemical mechanism involved, it was presumed th some fitness cost associated with pyrethroid resistanc may be responsible for the increase in Bt sensitivity.

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