Resistant Pest Management Newsletter

A Biannual Newsletter of the **Center for Integrated Plant Systems (CIPS)** in Cooperation with the **Insecticide Resistance Action Committee (IRAC)** and the **Western Regional Coordinating Committee (WRCC-60)**

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Letter from the Editors

The Resistant Pest Management Newsletter has now been published for 7 years and 14 issues. We still owe our success to over 200 authors and over 2000 subscribers in 70 countries all over the world. The IRAC Central committee and the Pesticide Research Center at Michigan State University have provided the funding except for a series of one time on short duration funding by IRAC-US, USDA CSREES and Ciba-Geigy. We appreciate all of the financial support, subscriber interest and author submissions.

This issue is the largest yet with four News and Review abstracts, 19 articles and numerous announcements. The Newsletter is available on the World Wide Web thanks to Michael Caprio at Mississippi State University. It is also accessible through the Internet.

"Thanks very much" to our current authors, IRAC Central and Michigan State University!

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Resistance Management from around the Globe

A Procedure for Monitoring Resistance in Cotton Pests

The adult vial test has become a standard method for monitoring insecticide resistance in cotton pests. The procedure was initially developed to monitor for resistance to pyrethroids in adult tobacco budworm (TBW), *Heliothis virescens* (F.) (Plapp et al. 1987). Recently, we modified the technique to monitor resistance to organophosphorus, carbamate, and cyclodiene insecticides in TBW (Kanga & Plapp 1992, 1995). We can also modify the technique to monitor for resistance to insecticides in the boll weevil, *Anthonomus grandis* Boh. (Kanga et al. 1995). Here we describe the method in detail to assist others who may face similar problems.

Glassware for Resistance Monitoring: We use 20-ml glass vials often used for liquid scintillation counts. Newly purchased vials are soaked overnight in soapy water, rinsed with acetone, and air dried. The vials are then baked at >100C for 4-8 hours to remove oils left over from the manufacturing process. Color coded dots (0.6 cm) are placed on the bottom of the vials to label insecticide and concentration.

Preparation of Insecticide Solution: Insecticides are prepared in acetone solutions. Anhydrous sodium sulfate was added to technical grade acetone to remove traces of water. Use of dry acetone should reduce insecticide breakdown and make vial preparation easier.

Insecticides and Working Solutions: We use technical grade samples of insecticide of known purity rather than formulated materials. To prepare stock solutions at 1 mg active ingredient per ml acetone, we add 10 ml acetone to every 10 mg pure material. If the technical material is 95% pure, we add 9.5 ml acetone to every 10 mg material. Solutions are stored in a freezer and warmed to room temperature before use.

Addition of Organic Acids and Treatment of Vials: Benzoic acid is put in vials that contain biodegradable insecticides such as organophosphates, carbamates or endosulfan. This acid increases stability of insecticide residues, apparently by protecting them from hydrolysis via reaction with water vapor (Kanga & Plapp 1992, 1995). For each vial prepared, 1 mg free acid (not the sodium salt) is added to 0.1ml acetone. Optimal results are obtained in range of 10:1 to 30:1 (w:w) benzoic acid: insecticide ratios.

After putting benzoic acid in vials, required concentrations of insecticide in 0.5 ml acetone are added. Vials are then laid on their sides in a hood and rolled gently for 1-2 minute intervals until dry. The process usually takes 12-15 minutes.

Insecticide Bioassay: TBW males, collected from pheromone traps early in the day, are brought to the laboratory and fed overnight on a cotton pad soaked in 10% sugar water. Only vigorous males are used in bioassays. Male moths are placed individually in insecticide-treated vials. As treatment controls, males are placed in vials treated with acetone only. These vials are kept at room temperature (25C) and susceptibility is determined after 24 hours exposure. Males unable to fly short distance (>1 m) when tossed in the air are considered susceptible to the insecticide.

Boll weevils are tested by a similar procedure (Kanga et al. 1995). Infested cotton squares are brought to the laboratory and placed in a cage for weevil emergence. Weevils are tested in groups of 2-5 per vial. All weevils tested are less than four days old. After 24 hours exposure to an insecticide, adults are considered susceptible if there is no leg movement when the snout is pinched with forceps or if they are unable to walk for 0.3m without rolling onto their back.

Selection of Diagnostic Dose: Determination of appropriate doses is an important consideration. If resistance is due to a single gene, three geneotypes of insects, homozygous susceptibles, heterozygotes and homozygous resistants, are possible. Rather than try to separate the three genotypes, we concentrate on identifying susceptible individuals only. Based on our experience, the appropriate dose to use for resistance monitoring may produce 80-90% or less mortality in a susceptible population but allow all resistant insects to survive (McCutchen et al. 1989). We determine the decline in mortality in a test population and compare observed mortality with a susceptible population. If mortality in a test population decreases from 90 to 80%, then 89% of the population is considered susceptible (homozygous) and 11% are considered resistant. Using the Hardy-Weinburg equation, we can estimate the proportions of resistant heterozygotes and resistant homozygotes. This strategy is considerably simpler than trying to determine doses that separate all three genotypes. If resistance is suspected in a field population, we recommend performing multiple sets of bioassays over time rather than single set screening large numbers of insects at once.

Shipment of Treated Vials: Insecticide-treated vials for resistance monitoring have been prepared in our laboratory and shipped to cooperators around the state. Each shipment is supplied with forms for record keeping. These cooperators collect insects from fields with insect control problems and screen them for resistance. Results are recorded onto the forms supplied with the vials and returned to a central data managing point for processing and calculation of resistance frequencies.

CONCLUSION: This described technique provides extension entomologists and crop consultants with a fast, inexpensive, and reliable method for monitoring cotton pests for resistance. The major advantage of this technique is that it uses field-collected adults and resistance can be determined overnight (Daly & Fisk 1993). No rearing of test insects is necessary as with most commonly used techniques.

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Consideration and Management of Pesticide Resistance by the U.S. Environmental Protection Agency

The views expressed in this article are those of the authors and do not necessarily represent those of the United States Government.

The problem of pest resistance to pesticides is a worldwide concern. The U.S. Environmental Protection Agency has considered the development of pesticide resistance and pesticide resistance management in its regulatory decisions. With a greater focus on use reduction of the higher risk pesticides, the EPA believes that it is very important to implement effective resistance management strategies. However, the EPA does not have an official policy or standard data requirements in place. This paper will consider: (1) how the Agency has considered pesticide resistance management under the Federal Insecticide Fungicide Rodenticide Act (FIFRA) when making emergency exemption decisions (e.g., oxytetracycline), special review decisions (e.g., EBDCs), and registration decisions (e.g., synthetic pyrethroids, and plant-pesticides producing Bacillus thuringiensis endotoxins); and (2) how the Agency is continuing to evaluate and refine the role pesticide resistance management has in the Agency's regulatory decisions.

The Role of Pesticide Resistance in EPA Regulatory Decisions: The EPA has considered pesticide resistance when making certain regulatory decisions. This paper will briefly summarize how the Agency has addressed pesticide resistance issues under the following sections of FIFRA : Sections 18 (Emergency Exemptions), 6 (Special Review), and 3 (Registration).

Pesticide resistance has been a factor in many decisions to grant "emergency exemptions" that allowed use of an unregistered pesticide in an emergency situation where significant economic loss would occur under a non- routine situation. In the last three years, more than 30% of the requests for emergency or crisis exemptions under Section 18 have been for the purposes of resistance management because resistant pest populations have rendered the registered alternatives ineffective. Typically, an emergency exemption is granted for use of one pesticide to use as a substitute for the pesticide to which pests have developed resistance. Some examples of recently granted emergency exemptions are: (1) cryolite to control Colorado potato beetle resistant to chlorinated hydrocarbon, organophosphate, and synthetic pyrethroid insecticides on potatoes in several states; (2) myclobutanil to control benomyl- resistant *Sphaerotheca macularis*, the causal agent of powdery mildew on strawberries in California; (3) oxytetracycline to control streptomycin- resistant *Erwinia amylovora*, the causal agent of fire blight on apples in Michigan, Washington, and Oregon; (4) lactofen to control paraquat- and diquat- resistant nightshade weeds in tomatoes and peppers in Florida; and (5) quinclorac to control propanil- resistant barnyard grass in rice in Arkansas.

The EPA has also considered pesticide resistance when making determinations of whether unreasonable adverse effects would occur if registered uses of a pesticide are maintained. This determination is a component of the Agency's Special Review process. One example where pesticide resistance played an active role in assessing the benefits during the special review process were for the ethylene bisdithiocarbamates (EBDCs) fungicides. These fungicides include mancozeb, maneb, metiram, and nabam. Two other EBDC fungicides, amobam and zineb, were voluntarily cancelled several years ago. EBDCs are major agricultural fungicides controlling several important fungal pathogens on over 40 fruit and vegetable crops. There are no reports of pest resistance under field conditions after more than 40 years of use. Upon review of the benefits for EBDCs, the Agency concluded that EBDCs are an important tool in fungicide resistance management. For example, EBDCs in combination with benomyl, function in resistance management by controlling apple scab (*Venturia inaequalis*), sooty blotch (*Gloeodes pomigena*) and fly speck (*Shizothyrium pomi*) on apples. EBDCs in combination with copper function in resistance management by controlling bacterial spot (*Xanthomonas vesicatoria*) resistance on peppers and tomatoes. The importance of EBDCs for pesticide resistance management was considered both qualitatively (decrease in fruit quality) and quantitatively (decrease in fruit yields) by the EPA in estimating the fungicide's benefits. The uses of EBDCs were maintained on numerous commodities, in part, because of the benefits of EBDCs in fungicide resistance management (1).

Historically, pesticide resistance has not been a consideration upon determining whether a new pesticide should be registered. The EPA has no formal policy or guidelines on how pesticide resistance management should be considered in making registration decisions. However, beginning in the late 1980s, in specific cases in which pesticide resistance development has been a concern, the EPA has worked with some pesticide registrants to develop appropriate pesticide label language to advise pesticide users on ways to avoid or delay the onset of pesticide resistance. Registration labels have included statements related to resistance management that include recommending the use of alternative pesticides if resistance were already a factor. In addition, the EPA has reviewed several pesticide resistance management strategies that were voluntarily submitted to the Agency by pesticide registrants.

One example of industry and EPA voluntary cooperation was the development of risk mitigation measures and use instructions to mitigate the development of resistance for synthetic pyrethroids. The industry (i.e., the registrants) formed a Pyrethroid Working Group (PWG), which developed programs that were reviewed by an OPP liaison group. The immediate issues were: aquatic organism risk mitigation and tobacco budworm resistance management. The synthetic pyrethroids include permethrin, bifenthrin, esfenvalerate, lambda-cyhalothrin, cyfluthrin, cypermethrin, fenproprathrin, zeta- cypermethrin, and tralomethrin. As a result of the PWG's efforts, the labels for synthetic pyrethroids include appropriate spray drift mitigation measures, a section on the development of resistance, and language indicating that the use of the product should conform to resistance management strategies established for the local use areas. If resistance is suspected, the label states that products with a similar mode of action, e.g., other synthetic pyrethroids, may not provide adequate control and that the user should consult with the local company representative or agricultural advisor for the best alternative method of control. As a result of these efforts, tri- state (Arkansas, Louisiana, and Mississippi) resistance management plan for cotton insect control has been developed by research and extension entomologists to control tobacco budworm populations.

Refining the Role the EPA Plays in Pesticide Resistance Management: The Agency is currently determining how to refine the role of pesticide resistance and pesticide resistance management in its regulatory decisions for all pesticides. In August 1992, the Assistant Administrator requested that an Office of Pesticide Programs (OPP) workgroup be formed following discussions at OPP's FIFRA Science Advisory Panel meetings and letters from Public Interest Groups regarding potential for development of pesticide resistance to *Bacillus thuringiensis* (Bt) foliar sprays because of the pending introduction of Bt plant- pesticides. At this time, the Pesticide Resistance

Management Workgroup (PRMW) was formed. The PRMW includes scientists from several scientific disciplines, e.g., plant pathologists, microbiologists, entomologists, weed scientists, biologists, and biochemists. The workgroup considers the EPA's role concerning the resistance management of conventional, biological, and genetically- engineered pesticides. The workgroup has had several discussions with registrants, representatives of the Insecticide Resistance Action Committee (IRAC), and other stakeholders on resistance management strategies.

Registration of Plant- Pesticides: The PRMW has identified seven elements that need to be addressed to develop an adequate resistance management plan. A subpanel of the FIFRA Science Advisory Panel (SAP) approved of these seven factors on March 1, 1995. These elements are: (1) knowledge of pest biology and ecology, (2) appropriate gene deployment strategy, (3) appropriate refugia (primarily for insecticides), (4) monitoring and reporting of incidents of pesticide resistance development, (5) employment of IPM, (6) communication and educational strategies on use of the product and (7) development of alternative modes of action.

The PRMW has reviewed plant- pesticide resistance management strategies which have been voluntarily submitted by the registrants. Reviews of resistance management plans that have been completed by the PRMW include: (1) the *Bacillus thuringiensis* (Bt) CryIIIA delta endotoxin produced in potato to control Colorado potato beetle (registered May of 1995); and (2) the Bt CryIA(b) delta endotoxin produced in field corn to control European corn borer (registered in August of 1995), and (3) the CryIA(c) delta endotoxin produced in cotton to control pink bollworm, cotton bollworm, and tobacco budworm (registered in October of 1995).

OPP used the workgroup's reviews of the resistance management plans to make recommendations to registrants to help them improve their management plans, and, when necessary, established conditions for registration of plant pesticides. The EPA believes that resistance management is critical to the long- term viability of plant- pesticides. For example, if no resistance management plan is implemented for Bt plant- pesticides, it is expected that widespread pest resistance would develop in less than 5 years after transgenic crops have been grown uniformly over large areas following registration. Because the pesticidal protein in Bt plant- pesticides, CryI delta endotoxins, are also widely used in a variety of Bt foliar spray products on many crops, resistance development to Bt plant- pesticides would also affect efficacy of foliar Bt products.

Workgroup Accomplishments and Proposed Bt Plant-Pesticide Registrant Task Force: The following list summarizes the PRMW's accomplishments on regulation and policy for pesticide resistance management:

Established a list of appropriate factors to be considered in developing a pesticide resistance management plan. This list was approved by the March 1, 1995 Subpanel on Plant- Pesticides of the FIFRA Science Advisory Panel.

Recommended reporting requirements for incidents of pesticide resistance development that are included in the revision of the adverse effects reporting rule (FIFRA Section 6(a)2 Rule, in draft at the time).

Recommended revisions to EPA policy to allow emergency exemptions to be granted under certain conditions for two or more unregistered pesticides for the purpose of avoiding or delaying the buildup of pest resistance (when resistance has not yet been documented). State pesticide regulatory agencies have requested these changes.

Recommended revising EPA policy to include resistance management criteria for issuing special local needs (FIFRA section 24(c)) registrations. EPA proposed a change in policy in the draft guidance for special local needs registrations in which EPA would allow a special local needs registration to avoid or delay the buildup of pest resistance under certain conditions (2). State pesticide regulatory agencies have requested pesticide resistance management be a part of the guidance document.

Recommended the development of screening criteria for when pesticide resistance management plans should be implemented for experimental use permits (FIFRA Section 5) and prior to registration of a new active ingredient (FIFRA Section 3).

Encouraging the development of a Bt plant-pesticide registrant task force to address, more uniformly, resistance management issues for Bt /corn and Bt /cotton.

Proposed Pesticide Resistance Screening Process and Request for Comments: The Agency believes that resistance management should be considered for all pesticides, but the workgroup is not recommending across- the- board data requirements for resistance management or specific labeling for all pesticides. A screening process is being considered to identify pesticides and pests which pose the greatest concern for the development of pesticide resistance and pesticide resistance management. At this early stage of development, OPP is considering the following criteria to identify pesticides which may require the development of a pesticide resistance management strategy as a condition of registration: 1) classes of pesticides with a known history of pesticide resistance; 2) target pests with a known history of pest resistance; 3) pesticides with new modes of action; 4) reduced risk pesticides which the Agency has determined required pesticide resistance management concerns, and; 5) new uses of pesticides which may dramatically increase the use of a pesticide and consequently pose a greater selection pressure on the target pest(s).

We are encouraging comments on these potential criteria. We would like to know which pests and classes of pesticides pose the greatest resistance management concerns so that the Agency can more clearly focus its resources. Please send your comments to the postal or electronic mailing address provided.

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Detecting Thiocarb Resistance in Australian *Helicoverpa armigera*

Thiocarb resistance was first detected in Australian *Helicoverpa armigera* (Hubner) in early 1993. Since then, the frequency of resistant individuals has increased and raised concern among growers of cotton, maize and grain legumes. The level of resistance is approximately 35 fold and confers cross resistance to other carbamates such as methomyl and carbaryl.

Our studies have shown that the mechanism responsible for thiocarb resistance is an insensitive target site. Resistant individuals posses a form of the neurotransmitter acetylcholine esterase (AChE), that is partially insensitive to carbamates. Resistant individuals can be either heterozygous or homozygous since the resistance mechanism is effectively dominant. Even with 70% of AChE inhibited, resistant individuals can survive exposure to thiodicarb. This resistance mechanism does confer a slight fitness deficit -- resistant larvae grow more slowly.

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Cross Resistant Patterns of Insecticide-selected Strains of Cotton Bollworm [*Helicoverpa armigera* (Hubner)]

Resistant strains of cotton bollworm (*Helicoverpa armigera*) were selected for fifteen consecutive generations on artificial diet and treated with one of the following insecticides: Fenvalerate, Deltamethrin, Cyhalothrin, Cyfluthrin, Cypermethrin, Fenpropathrin, Bifenthrin, Parathion, Monocrotophos or Methomyl. Resistance levels and cross resistance patterns were determined on larvae tested with the topical application method recommended by FAO. We also followed the development of resistance to Deltamethrin, Parathion, Monocrotophos and Methomyl in larva collected in fields from Liao Cheng, Shandong Province. Liao Cheng represents a major cotton producing area with severe pest resistance problems.

Tables 1 and 2.

Table 1. Levels of resistance and cross-resistance pattern for Helicoverpa strains selected with one of ten insecticides from 1993 to 1995.

Strain Selected with:		Cross Resistance Pattern									
		Fenvelerate	Deltamethrin	Cyhalothrin	Cyfluthrin	Cypermethrin	Fenpropathrin	Bifenthrin	Parathion	Monocrotophos	Methomyl
Baseline	LD50	1.9093	0.4387	0.54	0.5797	0.9316	1.1367	0.3245	5.7526	3.2023	6.1283
	RR	--	--	--	--	--	--	--	--	--	--
Fenvalerate	LD50	435.9726	3.4282	4.4862	5.3393	10.2631	12.7473	2.0442	126.8317	5.3457	7.1588
	RR	228.3	7.8	8.3	9.2	11	11.2	6.3	22	1.6	1.2
Deltamethrin	LD50	12.3358	10.4201	2.2671	2.9122	2.8638	6.8323	0.9131	12.2589	4.3571	5.0267
	RR	16.5	23.7	4.2	5	3.1	6	2.8	2.1	1.4	0.82
Cyhalothrin	LD50	16.8373	2.3969	19.054	4.5503	7.6277	9.4734	1.0035	11.1284	4.1926	5.5963
	RR	8.8	5.5	35.3	7.8	8.2	8.3	3.1	1.9	1.3	0.91
Cyfluthrin	LD50	15.7732	2.1249	3.6487	3.7388	44.4739	9.2965	1.1144	10.0263	4.3201	5.0982
	RR	8.3	5.3	4.5	32.5	7.2	8.2	3.4	1.7	1.4	0.83
Cypermethrin	LD50	17.9364	2.2149	3.6487	3.7388	44.4739	7.1125	1.5697	12.0283	5.3301	6.0008
	RR	9.4	4.8	6.7	6.4	47.4	6.2	4.8	2.1	1.6	0.97
Fenpropathrin	LD50	17.0472	2.222	2.4553	3.3969	5.9328	89.2706	1.8077	9.2385	4.3477	5.9449
	RR	0.89	5.1	4.5	5.8	6.4	78.5	5.6	1.6	1.4	0.97
Bifenthrin	LD50	10.3339	1.4608	1.7447	2.3849	2.8974	6.2771	3.3144	8.8703	3.6574	4.6111
	RR	5.4	3.3	3.2	4.1	3.1	5.5	10.2	1.5	1.1	0.75
Parathion	LD50	237.841	0.6199	0.975	0.9485	1.7171	2.2178	0.3156	153.283	3.7383	7.5981
	RR	124.5	1.4	1.8	1.6	1.8	1.9	0.97	26.6	1.2	1.2
Monocrotophos	LD50	2.6711	0.4833	0.8849	0.9847	1.3052	1.5001	0.3581	6.9541	116.2477	5.3211
	RR	1.4	1.1	1.6	1.7	1.4	1.3	1.1	1.2	36.3	0.86
Methomyl	LD50	1.7828	0.3678	0.5082	0.5307	0.8854	1.0613	0.2515	6.8545	2.9909	86.0818
	RR	0.93	0.83	0.94	0.91	0.95	0.93	0.77	1.2	0.93	14.3

Shaded boxes represent level of resistance for each strain towards the insecticide it was selected with.

Table 2. Levels of insecticide resistance in Helicoverpa collected in the Liao Cheng Area between 1984 and 1995.

Year	Strain	Pesticide	LD50 (ug/g)	Resistace Ratio
1984	Ching Chou	Deltamethrin	0.000314	1
		Parathion	0.0389	1
		Monocrotophos	2.91	1
		Methomyl	0.4967	1
	Hebei	Deltamethrin	0.001423	4.5
		Parathion	0.0496	1.2
		Monocrotophos	3.3068	1.1
		Methomyl	1.5994	3.2
	Liao Cheng	Deltamethrin	0.002274	7.2
		Parathion	0.2384	6.1
		Monocrotophos	22.7513	7.8
		Methomyl	2.5832	5.2
1985	Liao Cheng	Deltamethrin	0.005029	16
		Parathion	0.3306	8.4
		Monocrotophos	100.5	34.5
		Methomyl	5.3597	10.7
1986	Liao Cheng	Deltamethrin	0.029618	94.3
		Parathion	2.7742	71.3
		Monocrotophos	263.64	90.5
		Methomyl	40.61	81.7
1995	Liao Cheng	Deltamethrin	17.87	56910.8
		Parathion	98.62	2535.2
		Monocrotophos	300.84	103.3
		Methomyl	119.16	239.9
1995	Insecticide Selected Strain	Deltamethrin	10.42	33184.7
		Parathion	153.28	3940.3
		Monocrotophos	116.24	39.9
		Methomyl	86.08	173.3

Resistance Ratio = LD50/ LD50 (Ching Chou Strain)

CONCLUSIONS:

1. Each strain selected for resistance to one pyrethroid demonstrated cross resistance to the other six pyrethroids.

2. The strain selected for resistance to Parathion demonstrated resistance to Fenvalerate and vice versa.

3. There was no cross resistance between pyrethroid resistant strains and Monocrotophos.

4. Pyrethroids and Monocrotophos showed negative cross-resistance to Methomyl.

5. Deltamethrin resistance was detected in the Liao Cheng area in 1984. Resistance ratios in this field population rose to 94.3 in 1986 and soared to 56,910 by 1995. This is the same magnitude of resistance as was selected in the lab strain after 15 generations of selection.

6. The resistance ratio in the Parathion selected strain was 153.28-fold. In the Liao Cheng field population resistance reached 98.62-fold by 1995.

7. The resistance ratio in the Monocrotophos selected strain was 116.24-fold and resistance ratio in the Liao Cheng field population 300.84-fold by 1995.

8. The resistance ratio in the Methomyl selected strain was 86.08-fold and in the Liao Cheng field population 119.16-fold by 1995.

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Fenvelerate and Sumithion Mixtures May Delay the Resistance Development in the Peach-Potato Aphid

Field-collected peach-potato aphids [Myzus persica (Sulzer)] were reared for several generations in a greenhouse and then were divided into four colonies. The first colony (Fe) was selected with fenvalerate for 14 generations. The second colony (Su) was selected with sumithion and the third colony (Fs) with a mixture of sumithion and mixture (fenvalerate: sumithion = 3: 7) for 14 generations. The fourth colony (CK) was treated with water only. During selection, the rectified mortality rates of various groups were kept about 10 percent. Every two or three generations, the resistance level of each colony was bioassayed with the topical application procedure recommended by FAO. The CK colony was bioassayed with all insecticide treatments.

After 14 generations of selection, the level of resistance in the CK colony to each insecticide treatment remained almost unchanged. Meanwhile, the Fe colony developed 52.6-fold resistance to fenvalerate; the Su colony developed 11.1-fold resistance to sumithion; and the Fs colony developed only a 3.5-fold resistance to the mixture (see Table 1).

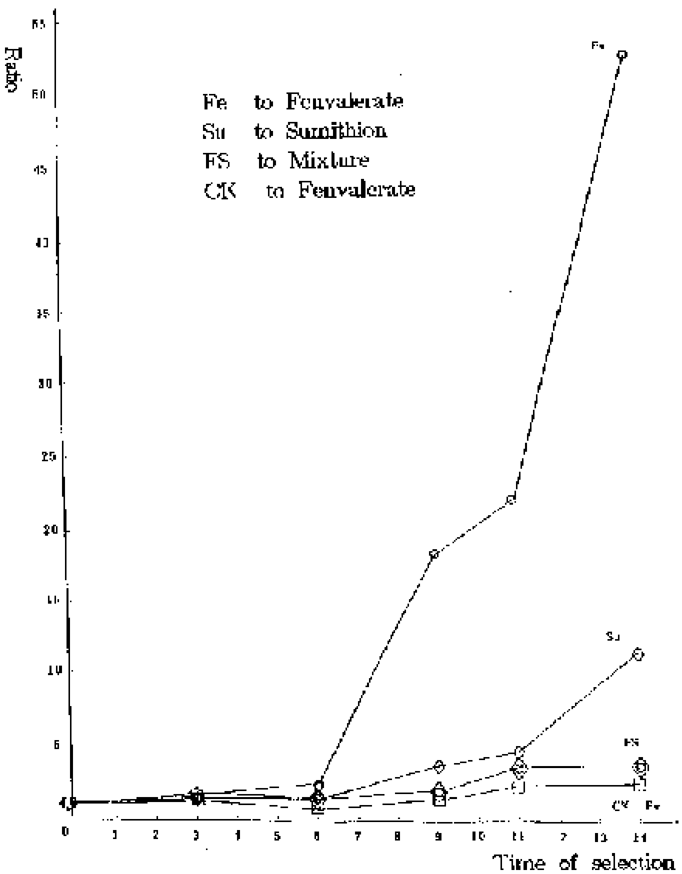
Table 1. Resistance development of various groups to corresponding insecticides.

Group	Week of Selection	Y = a+bX	LD50 (g/aphid) [L1-L2]	Ratio*
Fe	0	Y = 7.79+0.98X	1.39x10-3 [7.16x10-4 - 2.70x10-3]	1.0
	3	Y = 9.00+1.45X	1.71x10-3 [1.27x10-3 - 2.30x10-3]	1.2
	6	Y = 8.98+1.58X	3.02x10-3 [2.48x10-3 - 3.69x10-3]	2.2
	9	Y = 5.98+0.61X	2.52x10-2 [1.29x10-2 - 4.95x10-2]	18.2
	11	Y = 6.58+1.04X	3.03x10-2 [2.75x10-2 - 4.06x10-2]	21.8
	14	Y = 6.29+1.13X	7.30x10-2 [5.29x10-2 - 1.01x10-2]	52.6
Su	0	Y = 8.09+1.59X	1.15x10-2 [9.96x10-3 - 1.33x10-2]	1.0
	3	Y = 7.10+1.16X	1.53x10-2 [1.13x10-2 - 2.08x10-2]	1.3
	6	Y = 7.15+1.20X	1.63x10-2 [1.21x10-2 - 2.18x10-2]	1.4
	9	Y = 5.76+0.54X	3.94x10-2 [1.37x10-3 - 1.13x10-2]	3.4
	11	Y = 6.71+1.32X	5.01x10-2 [4.82x10-2 - 6.24x10-2]	4.4
	14	Y = 5.83+0.92X	1.28x10-1 [8.26x10-2 - 1.95x10-1]	11.1
Fs	0	Y = 9.86+1.75X	1.70x10-3 [7.85x10-4 - 3.68x10-3]	1.0
	3	Y = 8.96+1.44X	1.80x10-3 [1.35x10-3 - 2.42x10-3]	1.1
	6	Y = 9.52+1.70X	2.19x10-3 [1.78x10-3 - 2.71x10-3]	1.3
	9	Y = 8.67+1.45X	2.94x10-3 [2.35x10-3 - 3.68x10-3]	1.7
	11	Y = 8.41+1.51X	5.50x10-3 [4.40x10-3 - 6.88x10-3]	3.2
	14	Y = 7.53+1.14X	5.96x10-3 [4.36x10-3 - 8.16x10-3]	3.5
** CK	0	Y = 7.79+0.98X	1.39x10-3 [7.14x10-4 - 2.70x10-3]	1.0
	3	Y = 9.56+1.61X	1.48x10-3 [1.16x10-3 -1.87x10-3]	1.1
	6	Y = 8.99+1.33X	9.98x10-4 [7.62x10-3 - 1.31x10-3]	0.7
	9	Y = 7.40+0.89X	1.94x10-3 [1.33x10-3 - 2.81x10-3]	1.4
	11	Y = 7.14+0.83X	2.60x10-3 [1.75x10-3 -3.88x10-3]	1.9
	14	Y = 8.17+1.26X	3.11x10-3 [2.23x10-3 - 4.33x10-3]	2.2

* Ratio = LD50/LD50 (baseline) ** Response to Fenvalerate only

Figure 1.

Comparision of Resistance Evolution of Various Groups to Corresponding Insecticides



Therefore, we reach the conclusion that the mixture of fenvalerate and sumithion may delay resistance development of peach-potato aphids compared to exposure to either insecticide alone.

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Insecticide Resistance Management in *Helicoverpa armigera* (Hubner) in the Hebei Province, P.R. China

Hebei province is a large cotton-producing area in P.R. China. It has become difficult to control cotton bollworm, *H. armigera*, since late 1980's as a result of insecticide resistance. Our monitoring program in 1991 showed bollworm resistance to pyrethroids to be between 9.3 to 116-fold. In order to delay further resistance development, we designed an insecticide resistance management (IRM) strategy and in 1992 executed it in the field.

In general, the key measures of the strategy are as follows:

- Restrict the use of pyrethroids alone;
- Develop synergistic mixtures of insecticides and rationally apply them on the field;
- Choose different types of insecticides and apply them in rational rotation;
- Each insecticide is used no more than twice in one year;
- Choose the optimum time to apply insecticides that treat during periods of maximum egg hatch;
- Coordinate chemical control approaches of the pest in different crops;

- Educate farmers to properly apply and coordinate their activities in management of *Helicoverpa*.

This IRM strategy has proved to be useful. Since 1992, the resistance development has been significantly delayed in IRM demonstration areas. Between 1991 and 1994 *Helicoverpa* resistance to fenvalerate and cypermethrin was reversed in the IRM area while resistance increased 2.9 and 1.5-fold, respectively, in the control area (see Table 1). *Helicoverpa* resistance to deltamethrin and cyhalothrin increased in the IRM area by 1.8 and 1.1-fold, respectively; but in the control area, resistance increased at more than twice that rate (3.8 and 2.5, respectively).

Table 1. The change in response H. armigera to four pyrethroids from 1991-1994 in the IRM area compared to the control area.

Area	Insecticide	Resistance Ratio[1]		Overall Response[2]
		1991	1994	
Control	Fenvalerate	9.8	28.4	2.9
	Cypermethrin	13.7	20.9	1.5
	Deltamethrin	15.5	58.5	3.8
	Cyhalothrin	9.7	24.4	2.5
IRM	Fenvalerate	23.1	18	0.8
	Cypermethrin	27.6	21	0.8
	Deltamethrin	20.1	36.5	1.8
	Cyhalothrin	18.5	20.4	1.1

1 LD50(ug/g) of field population/LD50 of susceptible strain 2 LD50 (ug/g) detected in 1994/ LD50 detected in 1991; a value less than 1.0 = resistance reversion

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Insecticide Resistance in Beneficial Insects Associated with Stored Grain in the Southeastern United States

Field strains of three beneficial insects, *Anisopteromalus calandrae* Howard (Hymenoptera: Pteromalidae), *Bracon hebetor* Say (Hymenoptera: Braconidae), and *Xylocoris flavipes* Reuter (Heteroptera: Anthocoridae) were collected from farm storages in South Carolina and Georgia in the fall and summer of 1992 and 1993. Sensitivities of the two parasitoids and one predator to several common grain protectants were determined in laboratory bioassays and compared with sensitivities of susceptible strains reared in the laboratory for more than 20 years. Glass vial bioassays were used for *A. calandrae* and *B. hebetor* and a filter paper-petri dish bioassay was used for *X. flavipes* (Baker & Weaver 1993, Baker & Arbogast 1995). A summary of results for the three species with malathion, a common grain protectant, is presented in Table 1.

Table 1. Malathion sensitivity for laboratory and field strains of three beneficial insect species found in the stored grain ecosystem.

Beneficial species	Host/Prey	Malathion LD50*		RR
		Lab	Field	
A calandrae	*Sitophilus* weevils, In-kernal spp.	0.139	387	2,800
B. hebetor	Lepidoptera, Pyralidae	0.21	1.59	7.6
X. flavipes	Eggs & small larvae, numerouse spp.	51.4	1,700	33.1
		45.9	1,430	31.1

*mg AI/vial for A. calandrae and B. hebetor; mg AI/filter for X. flavipes; RR = resistance ratio

Significant levels of malathion resistance are present in field strains of all three beneficial species; however, the 2,800-fold resistance in the field strain of *A. calandrae* is most notable. Evidence from bioassays with inhibitors TPP and DEF indicate that a carboxylesterase may be involved in the malathion resistance in this strain. Field rates of malathion applied to wheat had no significant effect on longevity of the field strain of *A. calandrae*, parasitization of rice weevil larvae by *A. calandrae*, or fecundity of the field strain of *A. calandrae*.

Evidence from reciprocal crosses of the laboratory and field strains indicate that the malathion resistance in *A. calandrae* is an incompletely dominant trait. Backcrossing experiments to determine mechanism of inheritance are in progress.

We are continuing to collect field strains of biological control species in stored grain and to evaluate their sensitivities to new grain protectants. In view of efforts to reduce pesticide usage, it is hoped that these insecticide-resistant beneficial insects can be incorporated into pest management programs that combine both chemical and biological control technologies for stored grain insects.

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The Streptomycin Resistance Transposon Tn 5393

The dissemination of the streptomycin-resistance transposon Tn 5393 among the phytopathogenic bacteria *Erwinia amylovora*, *Pseudomonas syringae*, and *Xanthomonas campestris* is a powerful illustration of the ability of bacterial populations to evolve resistance through the recruitment of preexisting genes. Tn 5393 encodes the *strA-strB* aminoglycoside phosphotransferase genes that are also found in human and animal bacterial pathogens (Chiou & Jones 1993, Sundin & Bender 1995). Tn 5393 is typically plasmid encoded, but may also be chromosomally inserted. The location of Tn 5393 on different plasmids and chromosomes of phytopathogenic bacteria is retrospective evidence of the mobility of the element within and between populations. Gene transfer of Tn 5393 increases the chances for its association with superior genotypes which ultimately contribute to the persistence of the element.

Observations of the dissemination of Tn 5393 are similar to previous observations concerning the dissemination of antibiotic resistance transposons in clinical bacterial pathogens. Tn 5393 is found not only in phytopathogens, but is present in a wide variety of nontarget bacteria even from regions presumably never exposed to streptomycin through human usage (Sundin & Bender 1995). Also, current evidence suggests that Tn 5393 has been inserted into indigenous plasmids which are stable, adapted to their host and may encode other gene(s) which are beneficial to host fitness (Sundin & Bender 1994, McManus & Jones 1995).

The unwitting cooperatively of widely varied bacterial populations through the sharing of plasmid DNA adds to the complexity of the antibiotic resistance problem. Many characteristics of bacterial populations including large populations sizes, rapid generation times, and genome plasticity increase the chances for the selection of low-frequency events such as gene transfer and intracellular transposition. The results of ecological and genetic studies coupled with field observations suggest that the carriage of Tn 5393 is not ecologically detrimental to host organisms. The widespread dissemination of this transposon severely impacts the effectiveness of streptomycin in plant disease control.

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Evaluation of Three Bioassays for Detecting Resistance in Cocoa Mirid, *Helopeltis theivora*

Introduction: The mirid *Helopeltis theivora* Waterhouse is a key pest of cocoa in Malaysia. This mirid feeds predominantly on cocoa cherelles and pods and causes serious crop damage or loss during the cherelle stage (Muhamad & Way, 1995). Insecticide use continues to be the main method for commercial management of the mirid. However, the widespread use of insecticides against the mirid must be taken seriously as resistance problems may arise (Dzolkhifli et al., 1986; Liew et al, 1992). Therefore, experiments were conducted to evaluate different bioassay methods for detecting resistance in the cocoa mirid, *H. theivora*. These studies show that *H. theivora* resistance to the tested insecticides could be greater than what is indicated by bioassay results plus highlights the importance of verification of the bioassay method used.

MATERIALS and METHODS: Test Insects: Cocoa mirids were collected from three estates/ localities i.e. Serdang, Kelang and Sungai Tekam. The mirid was cultured with the technique described by Rita and Khoo (1983). Only the fourth and fifth instars of the F1 generation were used for bioassays.

Insecticides: Technical grades of gamma-HCH (99% a.i.), deltamethrin (99.5% a.i.) and cypermethrin (50 % and 90% a.i.) were used to prepare stock solutions in olive oil for time-response and residual bioassays. In the topical application bioassay, stock solutions were prepared in acetone.

Bioassay Treatments: The three following bioassay methods were evaluated: time-response on treated filter paper (FAO method - Busvine, 1980), insecticide residual on filter paper and topical application bioassays. Knockdown was recorded at certain time intervals for time-response bioassay; while mortality was recorded 24 hours following treatment for residual and topical application bioassays.

Controls were treated with the carrier solvent. Data was subjected to probit analysis (Finney, 1971) and the median knockdown time (KT50), median lethal concentration (LC50) and median lethal dose (LD50) was obtained for time response, residual and topical application bioassays, respectively.

RESULTS and DISCUSSION: Table 1 shows the susceptibilities of the Serdang, Kelang and Sungai Tekam populations of *H. theivora* to gamma HCH, cypermethrin and deltamethrin with 3 different bioassays. The median knockdown time (KT50), median lethal concentration (LC50) and median lethal dose (LD50) was obtained for time-response, residual and topical application bioassays, respectively.

Table 1. The susceptibilities of three cocoa mirid populations of the against gamma-HCH, Cypermethrin and Deltamethrin in three different bioassays.

Population	Time Response KT50 (min)			Residual on filter paper LC50 (%a.i.)			Topical Application LC50 (g/ml)		
	gamma-HCH	Cypermethrin	Deltamethrin	gamma-HCH	Cypermethrin	Deltamethrin	gamma-HCH	Cypermethrin	Deltamethrin
Serdang	94.2	584.1	142.2	0.28	0.53	0.33	26.7	2.52	2.58
Sg. Tekam	157.8	209.4	143.8	0.25	0.41	0.37	171.2	0.39	0.52
Kelang	97.4	209.4	200.5	0.36	0.22	0.22	34.5	0.36	0.67

The resistance factors (RF) were calculated by dividing each population response (expressed as KT50, LC50 or LD50) by the response of the most susceptible population. The resistance factor (RF) values of three cocoa mirid populations against gamma HCH, cypermethrin and deltamethrin are given in Table 2. When the Serdang population was assumed the population susceptible to gamma HCH, the Sungai Tekam population showed a RF value of 6.4 with the topical application bioassay, while the time-response and residual bioassays showed RF values of 1.7 and 1.9 respectively. For cypermethrin, assuming the Kelang population was the susceptible population, the Serdang population showed a RF value of 7.0 with the topical application bioassay while in the time-response and residual bioassays showed the RF value of 2.8 and 2.5, respectively.

Similar results were also obtained for deltamethrin (Table 2) when Sungai Tekam was assumed as the susceptible strain. The Serdang population gave a RF value of 5.0 with the topical application while the time-response and residual bioassays gave RF values of 1.0 and 1.5, respectively. These results show that the topical application bioassay is more sensitive for detecting resistance in the cocoa mirid to gamma-HCH, cypermethrin and deltamethrin compared to the time-response and residual filter paper bioassays.

Table 2. Resistance factor (RF)* values of three cocoa mirid populations against gamma-HCH, Cypermethrin and Deltamethrin in three different bioassays.

Population	Time Response			Residual on filter paper			Topical Application		
	gamma-HCH	Cypermethrin	Deltamethrin	gamma-HCH	Cypermethrin	Deltamethrin	gamma-HCH	Cypermethrin	Deltamethrin
Serdang	--	2.8	1.0	--	2.5	1.5	--	7.0	5.0
Kelang	1.0	--	1.4	1.3	--	1.7	1.3	--	1.3
Sg. Tekam	1.7	1.2	--	1.9	1.9	--	6.4	1.1	--

RF* = KT50, LC50 or LD50 of the least susceptible population

Although these results show that the topical application bioassay is more sensitive for detecting resistance in cocoa mirids, the time response bioassay (FAO method) is more rapid and simpler and uses fewer insects. Therefore, we recommend the time response bioassay and suggest that results be corrected by multiplying with a constant that may vary with the insecticide treatment.

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Probit Analysis of Correlated Data: Multiple Observations Over Time at One Pesticide Concentration

Often probit analysis is used to analyze data from bioassay experiments that assess pest resistance to pesticides (Finney 1964). Typically, entomologists expose insects to several insecticide concentrations, determine insect mortality at predetermined periods of time, and analyze the data generated with computer programs (Russell et al. 1977, Preisler & Robertson 1989). We developed a method for analyzing bioassay data when multiple observations are made on the same group of organisms at one insecticide concentration. Standard probit analysis techniques are not applicable to such serial time-mortality data because observations made on the same group of organisms at different times are correlated. However, serial time-mortality bioassays may be desirable when 1) test materials are limited, as might occur when few insects are available or when the experimental pesticide is available in limited quantities; or 2) when rapid mortality is important, as might occur with an insect that oviposites within a few days or in quarantine treatments.

A computer program written in Mathematica (reg.) language was developed to implement the new method. The program allows the option of the log-log, logit, or probit transformation of proportion insects killed, and allows the choice of a logarithmic transformation of time. All statistics required for complete reporting of probit-type analyses are provided by the program. Additional programs were written for testing equality of slopes and variances and for calculating relative potency of insecticides using information provided by our program, or any other probit program.

We have also written a program transforming probit-transformed (and logit- or complementary log-log-transformed) data back to the original units. The program calculates residuals and standardized residuals to aid in assessing goodness-of-fit of the regression line. Figure 1 shows probit-, logit-, and complementary log-log transformations of data describing the time required to kill a laboratory strain of the parasitoid, *Bracon hebetor* (Say) (Hymenoptera: Braconidae), with the LD99 of malathion determined for a field strain (Baker et al. 1995). The regression lines were fit, with and without, a logarithmic transformation of time. Only log-probit and log-logit transformations appeared to fit the data well. Backtransformation of the observed and predicted data indicate a much better fit of the regression line to the observed data when plotted in the original units (Figure 2). Thus, transformation may exaggerate the magnitude of residuals (differences between observed and predicted points) in the original units of measurement. Examination of residuals in the original units (Figure 3) also indicates that the log-probit and log-logit transformation result in a better fit compared to best fit the observed data. Standardized residuals indicate that the log-probit transformation results in residuals that are all within +/- 2 standard deviations of zero (Figure 4). Standardized residuals laying more than +/- 2 standard deviations of zero indicate possible lack of fit (Preisler 1988). Thus, the program allows the user to obtain data which may be plotted using a graphics program, to examine the fit of the regression line in the original units, and to examine residuals in the original units.

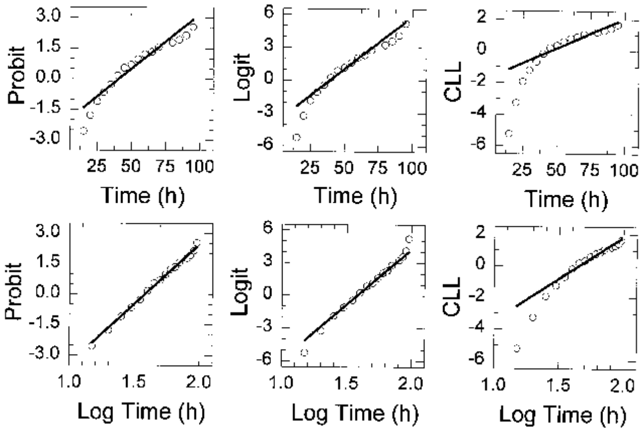

Figure 1. Observed (open circles) and predicted (line) transformations of proportion of the Savannah strain of Bracon hebetor killed over time by the LD99 of malathion.

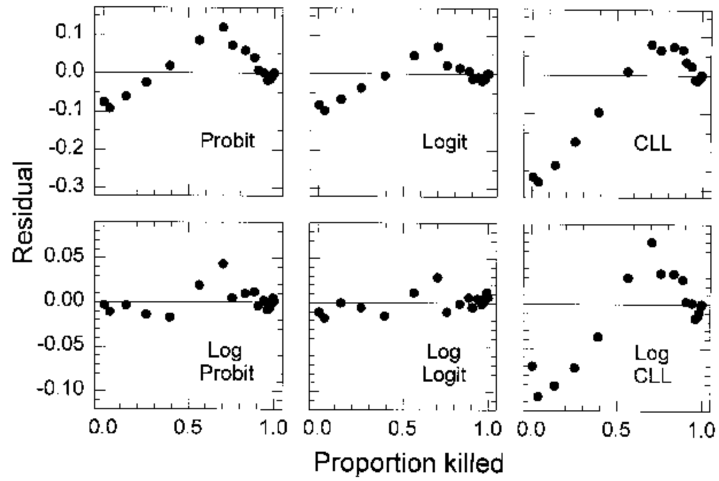

Figure 3. Residual (observed minus predicted) proportion of the Savannah strain of Bracon hebetor killed by the LD99 of malathion versus the observed proportion killed.

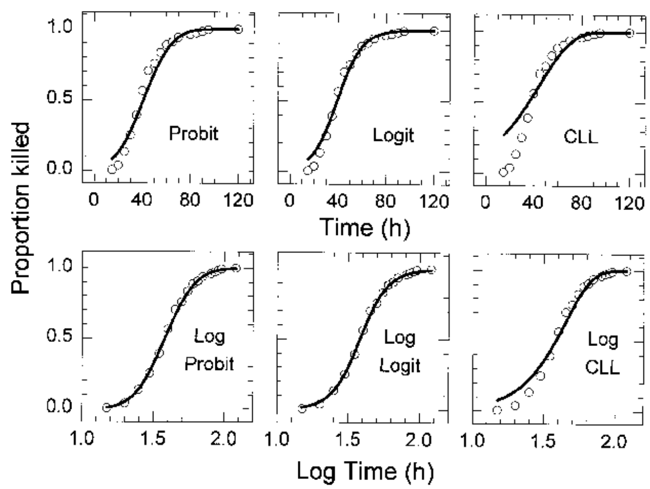

Figure 2. Observed (open circles) and predicted (line) proportion of the Savannah strain of Bracon hebetor killed over time by the LD99 of malathion.

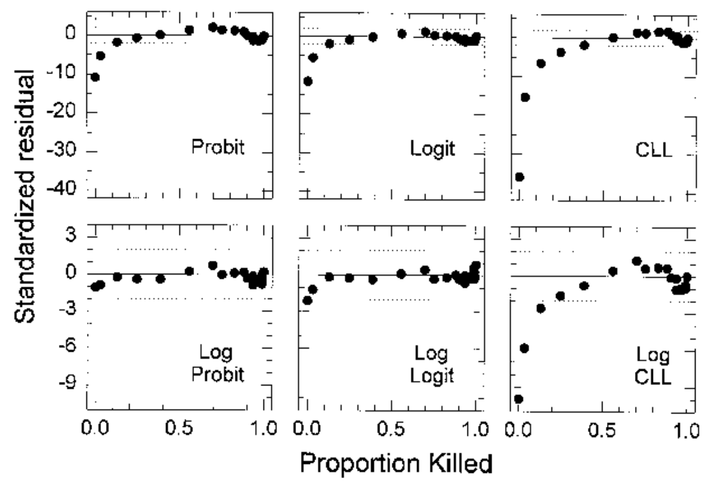

Figure 4. Standardized residual proportion of the Savannah strain of Bracon hebetor killed by the LD99 of malathion versus the observed proportion killed.

The computer programs may be obtained from the primary author. The probit program will allow easy analysis of serial time-mortality data, while the other programs may be used to aid in any probit-type analysis.

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The Effect of Pesticide Mixtures Versus Single Applications on Resistance in the Cotton Bollworm

In the major producing areas of China, farmers have adopted pesticide mixtures as the main means to control resistant cotton bollworm , *Helicoverpa armigera* (Hubner). In the laboratory, we selected colonies of resistant bollworm with three insecticides separately or in mixtures for 20 generations. We then compared the rate of resistance development between those colonies.

MATERIALS and METHODS: Bollworms demonstrating high levels of resistance were collected from Liaocheng cotton fields, Shandong Province. Between 1992-1993, *Helicoverpa* were reared on a synthetic diet that yielded pupal weights comparable to pupa collected in the field. C

olonies were exposed to one of two insecticide groups: Cyhalothrin, Phoxirn and Parathion-methyl or Cyfluthrin, Endosulfan and Quinalphos. Colonies were exposed to either each insecticide alone; mixtures of two insecticides, or all three insecticides. Each selection treatment was replicated three times.

We measured the LD50 values after every five generations. We selected larvae in each generation by treating them with the last known LD50 rate. We also determined efficacy of each insecticide/ mixture on 100 fourth and fifth instars.

RESULTS:
Insecticide Group #1 - Cyhalothrin, Phoxrin and Parathion-methyl: In Table 1 we see that resistance increased very slowly when *Helicoverpa* was exposed to a mixture of all three insecticides. In contrast, resistance increased rapidly when *Helicoverpa* was exposed to selection with only one insecticide. Meanwhile, intermediate increases in resistance occurred in each colony exposed to a two-insecticide mixture.

Table 1. Development of resistance in Helicoverpa in response to selection with seven insecticide regimes mixtures vs. single applications) for 20 generations.

Insecticide treatment[1]	Baseline LD_{50} (ug/g)	5th genertation		10th generation		15th generation		20th generation	
		LD_{50}	Ratio[2]	LD_{50}	Ratio[2]	LD_{50}	Ratio[2]	LD_{50}	Ratio[2]
Cyh., P., Pm.	0.1539	0.1864	1.2	0.2462	1.5	0.7233	4.7	0.8618	5.6
Cyh., P.	0.0458	0.0687	1.5	0.1053	2.3	0.3664	8	0.4625	10.1
P., Pm	0.2049	0.3688	1.8	0.6351	3.1	1.8031	8.8	3.012	14.7
Cyh. Pm.	0.257	0.4112	1.6	0.6939	2.7	2.1588	8.4	2.9041	11.3
Cyh.	0.0387	0.236	6.1	0.4721	12.2	0.7778	20.1	1.0255	26.5
P.	0.1504	1.2633	8.4	2.2259	14.8	3.6998	24.6	4.6473	30.9
Pm.	0.9217	7.0971	7.7	12.816	13.9	20.442	22.2	26.457	28.1

[1] Cyh. = Cyhalothrin, P. = Phoxim, Pm. = Parathion-methyl

[2] Ratio = LD50/LD50 baseline

Table 2 shows that control efficacy of the three insecticide mixture changed slightly after 20 generations of selection. In contrast, after 20 generations of selection with single insecticides, the control efficacies for those insecticides dropped to only 6-15%. Meanwhile selection with two-insecticide mixtures lead to a reduction of control efficacy of between 41-65%.

Table 2. Loss in control efficacy of *Helicoverpa* selected with the seven insecticide regime (mixtures vs. single applications) for 20 generations.

Insecticide treatment[1]	Baseline control	Efficacy %			
		5th genertation	10th generation	15th generation	20th generation
Cyh., P., Pm.	96	96	96	92	89
Cyh., P.	82	80	77	72	65
P., Pm	56	56	54	50	41
Cyh. Pm.	74	71	62	53	47
Cyh.	21	20	18	10	6
P.	34	29	25	19	10
Pm.	37	32	26	20	15

[1] Cyh. = Cyhalothrin, P. = Phoxim, Pm. = Parathion-methyl

Insecticide Group #2 - Cyfluthrin, Endosulfan and Quinalphos: From Table 3 and 4, we see the same general trends as detected for the first insecticide group. However, resistance development was slower and loss in control efficacy was less than in group #1 insecticides. In part, this can be explained by the fact that these pesticides are new products, some with new modes of action. Despite the novelity of these insecticides, *Helicoverpa* resistance to 20 generations of selection by a single insecticide led to resistance ratio of 15 to 23 and a drop of efficacy to between 14 and 60%.

Table 3. Development of resistance in Helicoverpa in response to selection with seven insecticide regimes (mixtures vs. single applications) for 20 generations.

Insecticide treatment[1]	Baseline LD_{50} (ug/g)	5th genertation		10th generation		15th generation		20th generation	
		LD_{50}	Ratio[2]	LD_{50}	Ratio[2]	LD_{50}	Ratio[2]	LD_{50}	Ratio[2]
Cyf., E., Q.	0.0632	0.0695	1.1	0.0821	1.3	0.1137	1.8	0.139	2.2
Cyf., E.	0.046	0.069	1.5	0.0966	2.1	0.2484	5.4	0.3358	7.3
E., Q.	0.1179	0.1886	1.6	0.3165	2.6	0.6838	5.8	0.896	7.6
Cyf., Q.	0.0384	0.0652	1.7	0.0921	2.4	0.192	5.0	0.2726	7.1
Cyf.	0.0401	0.2646	6.6	0.4211	10.5	0.6416	16.0	0.9022	22.5
E., Q.	0.216	1.1232	5.2	1.7928	8.3	2.3976	11.0	3.1536	14.6
Q.	0.1568	0.8624	5.5	1.1446	7.3	1.615	10.3	2.5715	16.4

[1] Cyf. = Cyfluthin, E. = Endosulfan, Q. = Quinalphos

[2] Ratio = LD50/LD50 baseline

Table 4. Loss in control efficacy of Helicoverpa selected with the seven insecticide regime (mixtures vs. single applications) for 20 generations.

Insecticide treatment[1]	Baseline control	Efficacy %			
		5th genertation	10th generation	15th generation	20th generation
Cyh., P., Pm.	98	98	97	95	95
Cyh., P.	92	90	86	81	74
P., Pm	90	88	86	82	77
Cyh. Pm.	92	89	86	83	80
Cyh.	42	35	30	27	14
P.	78	73	68	63	58
Pm.	83	79	74	68	60

[1] Cyf. = Cyfluthin, E. = Endosulfan, Q. = Quinalphos

CONCLUSIONS: In both insecticide treatment groups, resistance developed slower (control efficacy maintained longer) in a three-insecticide mixture compared to a two-insecticide mixture. Resistance developed most rapidly when *Helicoverpa* was selected with a single insecticide. These results support the decision of farmers to switch to insecticide mixtures as the main method to control resistant cotton bollworm populations.

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Controlling Weeds Resistant to 2,4-D in Russian Cereal Crops

Long-term application of 2,4-D herbicides in cereals throughout different regions of Russia has resulted in development of many resistant weeds species, particularly annual dicotyledous species such as *Polygonum* spp., *Matricaria perforata* Merat., *Stellaria media* (L.) Vill., and *Fumaria officinalis* L. These weed species have gained greater importance when using such cultivation practices such as ploughing without turning over the soil surface. This has increased infestation of these plants in cereals. Combinations of 2,4-D, MCPA with dicamba (Dialen, Diamet D), or the herbicides based on phenoxy-propionic acids (Duplasan KV) and dichlorprop (Duplasan DP) were used for controlling 2,4-D resistant species. The application of Basagran and its analogues was also effective and used in mixed plantings of cereals and legumes. Recently, annual grass weeds such as *Setaria glauca* (L.) Beauv., *S. viridis* (L.) Beauv., *Echinochloa crusgalli* (L.) Beauv., and *Avena fatua* L. have become a control problem in spring wheat and barley. Also, control of *Apera spicaventi* (L.) Beauv. and *Poa annua* L. have become a recent problem in winter wheat. Illoxan (prodifox) and Suffix BY are recommended to control annual grass weeds in spring wheat and barely. Topic is also recommended against these weeds in wheat. Puma Super is applied in spring wheat as well as in winter wheat. Today, herbicides that control wide-spectrum weeds (both annual weed grasses and dicotyledon weeds) have become popular. The herbicide, Assert, is one that is recommended for application in winter and spring wheat. We consider it to be a great bonus that herbicides such as Igran, Arelon (75% w.p., 50% c.s.), Tolkan, Dicuran and Ducuran Forte can be used in autumn on winter wheat as pre-emergence treatment or during the early growth stages and also used in spring on vegetable crops. These herbicides control annual dicotyledons, weed grasses and 2,4-D resistance weeds. A new group of herbicides, the sulphonil-ureas also control 2,4-D resistant weeds and thus show promise. Other promising herbicides such as Granstar, Harmony, Fenfis, Difesan, Kovbuy, Tresor (60% w.p., 60% w.d.g.) show no adverse effect on the following rotational crops and also effectively control *Sonchus arvensis* L. and *Cirsium arvense* (L.) Scop.

Tables 1 and 2.

Table 1. Decrease in weediness of spring barley sprayed with Satis. (18% WP) in 1993.

Herbicide	Rate (g/ha a.i.)	% contol or decrease in weediness[1]					
		Ural Exp. Stn.			Ukrainian Exp. Stn.		
		Galium aparine	*Galeopsis speciosa*	*Polygonum convolvulus*	*Polygonum convolvulus*	*Polygonum lapatitifiol*	*Stellari a media*
Satis	18	47	100	95	81	70	71
Satis	27	61	100	100	92	56	71
2,4-D	800	-14[2]	-60[2]	92	56	30	0
Control[1]	--	7.2	2	10	8	9	4

[1] Number of weeds per 1m^2

[2] A negative value indicates an increase in weediness

Table 2. Decrease in weediness in winter wheat sprayed with Difesan, (50% WS) and Granstar, (75% DF) in Rostov region, Russia in 1993.

Herbicide	Rate (g/ha a.i.)	% contol or decrease in weediness[1]				
		Polygonum convolvulus	*Galium aparine*	*Latuca tatarica*	*Chenopodium album*	*Crucifera*
Difesan	75	98	100	100	98	100
Granstar	22.5	75	20	63	75	100
Control*	--	30.0	1.0	6.8	18.3	12.0

*Number of weeds per 1m^2

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Monitoring Insecticide Resistance in Greenhouse Whitefly Adults in Beijing, China, 1991 to 1995

The greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) became an economical important insect pest of greenhouse vegetable and ornamental crops in the middle 1970's in Beijing, China (Zhu et al. 1981). The synthetic pyrethroids were the most effective insecticides for greenhouse whitefly control, when they were first introduced in the end of the 1970's (Zhang et al. 1981; Zou & Zheng 1988). But after several years of application, whitefly control with both fenvalerate and deltamethrin became very difficult in the greenhouse and in the field. Resistance levels for fenvalerate and deltamethrin reached 405.6 and 1,941.7-fold respectively, based on the dipping bioassay recommended by FAO in 1988 (Zheng & Rui 1992). In the late 1980's, beprofezin was to be used for whitefly control instead of pyrethroids (Zhu et al. 1992). From 1991 to 1995, we monitored whitefly resistance with an adult spray bioassay (Li & Zheng 1993; Zheng & Gao 1994). The results (Table 1) clearly showed that the resistant levels towards the two pyrethroids declined in a stepwise manner, whereas resistance towards dimethoate and beprofezin began to ascend.

Table 1. Change in toxicity towards different insecticides for greenhouse whitefly adults between 1991 to 1995.

Insecticide	Year	Slope +/- Se	LC50 (95% CL)(u/g)	TR[a]	Ratio[b]
Deltamethrin	1991	2.76 +/-0.25	185.2 (158.30-221.06)	1	28.4
	1992	3.15 +/-0.46	185.5 (164.25-209.55)	1	28,45
	1993	3.75 +/-0.31	98.54 (85.20-109.02)	0.5	15.11
	1994	1.82 +/-0.32	95.85 (88.63-103.66)	0.5	14.7
	1995	2.77 +/- 0.22	94.85 (40.30-142.78)	0.5	14.55
Fenvalerate	1991	1.85 +/-0.3	2677.95 (2308.43-3106.65)	1	68.95
	1992	1.73 +/- 0.38	1154.06 (953.01-1802.86)	0.4	29.71
	1993	2.75 +/-0.42	782.41 (702.55-871.75)	0.3	20.14
	1994	1.58 +/- 0.25	771.35 (663.74-897.36)	0.3	19.86
	1995	1.94 +/-0.38	534.09 (467.88-611.04)	0.2	13.75
Dimethoate	1991	1.67 +/-0.24	1748.64 (1454.45-2102.33)	1	1.32
	1992	1.44 +/-0.22	1649.55 (1315.41-2068.93)	0.9	1.24
	1993	1.90 +/-0.18	1461.08 (1229.51-5736.73)	0.8	1.1
	1994	1.69 +/-0.21	1870.09 (1552.02-2253.72)	1.1	1.41
	1995	1.67 +/-0.41	967.05 (869.76-1074.98)	3.2	4.23
Beprofezin	1991	1.89 +/-0.31	967.05 (869.76-1074.98)	1	--
	1992	2.33 +/- 0.5	947.44 (831.99-1103.42)	1	--
	1993	1.55 +/- 0.21	1666.59 (1357.60-2020.59)	1.7	--
	1994	2.32 +/- 0.33	1316.92 (1097.99-1579.79)	1.4	--
	1995	1.44 +/- 0.11	3900.92 (3321.01-4580.90)	4	--
Omethoate	1995	2.43 +/- 0.27	255.00 (226.00-285.00)	--	1.17
Malathion	1995	2.20 +/-0.22	35640 (22427-66772)	--	85.11

[a] TR = 1992-1995 LC50/ 1991 LC50

[b] Ratio = 1991-1995 LC50/ 1983 LC50 (Zou & Zheng 1988)

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Resistance Management of *Plutella xylostella* L. on Crucifers in Southeast Asia: Aspects of Implementation

INTRODUCTION: Cabbage is one of the most popular vegetables in Southeast Asia. It is grown widely and continuously throughout the year. One of the most serious pests of cruciferous crops is the diamondback moth (DBM), *Plutella xylostella* L. It can destroy an entire crop even when intensive, but improper, chemical control measures are used. This is due to its notorious resistance to a wide range of insecticides (Syed et al. 1989, Syed 1992). Since the 1970's, newly introduced products have remained effective against DBM for only 2-3 years. Even some *Bacillus thuringiensis* based products (Bt .) had suffered from the resistance development by DBM (Tabashnik et al. 1990, Hama et al. 1992).

Resistance is caused by misuse of products. Farmers in Southeast Asia tend to spray their cabbage crops from nursery to harvest intensively (3 to 5-day intervals). Previously, growers solved the DBM resistance problem by switching to new and more effective products. This is no longer easy or feasible. New insecticides are rare and expensive as result of the stringent selection criteria providing safety for users, consumers, beneficial arthropods and the environment.

Pest resistance imposes burden on the chemical industry (short life span of effective products that are expensive to develop), farmers (risk of crop failure and higher production inputs, e.g. more insecticides), consumers (risk of high residues in food), and the environment (effect on natural enemies, etc.). Therefore, insecticide resistance is undesirable and unacceptable to all concerned.

Aspects of Insecticide Resistance Management: Since 1990, new products have been introduced in Southeast Asia such as abamectin, diafenthiuron, fipronil, tebufenozide and chlorfenapyr. These products, introduced in close succession, have modes of action quite distinct from each other. They all have good activity on DBM (at least for the time being) and appear not to suffer from cross-resistance to organophosphates, carbamates, pyrethroids, or benzoylureas. This gives a good opportunity to avoid or delay the resistance development provided farmers do not continue to use only one highly efficacious product to control the DBM season after season.

A sound resistance management strategy is essential to preserve the efficacy of an insecticide. Each insecticide costs large sums of money and many years to develop for the benefit of the users, manufacturers, consumers and the environment.

Insecticide resistance management (IRM) has no general recipe. Each resistance case needs its own local solution. IRM tactics must be based on local knowledge on the pest susceptibility status and the insecticide usage habit of farmers.

Therefore, susceptibility/resistance monitoring is the basis for devising a practical IRM strategy.

Monitoring in Thailand: Our yearly monitoring in Thailand has shown that there are great differences in levels of susceptibility of different DBM populations to benzoylureas, diafenthiuron and Bt (Figures 1, 2, 3, and 4). These differences are related to the intensity of insecticide use by the growers. Ban Bua Thong and Kachanaburi are areas of intensive crucifer production that serve the high demands in Bangkok. Insecticides, usually the latest types, are used heavily. Resistance to abamectin may already have occurred (Figure 5). Pitsanulok is another area where the intensity of crucifer cultivation has increased in recent years. Abamectin and lufenuron showed high LC50 values in these three areas relative to others. Lufenuron has never been sold or used by farmers on cabbages. Most likely, the high LC50 values in these areas were caused by cross-resistance from other benzoylureas such as chlorfluazuron and teflubenzuron used extensively since the late 1980's. Figure 6 illustrates the trend of LC50 values for teflubenzuron in three selected areas. LC50 values for Ban Bua Thong and Kanchanaburi showed continuous rise since the monitoring started in 1990, whereas that of Takhli remained stable. DBM in Takhli and Chiang Mai are normally the most susceptible to the late generation insecticides: cabbages are not grown intensively in these areas and many newly introduced products are not available in the pesticide shops. However, the LC50 values for diafenthiuron (Figure 3) do not follow pesticide or cropping history. Since the compound has a mode of action different from all other products in use, cross-resistance is not expected. Of the three Bt products tested, Florbac showed the weakest activity, i.e. highest LC50 values in all the populations (Figure 2) and may indicate resistance as reported elsewhere (Tabashnik 1994). AGREE and Centari showed higher activity (lower LC50 values) than Florbac. Nevertheless, the LC50 was highest for AGREE in Ban Bua Thong and for Centari in Kanchanaburi.

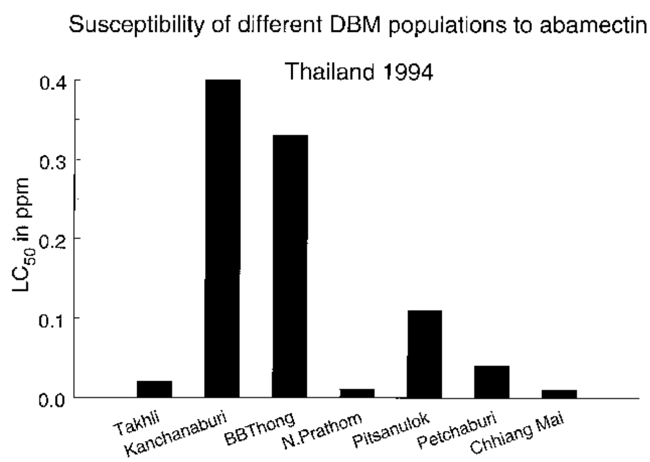


Figure 1.

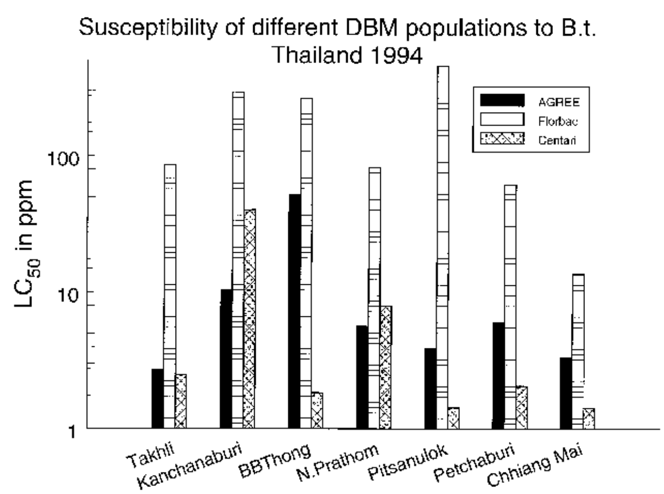


Figure 2.

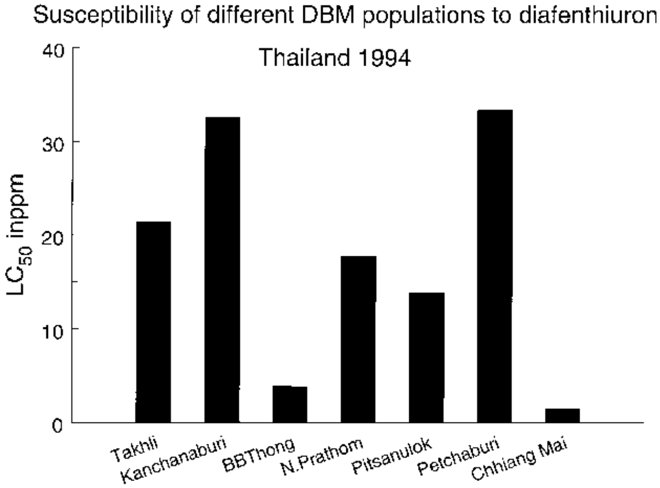
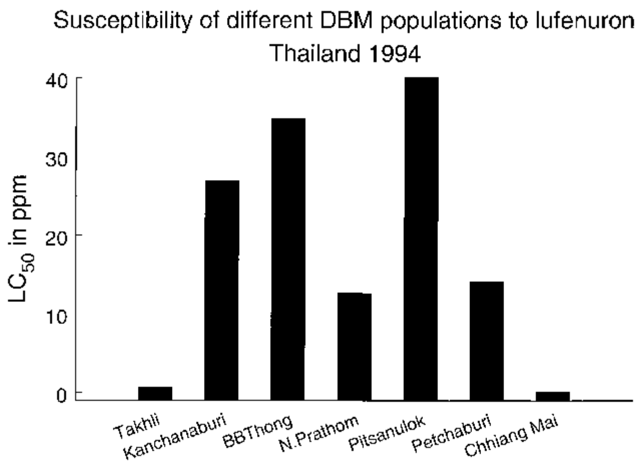


Figure 3.



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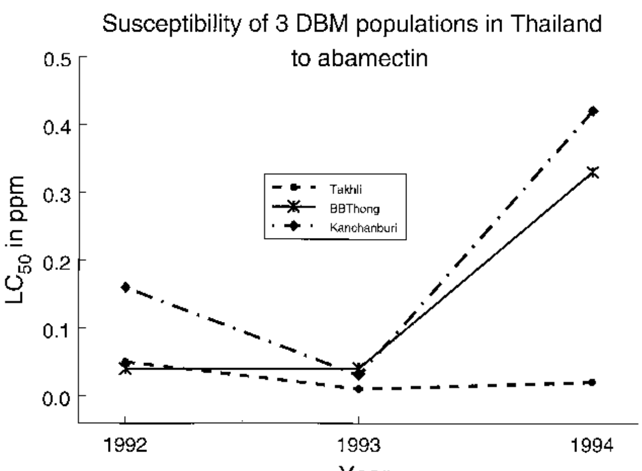


Figure 5.

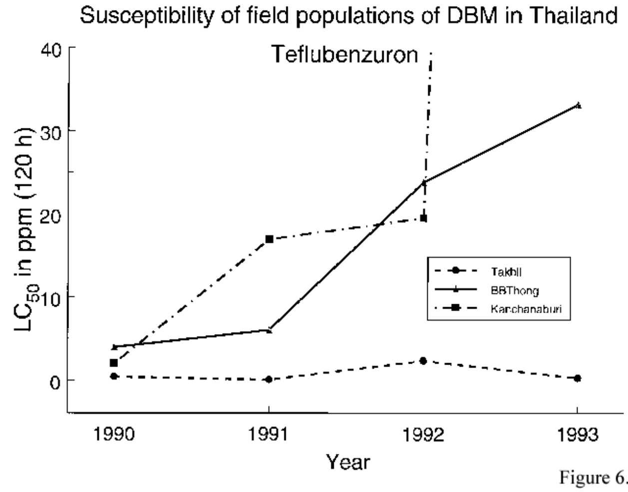


Figure 6.

Monitoring in Indonesia: The monitoring data on two benzoylurea products from Indonesia gave cause for great concern (Figures 7 and 8). DBM in Indonesia have been known to be susceptible to all benzoylureas. However in 1993, the LC50 for teflubenzuron in Pengalengan increased by more than 1,000-fold over the previous two years. The high LC50 was again confirmed in 1994. Also, Teflubenzuron LC50 for Lembang population remained low during the previous 3 years of monitoring but, showed a sudden increase (also ca. 1,000-fold) in 1994. Chlorfluazuron was introduced for use in vegetables in Indonesia in 1987 followed by teflubenzuron and flufenoxuron in subsequent years. These benzoylureas were used widely in Pangalengan. The sudden jump in teflubenzuron LC50 in 1993, that remained in 1994, seems to indicate a serious resistance problem. More data in 1995 are needed before any conclusion can be drawn.

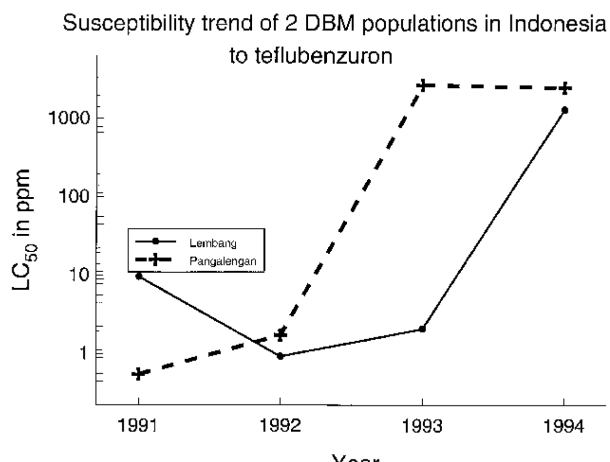


Figure 7.

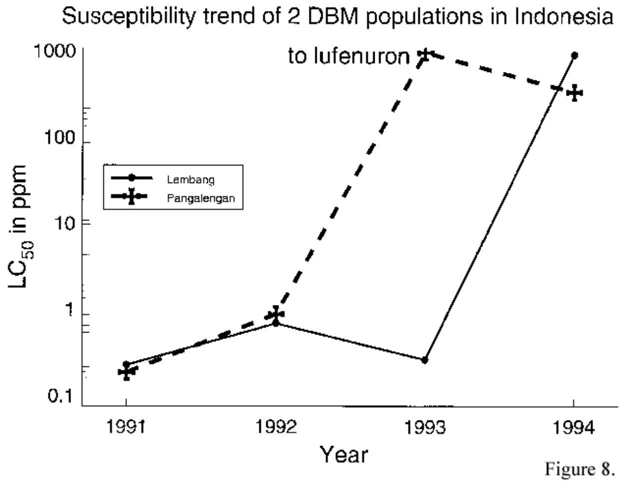
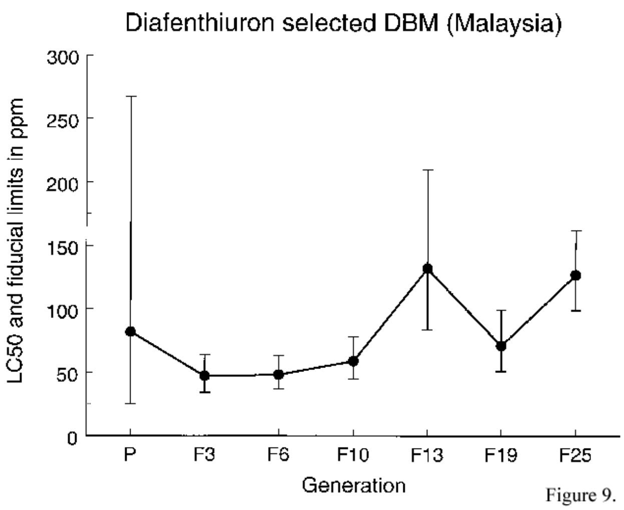


Figure 8.

Case Study: Our selection pressure studies with diafenthiuron were carried out in field-cages in Malaysia and in Thailand. In both cases, there was no observable resistance development by the tested DBM populations to diafenthiuron after 25 generations in Malaysia and 55 generations in Thailand (Figures 9 and 10).



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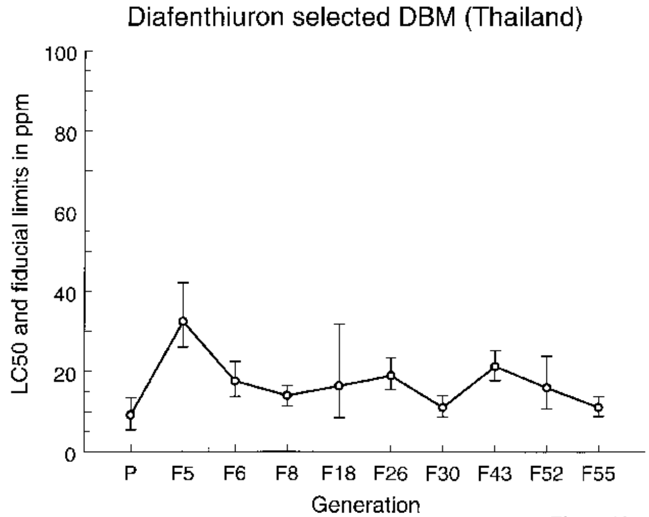

Figure 10.

Despite the lack of evidence of resistance development in this study, the ability of the DBM to develop resistance against diafenthiuron cannot be excluded. A sound anti-resistance strategy should unambiguously be recommended to farmers, i.e. always use diafenthiuron in alternation with any of the new products mentioned above. By the same token, each new effective products should never be used solely and continuously throughout the season year after year. They should be alternated among themselves. An effective Bt product should be included in the alternation regime.

IRM Implementation: The active participation of pesticide dealers and farmers is the key to success in the management of DBM resistance in Southeast Asia. Practical implementation of insecticide resistance management strategy (IRM) will involve closer cooperation between pesticide producers, dealers, users, advisers and regulators.

Farmers as well as pesticide dealers must learn to appreciate the value of judicious use of insecticides (the essence of IPM) and to carefully follow the label recommendations. Manufacturers should cooperate and coordinate to ensure that label recommendations are compatible with the IRM concept in order to avoid or delay the risk of resistance development by the DBM.

IPM minimizes the unnecessary or excessive use of insecticides, thereby reducing the selection pressure on the pest. IRM is thus a subset of IPM. IRM should be incorporated in all IPM concepts and policies. For the cabbage crop, IRM is inseparable from IPM and should be at the forefront of all IPM recommendations.

Successful implementation of IRM can occur in part through joint multilateral efforts between governments, industry, international agencies and consumer organizations to educate the farmers and pesticide dealers on the benefits of IRM. Regular training seminars for farmers and pesticide dealers together may be a shortcut to implementing an IRM tactic. Farmer participation in practical field trials, similar to that run by the Taiwan Agricultural Research Institute (TARI) (Cheng, E.Y., personal communication), is another method which could be tried throughout Southeast Asia. In the TARI approach, farmers are given a complete package of insecticides necessary to protect the cabbage crop from all pests. If mixtures are required, they are advised to mix a combination of two products (examples of specific combination clearly described) that are from different chemical groups. Those who break the rule will be excluded from the program the following season. Discrete and periodic checks of chemical residues on the crops are needed in order to enforce the rule. The publicity of the success that farmers experienced (e.g. better yield and quality for less chemical input) will be carried by word of mouth among farmer communities.

Short term demonstration trials will not have an impact on IRM since resistance only becomes evident after a long 'incubation period' covering many pest generations.

The agrochemical chemical industry through the Field Crops and Vegetables Working Group of the Insecticide Resistance Action Committee (IRAC) has worked out a preliminary practical IRM strategy for DBM. IRAC is now seeking support and cooperation to put the recommendation to test.

The active participation of pesticide dealers and farmers is the key to success in the management of DBM resistance in Southeast Asia.

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Potential "Probit-Type" Analyses for Rapid Assessment of Insecticide Resistance

Field collection of adequate numbers of individuals for bioassay is one of the major obstacles for consistent monitoring of insecticide resistance. This is particularly true for beneficial insects because of their size, relative scarcity, and cryptic habits. We have been working with insecticide resistant stored-product parasitoids for several years and have recently developed a method for a statistically-valid probit analysis of data collected as repeated counts in the same replicate container over time at a single dose. This has the potential to greatly reduce the number of individuals required to screen a recently collected population.

However, such an approach differs from those conventionally used because it compares the rate of kill from various populations at selected dose, rather than viewing mortality across doses at a specific time. Although the interaction between time and dose is well known for toxicologists, little specific information is available about how well time to kill can characterize resistance.

To test this idea, we used our data for a strain of the parasitoid *Bracon hebetor* that showed low-level resistance (resistance ratio 7.6 at the LC50, 9.7 at the LC99) to malathion (Baker et al. 1995). The data were fit to a probit model for correlated data (Throne et al. 1995a) and the output data were backtransformed to assess model fit at various lethal times (Throne et al. 1995b). Test durations for bioassays were determined for both populations at several dose multiples (from 1X

- 10X) of the LC99 for the reference susceptible strain and ranged from 35 minutes (7 counts at 5 minute intervals) for the susceptible strain at 10X their LC99 (Fig. 1) to 360 minutes (12 counts at 30 minute intervals) for the resistant population at 1X the LC99 for the susceptible population (Fig. 2). Bioassay details and mortality plots over time are reported in Baker et al. (1995). The resistance ratios calculated at the LT50 (Fig. 3) and at the LT99 (Fig. 4) both show consistent low level resistance that was approximately 3-fold regardless of the dose used for bioassay. While this differs from the published resistance ratio determined using dose response data, it does give a significant indication of resistance that could then be followed with a full dose-response assay, if required.

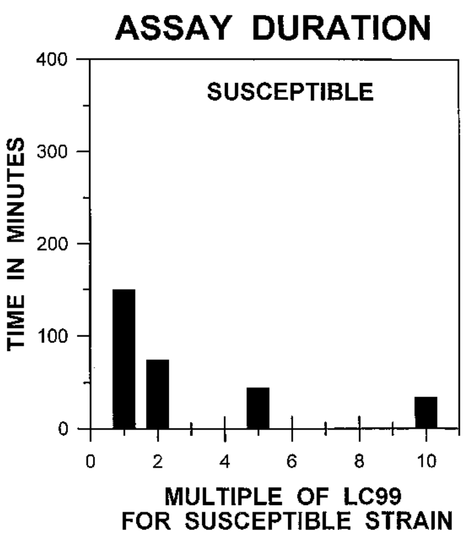

Figure 1. Bioassay duration for the susceptible strain at several concentrations.

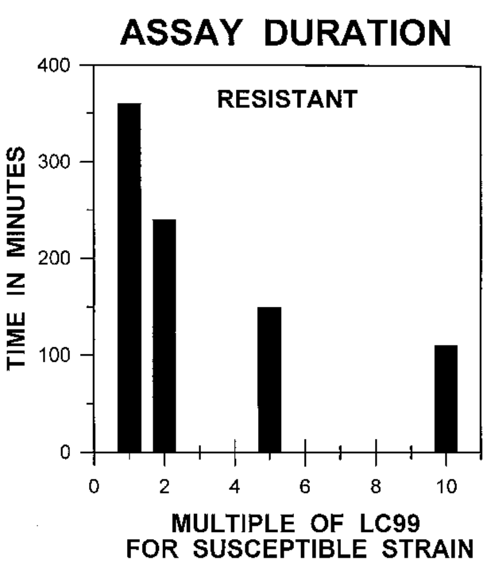

Figure 2. Bioassay duration for the resistant strain at several concentrations.

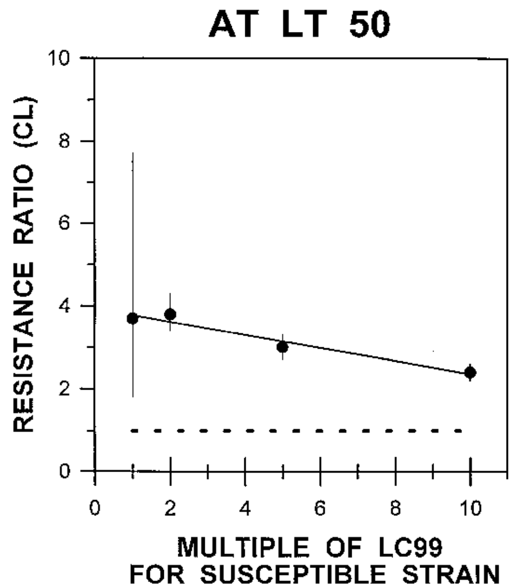

Figure 3. Resistance ratio for a malathion resistant strain of Bracon hebetor at the LT50.

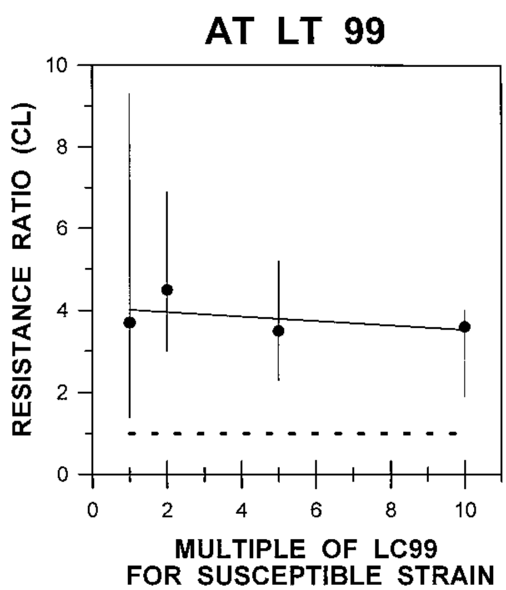

Figure 4. Resistance ratio for a malathion resistanct strain of Bracon hebetor at the LT99.

To apply this approach to a hypothetical screening situation, we might choose to use our 5X the LC99 dose, which would allow us to run the susceptible reference population for 50 minutes at 5 minute intervals, and set our bioassay duration for the test population to be that for our known population with low-level resistance, i.e . , the 150 minutes at 15 minute intervals shown in Fig. 2. If all of the members of the test population die within an hour, then the population is unlikely to be resistant. If only a few die within the 150 minute interval then the test population is likely to be more resistant than the low-level resistance population that the bioassay was based on. If the majority of the individuals tested have died by the end of the bioassay, then they are likely to be similar in resistance to the reference population with low-level resistance.

If the purpose of resistance monitoring is to measure changes in a population's sensitivity to a particular insecticide, such an approach may be very useful. However if a change is indicated and more precise information regarding the resistance level is needed, a dose-response assay should be run to provide more precise information on the actual resistance level in the units that are currently used. It still would be very convenient to use such an approach for routine screening since one could use a small number of individuals (for example, 5 replicates of 10 insects) and have the results in less than three hours. Due to the sensitivity of the assay, a concomitant bioassay with a reference susceptible population is necessary. This concomitant bioassay serves as a control for procedural errors or reference material inconsistencies that is frequently lacking in other screening studies.

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Constraints on the Evolution of Glyphosate Resistance in Weeds

Glyphosate is a post-emergence, nonselective herbicide used in weed control programs around the world since its commercialisation in 1974. Despite its widespread and long-term use, weeds have not evolved resistance to glyphosate. An examination of the literature on glyphosate-tolerant crops, the mechanism of action and glyphosate use, suggests that the lack of glyphosate resistant weeds may be attributed to two factors. First, genetic and biochemical constraints on the evolution of a mechanism of resistance appear to exist in higher plants. Second, the use pattern for glyphosate in agriculture may preclude the evolution of resistance in weed populations.

Three mechanisms of glyphosate resistance are generally assumed to be possible in plants: (1) overproduction of 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) glyphosate's site of action, (2) alteration of EPSPS, and (3) metabolic degradation of glyphosate (Dyer, 1994). Constraints associated with their evolution in weed populations may be preventing the occurrence of glyphosate resistance.

EPSPS overproduction confers too low of a level of resistance for plants to survive field rates of glyphosate (Kishore & Shah 1988, Shah et al. 1986). EPSPS alterations that confer resistance to glyphosate in bacteria are alterations in the active site of the enzyme (Padgette et al. 1991). In bacteria that produce EPSPS with a high degree of homology and identity to plant EPSPS, the alterations interfere with binding of phosphoenolpyruvate, the enzyme's normal substrate, and reduce EPSPS's catalytic efficiency (Kishore & Shah 1988, Padgette et al. 1991, Padgette et al. 1994). As a result, untreated transgenic plants with the glyphosate-resistant EPSPS exhibit significant reductions in fitness relative to plants with glyphosate-susceptible EPSPS (Comai et al. 1985, Fillati et al. 1987, Kishore & Shah 1988). Similarly, marked fitness reductions associated with an altered EPSPS may prevent the transmission of glyphosate resistance to succeeding generations in weed populations. Finally, metabolic degradation of glyphosate is improbable as a mechanism of resistance. Definitive evidence of its occurrence in higher plants, even at low levels, has not been demonstrated (Dyer 1994). Moreover, enzymes that degrade glyphosate, although found in numerous species of bacteria, have not been shown to occur naturally in plants.

In addition to constraints on the evolution of a resistance mechanism, two features of the use pattern for glyphosate in agriculture impede resistance evolution in weed populations. First, glyphosate has been and will continue to be used primarily for the control of perennial weeds. In general, the evolution of adaptation takes much longer in perennial than annual plants due to the lower reproductive effort (seed production) and seedling recruitment per growing season, as well as the increased generation time of perennials. As a result, the probability of evolution of resistance in perennial weeds is likely to be low.

Second, the use of glyphosate for annual weed control is almost always associated with the application of a second herbicide class that targets the major annual weed(s) in a field. The second herbicide is applied either as a tank mixture with glyphosate or as an in-crop treatment during the same growing season. Because the most abundant annual weeds in a field are the weeds most likely to evolve resistance (Jasieniuk et al. 1995; Morrison & Devine 1994), this practice of applying a second herbicide class, in addition to glyphosate, on the same weed population, reduces the likelihood of glyphosate resistance evolution (Jasieniuk & Maxwell 1994).

In summary, genetic and biochemical constraints associated with potential mechanisms of resistance, as well as the use pattern for glyphosate in agriculture, preclude the evolution of glyphosate resistance in weed populations. Although one can not state with certainty that resistance to glyphosate will never occur in weeds, it appears to be considerably less likely than resistance to many other herbicide classes.

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Comparative Sequence Analysis of Sodium Channel Genes from Agriculturally Important Lepidoptera

Pyrethroids are important insecticides for the control of many insect pests including the tobacco budworm (*Heliothis virescens*). Pyrethroid insecticides have been shown to act on the voltage-dependent sodium channel within the nervous system (Soderland & Bloomquist 1989). A change in the affinity for pyrethroid at the binding site on the sodium channel is presently the best supported mechanism for resistance (Pauron et al. 1989, Church & Knowles 1993). The precise region of the sodium channel to which pyrethroid insecticides bind to exert their neuotoxic effect is not known. To investigate regions of the sodium channel that may contain a mutation associated with resistance, we will perform a sequence comparison of the sodium channel gene isolated from pyrethroid resistant and susceptible strains of *H. virescens*. Reverse transcriptase polymerase chain reaction (RT-PCR) was used to amplify a 850 bp sodium channel gene fragment from a pyrethroid susceptible (sur) strain of *H. virescens*. This region contained the intracellular segment between repeat domains III and IV of the sodium channel (III-IV). The amplified fragment is 98% homologous to a region of the '*heliothis sodium channel para homolog*' (

hscp) isolated from the pyrethroid resistant PEG-87 strain of *H. virescens* (Taylor et al. 1993). This same region is also being sequenced from a pyrethroid resistant NII strain of *H. virescens* for sequence comparison. To produce a pure nerve insensitive pyrethroid resistant strain, a novel approach is being used. Nerve insensitive adult male and female insects are selected using a neurophysiological assay developed at Reading University (McCaffery et al. 1995). The offspring of crosses between two nerve insensitive parents are being used to produce pure nerve insensitive resistant strains. These insects will then be used in the sequence comparison of the III-IV fragment with the SUR strain. The III-IV region of the sodium channel has been shown to be important in the voltage-dependent inactivation of the sodium channel (Stuhmer et al. 1989, Vassilev et al. 1988). Voltage-dependent inactivation of the sodium channel is known to be effected by pyrethroids (Aldrich et al. 1989), so that this region may be a potential target site for pyrethroid insecticides. The present sequence comparison of the III-IV segment between a pyrethroid resistant and susceptible strains of *H. virescens* should give the first indication of whether an alteration in its structure is associated with resistance.

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Insecticide Resistance Mechanisms of the Greenbug, *Schizaphis graminum* (Homoptera: Aphididae)

The phenomenon of insecticide resistance in greenbugs remains a serious threat to small grain and sorghum production in the Midwest. Between 1988 and 1991, a rise in the incidence of greenbug control failures was noted several areas of Kansas. Clonal lines established from greenbugs collected from areas of control failure exhibit 20-30-fold resistance to organophosphate insecticides such as parathion (Sloderbeck et al. 1991). These resistant greenbugs displayed enhancement of general esterases based on polyacrylamide gel electrophoresis and staining with 1-naphthyl acetate. Two different patterns of esterase isozymes have been detected in resistant aphids. Type I aphids exhibit a single esterase band that is either absent in susceptible greenbugs or expressed at levels below the limits of detection. Type II aphids exhibit a different pattern of enhanced esterase isozymes with multiple darkly staining bands relative to the susceptible stain. Although a single band seems to predominate in this pattern, more than one band may be enhanced relative to the susceptible strain (Ono et al. 1994).

In addition to marked differences in electrophoretic mobility of esterase isozymes, the two resistant stains show striking differences in properties of general esterase activity measured spectrophotometrically from whole aphid homogenates (Ono et al. 1994a). Comparisons of activity toward a series of 1-naphthol esters varying in length of the acyl carbon side chain indicate a similar pattern of activity among susceptible Type I and Type II greenbugs. However, the Type II aphids consistently display 15-fold higher levels of activity in contrast to Type I which show only a 1.8-fold increase in activity relative to the susceptible strain. This pattern is consistent despite the observed differences in isozyme compositions.

Both esterase forms exhibit similar activity toward insecticide substrates such as parathion but are strongly inhibited by paraoxon suggesting that the mechanism of resistance does not involve true enzymatic hydrolysis (Ono et al. 1994b). Partially purified enzymes from resistant and susceptible strains exhibit similar elution profiles by ion exchange chromatography although the activity peak from the resistant strains is greater in both peak height and total area. Kinetic analysis of the resulting activity indicated that the Km of the esterase was identical for the resistant and susceptible strains, although the Vmax was consistently 305 fold higher in the resistant strain (Siegfried & Zera 1994). The results suggest that resistance is associated with over production of isozymes present in the susceptible strain rather than the presence of an enzyme with altered properties. Characterization of semipurified esterase preparations from resistant strains suggests that the two isozymes represent different genetic mechanisms that rely on similar processes to confer resistance.

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Pest Resistance to Pesticides and Resistance Management in the Former Soviet Union

Pesticide resistance is a great problem that reduces effective chemical control of agriculture pests. In the former Soviet Union during 1964 - 1994 pesticide resistance was registered in 40 populations arthropod species. The situation became the most complex in crops where pests were suppressed by intensive treatments with chlorine and organophosphate pesticides. Pesticide resistance was noted in:

-- 8 arthropod species in cotton: spider mites - *Tetranychus urticae* Koch., *T. turkestani* Ug. et Nik. (OPs, kelthane), aphids - *Aphis gossypii* Glov., *Acyrthosyphon gossypii* Mordv. (OPs, pyrethroids), whiteflies - *Trialeurodes vaporariorum* Wstw., *Bemisia tabaci* Genn. (OPs, pyrethroids, applaud), plant bug - *Lygus pratensis* L. (Ol's, pyrethroids), bollworm *Helicoverpa armigera* (Hbn.), (OCHs, OPs, carbamates, pyrethroids);

-- 8 arthropod species in apples: codling moth *Laspeyresia pomonella* L. (OPs, pyrethroids, OCHs, insegar), mites - *Panonychus ulmi* Koch., *Tetranychus viennensis* Zacher, *T. urticae* Koch. (OPs, kelthane), leafrollers - *Archips podana* SC., *A. xylosteana* L., *Adoxophyes reticulana* Hb., *Pandemis heparana* Den. u. Schiff (OPs);

-- 6 arthropod species in glasshouse vegetables: mite *Tetranychus urticae* Koch. (OPs, kelthane), aphids - *Myzus persicae* Sulz., *Aphis gossypii* Glov. (OPs, pyrethroids), whiteflies - *Trialeurodes vaporariorum* Wstw. (OPs, pyrethroids, applaud), thrips - *Thrips tabaci* Lind., *Franclinella occidentalis* Perg. (OPs, pyrethroids).

Resistance management is based on the decrease of toxic load on agrobiocenoses and the utilization of all positive/negative pesticide effects on arthropod populations. According to this concept, effective systems of control have been developed based on the following tactical methods:

-- pesticide use according to economic thresholds this reducing both acreage treated and rates of application;

-- earliest possible detection of resistance in pest populations in order to limit chemical usage before efficiency decreases;

-- pesticide rotation during the crop season based on different modes of action and spectra of activity (in response to knowledge of resistance mechanisms and cross -resistance activity);

-- integration of pesticides with microbial preparations and beneficial entomophagus arthropods;

--assessments of insecticide effects on the biotic potential of key pests.

As a result, effective systems of control have been developed and put into practice (See Table 1). These systems are highly effective for resistance pest population control. The use of these systems also leads to reduction of the pesticide use, the reversion of resistance levels or inhibition of resistance development pesticides used present low toxicity towards beneficial arthropods such as *Phytoseiidae*, *Chrysopidae*, *Coccinellidae*, *Anthocoridae*, *Miridae*, *Nabidae*, *Aphelinidae*, *Braconidae*, etc. That is why beneficial arthropods are an effective component of IPM in cotton, apple and glasshouse vegetables production.

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Resistance Management Reviews

Suggested Countermeasures for Insect Resistance to Insecticides in Thailand

In spite of the long history and many studies on insect resistance to insecticides, we are still bereft of excellent insecticides. The loss of any insecticide to resistance is very regrettable since the development of new insecticides requires much money and time. Furthermore, the losses or limitations placed on an insecticide effective for a key insect causes disruption for not only for the crop industries but also for national and public organization. The crop industry can not establish insect control programs if we can not recommend effective insecticides to growers. We have many reports on the insecticide resistance from past experimental studies. Based on these studies, the following suggestions are proposed for strategies to reduce insect resistance to insecticides.

1. Monitor important insect pests for insecticide resistance. Refer to FAO, 1979 and IRAC, 1990 for proposed insecticide susceptibility tests. Some improvements are still needed such as the establishment of standard insect strains and insecticide rates, better control of physical conditions (temperature, humidity, light, etc.), the development of a bioassay method suitable for IGR and B.t ., etc. It is also necessary to develop better networks of communication between of federal, state, university, industry and grower levels. After confirming the development of insecticide resistance, we must avoid selection for higher levels of resistance, cross resistance or more stable resistance.

2. Rotate insecticides that demonstrate no cross resistance. Insect resistance to any insecticide is a biological adaptation to control with that insecticide. Thus avoid, as much as possible, the continued use of the same insecticide. The choice of an insecticide with no cross resistance is difficult. First, collect as many insect pests from a field with high density. Then in the laboratory, select these insects over successive generations with the candidate insecticide. After the resistance ratio (LD50 value of selected strain / LD50 value of unselected strain) reaches 50 or more, then use it to screen for cross resistance with other insecticides. If resistance is not detected, researchers must again collect the insect from the field to isolate the gene(s) responsible for resistance. Note that differences can occur between laboratory strains and field strains, thus confirmation by field bioassays may be more accurate.

3. Employ tactics to delay the development of insect resistance to insecticides. Integrated pest management will contribute to the delay of insect resistance. However, more attention needs to be paid to resistance management tactics such as judicious and efficient use of insecticide and application of synergists.

Japan and Thailand have been working on a joint international research project on insecticide resistance in the diamondback moth (DBM) since 1978. Japan has donated instruments, growth chambers, hoods, screen net cages, mass rearing boxes, etc. to Thai researchers. Together we have developed techniques for mass rearing of DBM , egg and larval parasites, and laboratory bioassays for several insecticides. For monitoring DBM resistance in the field, we developed a yellow sticky trap technique. With Thai Government permission, we introduced a susceptible strain of DBM and used it to compare insecticide susceptibilities between field strains collected over 8 years. In 1987, all tested insecticides including OPs, pyrethroids, carbamates, nereistoxins, B.t .s and IGRs were ineffective in the laboratory and field bioassays with one exception -- abamectin. Fortunately DBM resistance is not stable to each insecticide. We conclude that rotations of insecticides without cross resistance together with continual field monitoring (yellow sticky trap) are promising countermeasures to DBM resistance to insecticides in Thailand (see Figure 1.)

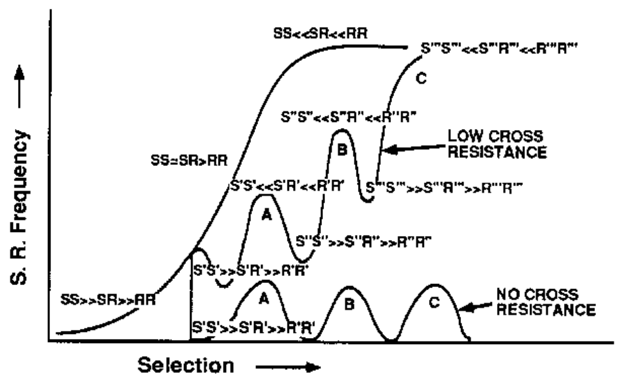

Figure 1.Development of resistanct to insecticide

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Resistance Management of Colorado Potato Beetle in Croatia, 1965 to 1995

The Colorado potato beetle (CPB) was discovered in Croatia in 1947. Now the beetle has spread over the whole territory with the exception of some small islands in the Adria. We proved CPB resistance to DDT and lindane started in 1965 (Maceljski, 1967, 1968), 16 years after widespread use of chlorinated hydrocarbons. The resistance to kelevan, an insecticide similar to the chlorinated hydrocarbons, was established in 1972 after 4 years of use. Carbaryl resistance at low levels was reported in 1973, but resistance at high levels for most carbamates was reported in 1986 after about 18 years of moderate use. Resistance to organophosphorous insecticides was established in 1986 after 16 years of use, and to pyrethroids in 1987 after only 7 years of widespread use (Maceljski & Igrc 1992-1994, Maceljski 1995a).

From 1986-1990, 42 populations of CPB were sampled and bioassayed. From 1991-1995, 56 additional populations were bioassayed. The bioassay used was very similar to the method (No. 7) recommended by IRAC, and after 1990 was the same as the IRAC method (No. 7).

At the end of 1990, resistance to OP, OC and pyrethroid insecticides had developed on about 5% of all potato fields in the northern (continental) Croatia. At the end of 1995, resistance to OP and OC insecticides had spread to 70% and pyrethroids to 30% of all potato fields in northern Croatia (Maceljski 1995b). It should be pointed out that practically no significant resistance was detected in southern (Mediterranean) Croatia. Hence at this time, the resistance is a phenomenon in northern Croatia only.

CPB resistance management has met some difficulties. In Croatia, each land owner is extensively growing potatoes. Therefore, many hundreds of thousands of small potato fields are dispersed all over Croatia. Crop rotation does not reduce the attack of CPB which is severe each year. In contrast, the small plot size allows for mechanical control by collecting overwintering adults and egg masses. No natural enemies are present to limit CPB abundance in Croatia. Therefore, CPB resistance management is based primarily on chemical control with rotation of insecticides including Bacillus thuringiensis tenebrionis (B.t.t .) based insecticides.

Potato growers usually spray 3-4 times in one season, often applying a much higher dosage than indicated by the label. We are trying to reduce the number of treatments to 1 or 2 by postponing the first treatment until the first larva appears and by recommending insecticide use only when more than 15 larva per plant are present on more than 20% of plants. We strongly recommend growers collect overwintering adults and egg masses prior to the first insecticide treatment.

We also recommend growers rotate insecticide groups. Insecticides from groups with similar modes of action should never be used successively or more than twice in a season. In regions where widespread resistance occurs, we recommend at least one application of the following groups: nereistoxins (bensultap, tiociklam), IGR's (teflubenzuron, hexaflumuron) and B.t.t. s. To date, no resistance to these insecticides (larvicides) has been detected. We have established that in normal climatic conditions, IGRs have a much longer residual action (at least 22 days) than any other insecticide. However in rainy weather (like in 1995), this residual action decreases to the range of other chemicals. B.t.t. s have the shortest residual action (max. 8-12 days) (Maceljski, 1995a). The initial and residual action of B.t.t. s and the initial action of IGRs can be substantially increased by adding a sublethal dosage (5-10% of the normal dosage) of other chemical insecticides.

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Monogenic Models in a Heterogeneous Environment

As scientists we search for robust patterns in nature. Complex detail interferes with a simple and presumed robust explanation for phenomena and shapes many of our scientific debates. For example, a widely held thesis seems to be that insecticide resistance is due to the effects of one or a few major genes becoming fixed at high frequencies within a population and allowing survival under uniformly high levels of exposure to the toxin. Although appealing in its simplicity, supportable with chosen data, and leading to obviously important and fundable lines of investigation, this thesis ignores several important considerations. Our purpose in this discussion is to illustrate some of the complex details that erode the thesis described above. We address in particular how environmental heterogeneity affects the evolution of insecticide resistance by introducing behavioral effects, violating assumptions of the single gene model.

The single gene model of resistance permeates the literature. Describing the mechanism, finding the gene, and understanding cross resistance patterns have all been primary goals of empirical work. If resistance truly is regulated by a single gene the results have practical utility. They may allow design of an insecticide rotation scheme that permits strong, regular selection pressure and temporary increases in resistance allele frequency but without control failures. However, lack of fitness costs to resistance or negative cross-resistance and operational constraints on insecticide rotation may interfere with finding a suitable rotation. Furthermore, a wide variety of physiological mechanisms of resistance have been identified this variety cannot be ignored or assumed to be mutually exclusive. Single-gene models assume that adaptation is a one-time event. If a resistance allele becomes fixed and selection pressure continues, nothing changes. But this is true only for a single mechanism and a gene that does not interact with other genes or the environment. Otherwise, adaptation can continue, modifiers change in frequency and correlated traits change. Resistance management schemes that ignore the possibility of these further changes could easily fail.

Even in cases where resistance may be monogenic, assuming that resistance begins and ends with a single gene, leads to an inappropriate focus on the organism independent of its environment. In systems where the environment can be considered uniform, the single gene assumption may make sense. But in reality spatial distributions of insecticidal toxins are remarkably heterogeneous. Research on pesticide application technology in agriculture indicates that a wide variation in concentration within crop canopies is typical. The underside of treated leaves can be entirely devoid of deposits and deposit amounts on leaves at the bottom of a crop canopy can be 100-fold less than those at the top. Deposit degradation, influenced by extremely variable microclimatic conditions, further exacerbates the spatial heterogeneity in toxin concentration. Despite variation in toxin concentration, we typically achieve satisfactory control by increasing field application rates to maximize mortality at the lowest concentrations. However complete control or 100% mortality of the target pest, is a very rare event and we certainly would not presume that it is rare because of resistance. Rapid deposit degradation, differential susceptibility of insect life stages, and our inability to deliver a lethal dose to every square centimeter of the canopy even at the time of the application all result in the norm being a functionally heterogeneous distribution of insecticidal toxins.

Given current technology, the assumption of selection occurring in a uniform, high dose environment typically is incorrect. The few systems where a uniform lethal dose is consistently achieved tend to be inefficient (extreme overdosing), unsustainable (if new insecticidal chemistry is not unlimited), and should not be readily accepted. Furthermore a refuge from selection, toxin heterogeneity by design, is a cornerstone of many resistance management programs.

A consequence of heterogeneous toxin distributions is that target insects have the opportunity to avoid toxin deposits through behavioral responses. Predicting the impact of a refuge on selection pressure, for example, requires an understanding of both behavior and physiology. Physiological mechanisms of resistance still are important because selection on these mechanisms occurs outside of the refuge. However if behaviors that result in movement across the refuge borders do not occur the refuge becomes irrelevant. Furthermore, understanding any relationship between behavioral patterns of movement across refuge borders and resistance becomes critically important.

Studying larval diamondback moth, we have demonstrated that behavioral responses to permethrin occur before contact (Lin et al. 1993) and reduce contact with droplet deposits on a treated surface (Head et al. In press). Behavioral response, therefore, alters the dose acquired by the insect. Seen in this light it is clear that behavior shapes selection for physiological mechanisms of resistance. Colorado potato beetle responds to Bacillus thuringiensis delta-endotoxin only after ingestion (Hoy & Hall 1993). A consistent dose, the amount consumed before toxic effects in the midgut begin, is acquired before behavioral responses occur. Subsequent behavior may determine subsequent endotoxin ingestion, but the impact of behavior on dose acquisition appears less immediate than in the case of diamondback moth and permethrin. The different behavioral responses seen in our two model systems could shape selection for physiological mechanisms of resistance in different ways.

Plant compounds that play a role in protection from herbivores also are rarely distributed uniformly within plants. The interaction between behavioral and physiological traits that permit survival in insects on toxic plants seems to have a long evolutionary history that cannot be ignored. For example, behaviors like leaf trenching and stem cutting reduce exposure to toxic compounds and influence selection for physiological adaptation to them.

Behavior has often been considered to be important only to the extent that it contributes as a mechanism of insecticide resistance. Because most cases of resistance demonstrate an improved capability for surviving an acquired dose, through physiological mechanisms, behavior is considered unimportant. Behavior of resistant insects has occasionally been quantified to verify that it does not contribute to their survival, at least to the extent that the physiological mechanism does. The the two traits are often considered to be separate issues and arising from separate mechanisms. But stimulus-dependent behavioral responses do not occur without exposure and toxic effects. If the insect does not encounter the toxin, it will not respond to it. Conversely if the insect responds behaviorally, it has been exposed to the toxin and toxic effects are taking place. Toxic effects vary with the insect's level of tolerance. Therefore, correlation between behavioral and physiological responses to a toxin can be expected.

We have measured the genetic correlation between behavioral and physiological responses of diamondback moth to permethrin (Hoy et al. 1991, Head et al. 1995) and of Colorado potato beetle to Bacillus thuringiensis delta-endotoxin (Hoy & Head 1995). Our results demonstrate that the magnitude of this correlation varies and depends on genetic variation in the two traits. We use quantitative genetics methodology. Quantitative genetics is sometimes considered an inappropriate tool for resistance research because resistance is considered to be monogenic. For us, the most important advantage of these techniques is that they require no assumption about the number of genes influencing the traits of interest. Both behavioral and physiological traits are described by quantitative measures. We typically use a measure of distance moved in response to the toxin as the behavioral trait and proportional mortality within a family after administration of the population LD50 as the physiological trait, both continuous and quantitative characters. The mathematical models underlying quantitative genetics are appropriate for estimating genetic variation in these traits and genetic correlation between them regardless of the number of genes governing either trait. Behavior, which one might expect to be influenced by many genes, can be correlated with physiological resistance governed by a single or multiple genes if the single or multiple genes governing resistance also influence behavior.

Consider the implication of dose being altered by behavioral response and behavioral response being correlated with physiological resistance. Selection on behavioral response, which we have determined to be heritable (Hoy et al. 1991, Head et al. 1995, Hoy & Head 1995), is possible. Behavioral responses result in survival if they reduce dose to below an individual's threshold (this requires suitable spatial distribution of the toxin). Selection on behavior will have an effect on the correlated physiological trait. Depending upon the sign of the correlation, behavioral response could increase survival of either more resistant or more susceptible insects. We have measured both negative (Hoy et al. 1991, Head et al. 1995) and positive (Hoy & Head 1995) genetic correlations between behavioral responsiveness and physiological resistance. Conversely the spatial distribution of the toxin determines the extent to which behavioral responses and their correlated effects on physiology occur. Therefore, understanding behavioral responses to toxins, how they are affected by spatial distribution of toxin, and their genetic correlation with physiological resistance is essential to understanding and successfully manipulating selection pressure for physiological mechanisms of resistance.

The indirect effects of behavior on resistance we suggest become very important when designing a refuge from selection for resistance. A recent topic of concern has been the usefulness of a mixture of transgenic (expressing Bt endotoxin) and non-transgenic seed to provide a refuge for susceptible insects within a crop. Single gene models have been used to predict the impact of movement in seed mixtures, but without any of the behavioral effects discussed above. Any impact on resistance of insect movement within the seed mixtures has been attributed to changes in effective dominance of a single resistance gene in these models. Behavioral responses to the different plants and their correlation with resistance are clearly relevant and deserve our attention. A model better suited to the added complexity will be required.

Dismissing all of the above by contending that relative to "the gene responsible for resistance" any " modifiers" such as correlated behavioral, physiological or ecological traits, are of minor importance and can safely be ignored is dangerous. The consequences of assuming simple Mendelian segregation when it is not occurring can be severe. Monogenic models have allowed a very useful systems approach to resistance management. Further progress in understanding and manipulating the evolution of resistance seems to require relaxation of the assumptions dictated by monogenic models and a willingness to delve into the complex reality of organisms interacting with a heterogeneous environment.

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Resistance Management News

Herbicide Resistance in Plants: Biology and Biochemistry

Edited by: Stephen B. Powles and Joseph A.M. Holtum
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The late 1980's saw an explosion in the amount and diversity of herbicide resistance. Herbicide resistance poses a threat to crop production in many countries. The rapid escalation in herbicide resistance worldwide and resistance at the population, biochemical and molecular levels are the foci of this timely book. Leading researchers from North America, Australia and Western Europe present lucid reviews that consider the population dynamics and genetics, biochemistry and agro-ecology of resistance. Resistance to various herbicides is discussed in detail, as well as the mechanisms responsible for cross resistance and multiple resistance. This reference is invaluable to those interested in the evolution and ability of species to overcome severe environmental stress.

For more information on this publication, write to the address below for details. Be sure to include your full mailing address, mention Catalog no. L713 and the title above.

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Summer Institute on Global Pest Resistance Management

The Third Annual Summer Institute on Global Pest Resistance Management hosted in July by Michigan State University was attended by sixteen researchers from Pakistan, USA, Canada, India, Malaysia, The Netherlands, Nigeria, South Africa, Switzerland and Venezuela. Pest resistance is not only a general phenomenon exhibited by arthropods, weeds, nematodes, rodents, plants and human pathogens, but it is a widespread global phenomenon. This is reflected by the makeup of the 58 Summer Institute alumni representing entomologists, weed scientists, plant pathologists, plant breeders, toxicologists and sociologists from 29 countries around the globe.

The Summer Institute is a 2-week training workshop designed to provide researchers with the most current concepts and principles of pest resistance management through classroom instruction, informal discussion and field/laboratory demonstrations. The success of this workshop can be attributed to over 30 instructors (entomologists, weed scientists, plant pathologists, horticulturists and sociologists) and the continual interaction between instructors and participants from around the world. Participants are provided with critical literature, networking capabilities and hands-on resistance management experience. The primary goal of the workshop is to "Train the Trainers" whom we trust will promote basic knowledge and needs of pest resistance and resistance management to other researchers, end-group users (the growers), and agricultural policy-makers. This workshop is but one step in the overall global education process.

Arrangements are underway to organize the Fourth Annual Summer Institute on Global Pest Resistance Management. The Summer Institute will be held between July 8 to July 19, 1996 in East Lansing, Michigan, USA. For further information on this workshop, please contact Michael Bush or Mark Whalon.

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Abstracts

Molecular Genetics and Evolution of Copper Resistance in Bacterial Plant Pathogens

Related copper resistance operons have been described in recent years from different genera of bacteria from agricultural environments where copper compounds are applied to plants for disease control and fed to livestock as dietary supplements. Although similar in overall structure, copper resistance operons from Pseudomonas syringae, Xanthomonas campestris, and Esherichia coliare diverged considerably at the sequence level in their functions, and at the level of metal-induced gene expression. The operons are likely of ancient origin, related to indigenous bacterial multicopper oxidase systems. In plant pathogens, there is evidence for the recent spread of copper resistance genes among closely-related bacterial strains and species, but not between different bacterial genera. The evolutionary-related copper resistance operons in different genera have highly specialized regulatory mechanisms that determine copper-inducible, and sometimes zinc-inducible, expression in their host genus. No expression of copper resistance was observed when copper resistance genes from one genus were transferred to another, suggesting that regulatory mechanisms may restrict horizontal transfer among bacterial taxa.

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Evidence of Selection for Strains of Uncinula Necator Resistant to Fenarimol and Triademefon by Repeated Applications of Fenarimol

Triadimefon and fenarimol have been used since 1982 and 1989, respectively, to control grape powdery mildew (Uncinula necator) in California. Resistance in U. necator, affecting fungicide efficacy, was observed to triadimefon but not to fenarimol five years after introduction. To determine whether fenarimol can be used in control programs without the risk of increasing resistance to triadimefon, a container-held population consisting of a mixture of U. necator samples was subjected to applications of fenarimol at 5 mg/l and evaluated for resistance to each fungicide. Resistance was determined by measuring conidial germ tube lengths 72 hours after inoculating conidia onto leaf discs separately treated with a range of concentrations for each fungicide. After two fenarimol applications, conidial germ tube lengths of the treated population were significantly larger compared to those of the untreated population at all concentrations of fenarimol and triadimefon applied to the leaf discs. This study indicates that repeated applications of fenarimol may increase resistance levels to triadimefon of surviving U. necator.

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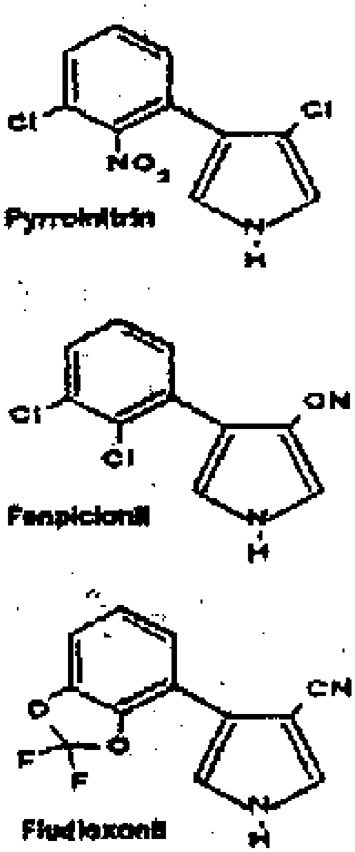
Discovery and Biochemical Mode of Action of the Phenylpyrrole Fungicide Fenpiclonil

CGA 142705 (fenpiclonil) and CGA 173506 (fludioxonil) are the first phenylpyrrole fungicides commercially developed by CIBA-GIEGY AG, Basel. Fungi among Ascomycetes, Basidiomycetes and Deuteromycetes are sensitive. Benzimidazole and dicarboximide-resistant field isolates are not cross resistant with phenylpyrroles.

Fungicide activity of phenylpyrroles was first recognized in pyrrolnitrin, produced by several Pseudomonas species. The use of these bacteria in biological control programs has been investigated, but never gained large scale application. Pyrrolnitrin was not commercialized as a fungicide in agriculture because of its low stability in the light and its difficult and inefficient full synthesis. One-step-synthesis of phenylpyrroles became feasible when a strong electron-withdrawing cyanide group was introduced in the 3-position of the pyrrole ring. Since stability was improved as well, the cyanopyrroles were the basis of the now commercialized phenylpyrroles -- fenpiclonil and fludioxonil.

The biochemical mode of action of fenpiclonil has been studied in the fungus Fusarium sulphureum (Schlecht). We recommend fenpiclonil for control of F. sulphureum , one of the casual organisms of damping-off in cereals. When monitoring the interference of fenpiclonil with fungal metabolism, the accumulation and incorporation of glucose into fungal glycans appeared to be most strongly inhibited. However, when examining the elimination of carbon dioxide from glucose and the behavior of various glucose analogues, the most plausible mode of action is that fenpiclonil inhibits the trans-membrane transport associated with glucose phosphorylation.

Figure 1.



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Adaptive Fitness in Herbicide-Resistant Weeds

Resistant weeds selected by repeated herbicide application have evolved a wide variety of physiological and biochemical adaptations to escape injury. Resistance mechanisms described so far include altered target sites, increased herbicide metabolism rates, sequestration, altered uptake and translocation patterns, and reduced metabolic activation of proherbicides. In addition to conferring a selective advantage in agricultural fields, resistance mechanisms may also lead to subtle changes in the plant's physiology and ultimate fitness. The first reported cases of resistance to triazine and related herbicides demonstrated that resistant populations suffered from substantial fitness losses. However, most subsequent cases show that fitness is not necessarily impaired in populations resistant to other herbicides. In fact, some mutations for herbicide resistance may indirectly confer a fitness advantage. Several sulfonylurea-resistant Kochia scoparia accessions selected by field use of chlorsulfuron displayed an altered germination phenotype. Germination rates were significantly faster than in susceptible accessions at low temperatures (4deg. to 8deg. C), perhaps due to altered feedback inhibition properties of an insensitive acetolactate synthase target enzyme. In another case, anther exertion and pollen release was significantly delayed in diclofop-resistant Lolium multiflorum compared to wild type plants, leading to a decreased rate of resistance evolution under most conditions. These and other examples illustrate that selection for herbicide-resistant populations may concurrently select for altered and unpredictable fitness characteristics.

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Resistance Management Strategies for Transgenic Cotton in Australia

Transgenic cottons expressing insecticidal proteins from Bacillus thuringiensis subsp. kurstaki (B.t.) will soon be commercialized in Australia. Given the strong selection pressure which will be applied to field populations of the target pests (Helicoverpa spp.) by B.t. cotton, the potential for the rapid evolution of resistance is a real concern. Pre-emptive resistance management strategies, based on the use of refugia to maintain susceptibles in local populations and high expression of B.t. toxins in the plants sufficient to kill all susceptible and most heterozygous resistant larvae, are now being researched. Empirical data from field studies on the efficacy of B.t. cottons in Australia and of various refugia options will be presented. Consideration of resistance risks with minor Lepidopteran pests will also be highlighted. The refugia/high dose strategy will be integrated with other tactics (cultivation of crop residues, monitoring of B.t. resistance levels, strategic use of non-disruptive chemicals, future pyramiding of other insecticidal proteins) to provide a basis for sustained exploitation of transgenic technology in the Australian cotton industry.

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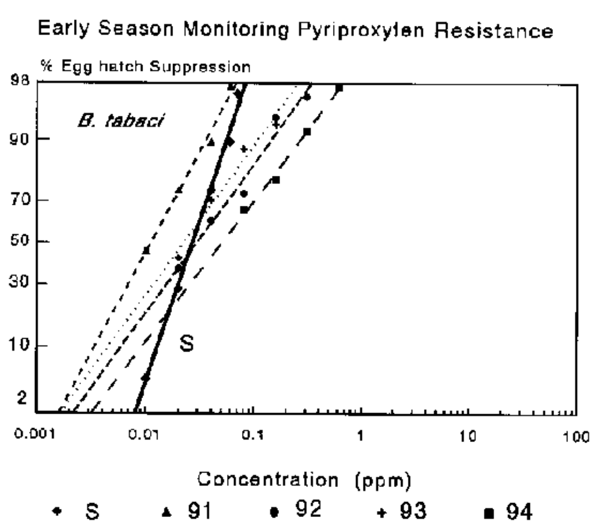
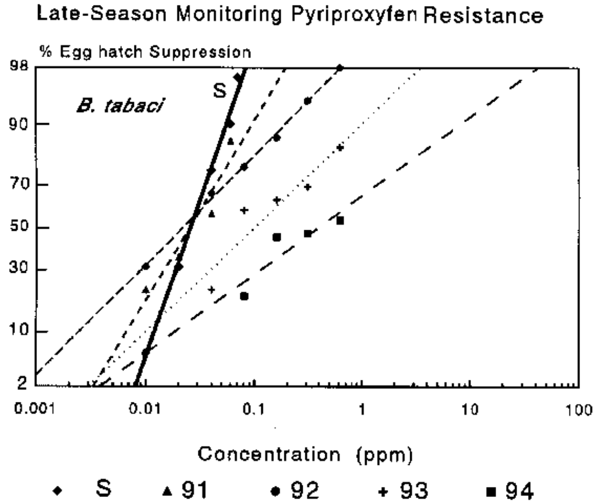
Israeli IRM Strategy - Insecticide Resistance Dynamics of Key Pests

The Israeli strategy was introduced in cotton in 1987 to reduce resistance to insecticides. The strategy focused primarily on whitefly, Bemisia tabaci control with novel insectiicdes such as buprofezen, pyriproxyfen and diafenthiuron. Benzoylphenyl ureas were used to control lepidoptera pests. Each insecticide was used only once during one pest-generation then alternated with another having a different mode of action. Extensive resistance-monitoring programs were conducted. Base-line bioassays for susceptibility of key pests to the most important novel insecticides were carried out prior to the resistance-monitoring in field populations.

The seasonal trends in susceptibility to buprofezin and pyriproxyfen in B. tabaci field populations were monitored from June (prior to treatment) through late summer. A slight increase in tolerance was observed. Due to the restricted use of the novel compounds and a reduction of selection pressure, those insecticides could be applied in the following season when the pest populations were most susceptible to both compounds. In contrast after successive applications, high to moderate resistance levels to pyriproxyfen and buprofezin in B. tabaci were detected in some ornamental greenhouses. This resistance level was slightly decreased after two years of non-use in these greenhouses. Restricted use of benzoylphenyl ureas did not appreciably alter lepidopteran susceptibility to chlorfluazuron, although, some tolerance to teflubenzuron was observed after five year of use. The rational use of insecticides has maintained the susceptibility of peststhese groups of insecticides and substantially reduced insecticide applications.

Figures 1 and 2.





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Symposia

IRAC US Meeting Minutes

The third meeting of the year was held on September 7, 1995 at the DowElanco Education and Training Center in Indianapolis, Indiana, USA.

Educational and Public Relations Program - At the last meeting, IRAC US committed $20,000 to the educational and public relations proposal. Cotton Incorporated and the National Cotton Council each committed $5,000 toward this. Additionally, IRAC Central will contribute one half of the development costs for the brochure. As a consequence, IRAC US was able to proceed with the educational and public relations program.

Julie De Young/Fleishman-Hillard presented a final draft of the brochure asking for any additional comments before printing. A few changes were made to the final draft. The earlier draft had been circulated to several university extension/research personnel and the IRAC committee members. Plans were to print 10,000 brochures and 5,000 posters. There was a motion to increase the brochure quantity to 25,000. There was a unanimous decision to do so. The poster quantity will remain at 5,000. These go to print prior to October 31, 1995.

The topic of distribution of these materials was discussed at length. It was suggested that a brochure be included in the registration packet for attendees at the Beltwide Cotton Conference and the regional Entomological Society of American meetings. Additionally, it was suggested and agreed that a postcard be included allowing for the ordering of additional brochures and posters. It was also suggested that committee members contact appropriate individuals within their own organization for distribution and possible contribution towards additional printing costs if a company's logo were to be added.

Julie De Young/Fleishman-Hillard presented an outline for a resistance management education package. The target audience would include extension specialists, county agents, crop consultants and agrochemical dealers. This group would then use the material to train growers, scouts, and students. The recommended contents include:

- Moderator's Guide
- IRM brochure
- Quiz
- Research report reprints
- Bibliography for additional information
- List of contacts for additional information
- IRAC background information
- "Resistant Pest Management" Newsletter

- Order form/reply card

The target date for completion is January 1, 1996.

Assignments: Frank Carter/National Cotton Council will contact the registrations committee regarding inclusion of the brochure in the registration packet for attendees of the Beltwide Cotton Meeting and will arrange to transfer the necessary quantity of brochures if approval is granted. Additionally, Frank Carter will obtain a mailing list of cotton gins. IRAC US members are to contact their own companies regarding distribution of materials. Pat O'Leary/Cotton Incorporated will order a sufficient quantity of brochures/posters for distribution at a consultant/extension meeting to be held in late October. Additional distribution will be further discussed at the next meeting.

Gary Thompson/DowElanco and Julie De Young/Fleishman-Hillard will solicit existing material for the education package from the Pesticide Research Center and the University of Reading Short Course. All members were requested to forward existing material to Julie De Young.

Membership - Monsanto and BASF will become members of IRAC US starting in 1996. The BT Working Group will be invited to attend our meeting with possible interest in meeting with IRAC US as one group in the future.

NAICC - The National Alliance for Independent Crop Consultants has allocated a three-hour time block on January 24, 1996 in Orlando, FL for IRAC US to develop a symposium/panel discussion with the subject of resistance management. At our last meeting, we proposed a list of topics and speakers. NAICC was surprised at the emphasis on university speakers and indicated that they would prefer debates on different resistance management methods and something substantiative for day-to-day implementation. The committee proposed that Ian Watkinson/Gowan provide an industry presentation. Also, it was suggested that Stan Nemec, private consultant, be considered as a speaker. Also, a topic to be considered would be futuristic solutions (test kits) with Mike Roe from North Carolina State University as a potential speaker. Subjects such as high rate/low rate and tank-mixes/rotations were suggested as well as with a panel discussion.

Assignments: Ian Watkinson/Gowan and Don Allemann/CIBA will firm up a program/speakers and provide this to NAICC. Pat O'Leary/Cotton Incorporated will pass on information learned from the Cotton Inc. sponsored consultant/extension meeting scheduled for the end of October.

Beltwide Cotton Conference - A four-hour time block has been set aside for insect management in the Production Conference on January 9, 1996. Frank Carter/National Cotton Council is in charge of developing a program. Resistance management would be addressed under the following proposed topics: 1) IRAC educational package introduction, 2) whitefly resistance management, and 3) tobacco budworm resistance management.

Assignment: Gary Thompson/DowElanco will make the presentation on the IRAC educational package and will assist Frank Carter/National Cotton Council in finalizing the program/speakers.

USDA - IPM Symposium - The USDA is organizing an IPM symposium for February 27-29, 1996 in Washington, DC. It was suggested that IRAC US contact the program chairman regarding involvement. Assignments: John Lublinkhof/AgrEvo will contact Barry Jacobsen, program chairman, regarding a poster on IRAC US function and purpose.

EPA and ACPA - A conference call had been pre-arranged for the group to discuss resistance management issues with Sharlene Matten, EPA and Ray McAllister, ACPA.

A future seminar had been suggested for the spring of 1996 that would be an extension of our introductory educational seminar held in October 1994. The purpose would be educational, i.e. educating EPA on resistance management issues. University speakers are being considered. Sharlene Matten will propose a date.

A copy of the EPA fact sheet on BT corn was circulated prior to the conference call. The committee felt that the EPA was very stringent regarding resistance management requirements. This concern was voiced to Sharlene Matten. She indicated that this resulted from negotiations with the registrants. The committee was also concerned that this would be precedent for regulatory mandates on new insecticide registrations. She indicated that their intention is to not "dictate" resistance management requirements, but that the statements regarding resistance should be the responsibility of the registrant (voluntary) and that this would encourage flexibility. She further indicated that current resistance management statements are not specific enough, are too vague and improvement is needed. The sharing of strategies should be a first step to improvement.

After the conclusion of the conversation with Sharlene Matten, IRAC-US continued discussions with Ray McAllister/ACPA. It was suggested that IRAC US draft a position statement ("white paper") regarding handling the whole subject of resistance management on labels. This statement will be presented to the registration committee at the ACPA who in turn could influence the EPA.

Assignments: Don Alleman/CIBA will draft a "white paper" and will solicit the entire committee for input. Then the "white paper" will be sent to the ACPA registration committee.

Future Meeting - The next meeting will be held on January 8, 1996 from 1:00 - 6:00 pm at the Opryland Hotel in Nashville, TN.

Assignments: Gary Thompson/DowElanco will invite IRAC Central to participate in this meeting. Frank Carter/National Cotton Council will obtain a meeting room. David Marsden/DuPont, Joe Hope/Rhone Poulenc and Dick Pence/Merck are to contact Betsy Beers/Washington State University, Nick Toscano/University of California and James Ottea/Louisiana State University, respectively, regarding updates on the funded research projects. Ideally, the researchers would be able to provide a verbal summary at our next meeting. If not, the assigned committee member will be responsible for obtaining an update and presenting this at our next meeting.

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IRAC-Poland Potato Minutes

The IRAC Field Crops and Vegetable Working Group (FC & V) has sponsored a project in Poland with three cooperators to begin to define the extent of resistance of Colorado potato beetle (CPB) to some commonly used pesticides with the ultimate objective of defining a strategy for resistance management. Cooperating researchers include Dr. Maria Pawinska of the Institute of Plant Protection in Bonin, Dr. Anna Przybysz-Szczesna of Institute of Organic Chemistry in Warsaw and Mr. Pavel Wengorek of the Institute of Plant Protection in Poznan.

Up to the late 1960's, DDT was the major product for control of CPB in Poland and in many areas resistance to this product became widespread. In 1970, chlorfenvinphos was introduced and was effective for 15 years until the first field failures occurred in 1986; since that time the use of chlorfenvinphos has been declining. Synthetic pyrethroids were introduced in 1980 and have been widely used. Reports of poor performance only began to be recorded in 1992. One of the most recent products introduced into Poland for the control of CPB is bensultap which is becoming very popular with Polish farmers.

In recent years, potato acreage in Poland has fallen due to the changes in the market economy, but as the economy improves it is believed that acreage will increase. Therefore, it is important that the resistance situation be monitored and managed.

It is with this background that IRAC-FC & V have sponsored a research program in 1995 to:

1. Test the validity of using IRAC #7 test method (leaf-feeding bioassay) on CPB larvae to monitor resistance, compared to topical application to adults. Chlorfenvinphos, cypermethrin and bensultap will serve as representative standards from their respective chemical groups.

2. Develop the technique with a "discriminating dose for widespread monitoring."

3. To define a resistance management strategy for CPB in Poland.

At the recent FC & V Working Group meeting held in Berlin on the 13th of October 1995, our Polish cooperators summarized their findings as follows:

1. IRAC #7 was easy to use and produced consistent results for all the products.

2. This data can be used to define a discrimination dose.

3. Preliminary evaluation of the results indicates that there are "hot spots" of resistance to both pyrethroid and organophosphate products.

Resistance is associated with past pesticide use patterns.

This study will be sponsored further by IRAC in 1996. We seek to define the extent of resistance to the standard products and begin the synthesis of a resistance management strategy.

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Announcements

It has been brought to my attention that there were a couple of mistakes in the last newsletter. Hopefully these corrected versions will help clear things up.

First, in the editorial by Rick Roush the words 'the resources' were left out of the second paragraph, second line. It should have read like this....

"There is insufficient space here to review these debates, but it seems clear that the EPA is reluctant to use regulation to help delay resistance to pesticides. In the EPA's defense, it may not have the resources to do so. EPA has made some modest Proactive efforts in avoiding resistance, such as requesting resistance management statements from some companies developing transgenic plants and more traditional pesticides. Nonetheless, in the absence of any efforts to enforce or facilitate the adoption of resistance management plans, such documents clearly have little more than public relations or educational value. "

Second in the article "Consequences of Shared Toxins in Strains of Bacillus thuringiensis for Resistance in Diamondback Moth" in the Resistance Around the Globe section the words 'to which' were replaced with 'that' in the last sentence of the article. The last sentence should read as....

"To avoid this dilemma, industry should avoid introducing Bt products with shared toxins to which populations have already developed resistance."

Also, if you would like to submit an article to the Newsletter the next issue will be coming out in late June- early July. Submissions should be to me no later than May 20th, 1996. Submissions for articles can be done via disk (preferred) from any IBM software package, via e-mail to the address below, or any hard copy of text and/or graphics. Please try to keep your articles under 4 single spaced pages.

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