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)**

Vol. 12, No. 1 (Fall 2002)

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Letter from the Editors

We would like to invite our readers to comment in the Perspectives Forum section of the newsletter on the communication of resistance issues. This resistance management arena is important and is often overlooked, especially in the early period of resistance or in a resistance outbreak situation.

In addition, it is difficult to accomplish the communication of resistance information from such varied compass directions around the globe. This difficulty arises for three reasons. First, by the time refereed journal articles are published, resistance is often old news and it is too late to initiate management strategies. Second, methods of identifying resistance vary and are often under scientific and practical scrutiny. These methods are frequently and forcefully debated, particularly in the early stages of resistance reporting. Third, a public/private/policy rift can often result from the miss-reporting, false reporting, or early reporting of resistance.

These difficulties in resistance communication pose a number of questions. What have we learned in reporting resistance? How effective are these systems? How could they be more effective? What are the technical parameters around which these systems should be developed and expanded? How can we maintain both security and validity in reporting and making data available? Which experts ought to evaluate the information? Which information should be communicated and when? How can modes of reporting be used locally, regionally, nationally, and globally in resistance management?

These questions require input from the many areas of resistance mangement in order to ensure effective communication. For this reason, we encourage you to visit the Perspectives Forum and let us know how you feel about resistance communcation methods and issues.

Lastly, besides the newsletter, a second web site has recently become available to aid in resistance reporting:

WeedScience (http://www.weedscience.com).

This site involves an international survey of herbicide resistant weeds.

Both web-based systems are in the process of developing spacial mapping and near real-time reporting features. We encourage you to spend some time perusing both the newsletter and the WeedScience site to discover the resistance-related information they offer.

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Resistance Management Reviews

Solving the Pest Resistance Puzzle
by Len Richardson

The USDA funded group IR-4 is responsible for developing the pesticide residue data needed to support new EPA pesticide tolerances and registrations covering most crops grown in California -- the so-called "minor use" crops. In just the last two years IR-4 has helped produce the data needed to establish more than 1,000 new tolerances, each covering a pesticide-crop combination. About 80% of these tolerances cover products EPA has approved on a fast track as "reduced risk" products.

There has been a flood of new and safer pesticides entering the market the last few years, leading many commentators to speak fondly of a new "Golden Age" of pest management. With the EPA about to drop the Food-Quality-Protection-Act-hammer on some organophosphate insecticides, this flurry of innovation comes none too soon. But many of the new products act through very specific mechanisms, increasing the odds that target pests will learn to live with them, a trend that increases your costs and limits your options.

Indeed, resistance is the dark cost cloud on the horizon of this pest management Golden Era. To manage resistance everyone involved -- growers, IPM specialists, researchers, the industry, and regulators - must ramp up their collective skill and attention to resistance management. Fortunately, solving the resistance management riddle might get easier:

- Progress is being made via biotech to understand the genetics behind resistance, a key step in identifying new resistance management tools and strategies.
- Thanks to Mark Whalon of Michigan State University, the popular "Resistant Pest Management" newsletter is back at http://whalonlab.msu.edu/rpmnews/. Even better, the USDA has made a down payment to MSU to develop and update a searchable, public database on insects resistant to pesticides.
- A major USDA-funded IPM project involving vegetables in Florida and Wisconsin is focusing on two pressing resistance management challenges -- saving the efficacy of the nicotinoid insecticides (Admire, Platinum, Actara) and the strobilurin fungicides (e.g., Quadris).
- The EPA has launched a voluntary resistance management pesticide labeling initiative. The $64,000 question is will industry use it? If not, EPA will likely drop the voluntary part.
- At least some pesticide makers are placing useful, albeit "soft touch" resistance management suggestions and/or restrictions on product labels.
- The world's leader in "reduced risk" pesticides -- Syngenta - shocked the industry (especially Monsanto) when it unilaterally announced tough farmer strategies for preserving the efficacy of glyphosate herbicides.

These positive steps are pieces of an incomplete resistance management puzzle. It is everyone's puzzle to crack if we want to cut costs and sustain this new "Golden Era" of pest management.

Mapping Tsetse Targets in Botswana

By Dr Terry Mabbett

Eleven million square kilometres of Africa is afflicted by tsetse flies (*Glossinia* spp.) and the parasites that they carry and transmit. The flies themselves are harmless but as soon as they pick up the trypanosome parasite from a person or animal during a blood meal they become lethal vectors of trypanosomiasis known in man as 'sleeping sickness.'

These vector insects were traditionally controlled by blanket spraying of chemical insecticides but more enlightened control strategies are now employed. Artificial baiting techniques using custom-designed targets to mimic the host and exploit behavioural responses in the tsetse fly are now the order of the day.

Targets are deployed in the field and are comprised of blue and black cloth, colours that elicit attractive responses and landing responses, respectively, from tsetse flies. They are supplied with odours from sachets of specific semiochemicals placed in pockets sewn into the cloth. These are synthetic chemicals based on those identified in animals from research into the chemical composition of cow's breath that contains chemicals to which tsetse flies are attracted.

The most commonly used chemicals are octanol and methyl ethyl ketone (MEK) that are deployed in lures to provide specific release profiles. To the tsetse fly the target looks, smells, and feels like a cow. It lands on the target to achieve a blood meal but instead picks up a lethal dose of deltamethrin or alphacypermethrin insecticide with which the target has been sprayed.

Many African countries have their own highly organised departments of tsetse research and control including Botswana in southern Africa where the *Glossinia morsitans* group of species is present. They are major vectors of animal trypanosomiasis and some species are vectors of sleeping sickness.

Patrick Kgori from Mochudi near Gaberone, the capital city Botswana, worked in the Insecticides Section of the Ministry of Agriculture's Tsetse Control Programme before coming to the United Kingdom to study for BSc and MSc degrees. For the latter he chose the MSc Pest Management course at Imperial College Silwood Park which combines taught courses and research components. For his research project Patrick integrated two state-of-the-art technologies, GPS (Global Positioning Systems) and GIS (Geographic Information Systems), in the development of an efficient and environment-friendly target management system of artificial bait techniques for tsetse fly control in the Okavango Delta of northern Botswana.

"It is a large, remote, and dynamic environment," says Patrick. "With a game management area surrounded by agricultural land where farmers raise cattle, the Okavango presents a highly fragmented tsetse habitat structure," he adds. "Access is severely restricted by conditions that can change virtually every year with the rains and the floods which makes ground based transport difficult. Efficient application of the odour bait technique or chemical-impregnated 'target' screens for tsetse control is therefore constrained. Targets are easily lost and it is practically impossible to run an efficient programme of target maintenance under these conditions. Hence the need for a GPS/GIS management system," he says.

Mapping based software utilising satellite imagery gives a good idea as to what is the most likely suitable habitats for tsetse flies. Surveys are carried out to confirm the presence of flies and targets deployed. The software also records the position of each of the targets and these are overlaid on the base map to allow easy location and routine maintenance. This will include sachet replenishment, re-spraying with insecticide, and even re-building following damage by elephant and other large game. A colour coding system plots the progress of target management and shows those areas that are due for maintenance.

"GIS," says Patrick, "takes GPS points (geo-references on the form of co-ordinates) and overlays the data on base maps, in precisely the same location as it would be on the ground. Therefore what you see using the GIS management system is a 'birds-eye' view of what you would see on the ground. GIS is a judgement tool (identification of prime tsetse areas for target deployment) and a monitoring tool (target maintenance)," says Patrick, "allowing the latter be achieved with minimal vehicle damage to the environment by mapping tracks and plotting them on the map."

By using the GIS management system, Patrick was able to define the tsetse distribution limit throughout Okavango with the deployment and maintenance of approximately of 25,000 targets.

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Pinpointing Parasitoids for *Plutella xylostella*

By Dr Terry Mabbett

Plutella xylostella (diamond-back moth), the ubiquitous Lepidopteran insect pest of brassica crops, is a continual focus for IPM (Integrated Pest Management) studies. With the capacity to wipe out crops of cabbages, cauliflower, and broccoli, it has traditionally been controlled by intensive application of organophosphate and pyrethroid insecticides. This has invariably been accompanied by the well-documented problems of insect insensitivity (resistance) and pest resurgence, as well as potential insecticide hazard for spray operators and consumers alike. These ongoing problems are especially acute in the Asian/Pacific Region.

Ian Hatherly, a postgraduate student on the 2000/2001 MSc Pest Management Course at Imperial College Silwood Park in the United Kingdom investigated the use of various food sources as a means of boosting the activity of *Diadegma semiclausum* (Hymenoptera: Ichneumonidae). The field trials were carried out in commercial cabbage fields in Queensland, Australia, where *Diadegma semiclausum* is an important wasp parasitoid of *P. xylostella*.

Hatherly used food preference studies conducted in rearing cages to compare the effect of two flowering plants belonging to the Brassicaceae (*Lobularia maritime* [sweet alyssum] and *Rapistrum rugosum* [turnip weed] a common weed of cabbages in Queensland), and cabbage leaves treated with Envirofeast ™, a commercial food supplement.

Both sweet alyssum and turnip weed significantly increased the longevity of the parasitoid compared with insects reared on untreated cabbage leaves and leaves coated with the food supplement. Sweet alyssum also increased the longevity of the insect pest species compared with untreated cabbage leaves. The fecundity of individuals of both the parasitoid and the pest insect was increased significantly by sweet alyssum compared to those kept on untreated cabbage leaves. Egg viability of *P. xylostella* was unaffected by the presence of sweet alyssum.

Extension of these cage-conducted choice experiments into field trials clearly indicated that parasitism rates of *P. xylostella* by *D. semiclausum* is increased by intercropping cabbages with sweet alyssum and turnip weed. Whether or not the intercropping of brassica crops with either of these flowering plant species, which also belong to the Brassicaceae, could be a viable proposition clearly depends on whether the presence of sweet alyssum or turnip weed favours the parasitoid more than the pest. For *R. rugosum*, which is a common weed in cabbage fields in Queensland, the possible negative effect of clean weeding on the parasitism rates of *P. xylostella* by *D. semiclausum* appears to warrant further investigation.

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Host Plant Selection by Root Fly Pests of Brassicas and Alliums

By Dr Terry Mabbett

Mixed cropping in which two or more crops are grown in the same time and place is an age-old 'art' based on sound science. It is still widely practised on low-input smallholdings in the tropics, but largely ignored in the intensive field vegetable systems of temperate agriculture. With increasing movement towards integrated pest management, and away from reliance on stand-alone insecticide applications, there is renewed interest in the use of so called 'companion crops' to disrupt the feeding and breeding of specific field vegetable insect pests.

Helen Billiald, postgraduate student on the MSc Pest Management Course at Imperial College, Silwood Park, looked at this strategy for the management of *Delia radicum*, (cabbage root fly) and *Delia antiqua* (onion root fly). Cabbage root fly attacks a wide range of brassicas including cabbages, cauliflowers, and

brussel sprouts, and onion root fly is a serious problem on shallots and leeks as well as onions.

Twenty-four non-host plant species evaluated in both laboratory experiments and main field cage trials included bedding plants, spanning a wide range of canopy architectures and leaf colours, weed species commonly associated with these crops, and aromatic plants including marigolds (*Tagetes* sp). Marigolds are commonly touted as 'good' companion plants as their distinct odour, caused by plant volatiles, is considered to deter pest insects from landing on host plants in the immediate vicinity.

In laboratory experiments neither cabbage root fly or onion fly was deterred from landing on the foliage of aromatic plants or the adjacent host plants, dispensing with the 'chemical deterrence' theory. More detailed observations and records showed that female flies remained longer (188-403 seconds) on the leaves of non-host plants than on the leaves of host plants (70-80 seconds). And, contrary to earlier reports, landing on the leaves of non-host plants did not induce the flies to emigrate sooner.

For the main field cage trials in which the host plant species was surrounded by four plants of the same test species, host plant location by cabbage root fly and onion root fly was disrupted by, respectively, 20 and 14 of the 24 test species. But the 'companion' and other aromatic plants were no more effective at disrupting the oviposition of either fly than were any of the other non-host plants.

The number of eggs laid by cabbage root flies (but not onion root fly) on its host plant decreased with increasing height of the surrounding non-host plants. For onion root fly numbers of eggs laid on host plants decreased with increased leaf area of the surrounding non-host plants. The number of non-host plants surrounding the host plant species was the critical factor that determined the success of host plant location for both cabbage root fly and onion root fly. Effective disruption of host plant finding was only achieved when the ambitus of the host plant was completely surrounded by non-host plants.

These findings have some important implications for growers of field crop vegetables and especially those moving towards organic production systems in which the mixed cropping of, for instance, salad/bulb onions and bedding plants like marigolds could be a viable proposition. The findings that related host-finding disruption by non-host plants of common weed status show that the concept of clean weeding, especially using applications of herbicide, may need to be re-assessed.

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International Regulations are Standing in the Way of Insecticide Resistance Management of the Colorado Potato Beetle in Alberta, Canada

By Mark S. Goettel

The Colorado potato beetle, *Leptinotarsa decemlineata*, is considered one of the most destructive foliage feeding pests of potatoes worldwide. The application of insecticides has been the primary method used to control this pest. However, widespread and repeated use of chemicals as a control method has resulted in selection of insecticide resistant populations. A strategy to avoid, or at least delay selection of resistant populations is to rotate between chemical classes of insecticides because each class has a different mode of action. However, in most potato producing areas in North America, nearly all previously effective insecticides are no longer capable of reducing beetle populations. Consequently, this strategy is not possible. Most North American producers must rely on only 1 or 2 recently registered chemicals, and of course, this is a recipe for further selection of resistant populations.

An exception is in Alberta, Canada where beetle populations are still susceptible to all conventional chemicals registered against the beetle (Noronha et al 2001). Products from each chemical class are readily available and producers are urged to rotate between these classes as an insecticide resistance management strategy. Unfortunately, international regulatory constraints are hindering this. Because most of the chemical products are no longer effective in the United States, insecticide companies have allowed registrations of ineffective products against the beetle to lapse in that country. And because regulations in the U.S. prohibit use of a non-registered chemical insecticide against a pest of a food product that will be

imported into the U.S., potato processors in Alberta are restricting the use of many still effective products in southern Alberta because much of their products are exported south.

Import restrictions of food products treated with insecticides not registered in the importing country are aimed at ensuring the safety of the food supply. In this case, they are forcing producers to abandon resistance management through the rotation of chemicals, not because of safety issues, but because these chemicals are no longer effective in the importing country!

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Resistance Management from Around the Globe

Historical Records of Field Cotton Leafworm (*Spodoptera littoralis*) Resistance to Conventional Insecticides as Influenced by the Resistance Programs in Egypt from 1950-2002

INTRODUCTION The cotton leafworm, *Spodoptera littoralis* (Boisd) is a key polyphagous pest in Egypt. Without a hibernation period, cotton leafworm (CLW) is active year round, attacking cotton as well as more than 29 hosts from other crops and vegetables. The rate of CLW infestation can reach up to 50,000 egg-masses/acre, causing severe damage to leaves, buds, flowers, and bolls.

Hand picking CLW egg-masses is a reliable practice as well as a safe approach for control, particularly in the first generation of CLW on cotton in Egypt (El-Badawy et al 1980). However, this process is not enough to control CLW due to its overlapping generations. In addition, when cotton grows too big, this process becomes too difficult. Consequently, the Ministry of Agriculture (MOA) has had to spray the cotton crops every year despite hand picking.

The cotton leafworm has the ability to develop relatively quick resistance to most conventional insecticides. Several publications have confirmed a significant difference between the LC50 of the field and the laboratory strains (strains without exposure to any insecticide). These differences have indicated either a tolerance or a real resistance in the field strain to the conventional insecticides from organophosphates (OP), pyrethroids (PY), or carbamates (CAR) (Maher 1975, Yehia et al 1985a &b, El-Dahan et al 1985, El-Said and Sammour 1991, Rashwan et al 1991-92, El-Barmawy et al 1991-92, El-Sebae et al 1993, Allam et al 2000a&b).

PROGRAMS Historical records of low field performance of products or of cotton leafworm (CLW) resistance during 1950-2002 are presented in Table 1. R. M. Sawiki, in his local reports in Egypt, has indicted that field failure is the real criterion of resistance rather than the change in response to an insecticide. The following account details the history of resistance programs for cotton leafworm in Egypt.

Historical Records of Products of Low Field Performance or of Cotton Leafworm Resistance

Chemical Name	Year of Field Problem	Introduction	Cancellation	Return of Use
DDT	1956	1952	1956	-
Toxaphene	1961	1955	1961	-
Lindane	1964	1952	1970/1971	-
Endrine	1965	1961	1970/1971	-
Carbaryl	1965	1961	Cancelled from CLW,1966	-
Trichlorfon	1965	1961	1965	-
Fenitrothion	1967/71	1967	1970/71	-
Azinophosmethyl	1968	unknown	1968	-
Methy parathion	1966	1965	1969	-
Phosfolan	1972/73	1967	1973	-
Mephosfolan	1972/74	1967	1973	-
Monocrotophos	1971/ 72	1967	1997	-
Leptophos	1974	1972	1975	-
Methamidophos	1975	unkown	1997	-
tetrachlorvinphos	1976/ 77	1976	1977	-
Profenphos	1977	1976	still in use	-
Methomyl/Triflumuron	1985/ 86	1984 /85	1984 / 85	-
Cyanophos	1990-92	1988?	1993/94	-
Primiphos ethyl	1988?		1988?	-
Cypermetrin	1993	1975	1993	1999*
Alphacypermetrin	1993	1975	1993	-
*Back due to very low cost				

No Program (1950-1978)

During the 1950-1978, there was no CLW resistance management program. The Ministry of Agriculture sprayed the same product, e.g. trichlorfon, toxaphene, or carbaryl,3-4 times via airplane in the same area within the same season. Several products showed high levels of resistance (field failure) and have since been cancelled from the official cotton-spraying program against cotton leafworm: DDT, toxaphene, lindane, endrin, carbaryl, trichlorfon,

fentrotion, methyl parathion, leptophos, azinophos methyl, monocrotophos, tetrachlorvinphos, mephosfolan, and phosfolan, (Maher 1975, EL-Sebae et al 1993).

Cross-resistance was common and was detected between toxaphene and endrin, monocrotophos and trichlorphon, methyl parathion and tetrachlorvinphos, and carbaryl and methomyl (Maher 1975).

Egypt lost 50% of the national cotton yield due to countrywide resistance of the CLW to toxaphene in 1961. Toxaphene was used in four successive sprays in the same season at very high rates of 4L/acre in comparison to 1.5L/acre in 1956. An amount of 54,000 metric tons of the active chemical was used during 1956-1961 (EL-Sebae et al 1993). In 1961, Maher (1975) indicated that the total numbers of collected egg-masses of CLW from an area of 2 million acres was close to 10,000 million. The total treated area for CLW was 2,764,007 acres according to MOA records. Therefore, some areas were sprayed 1-4 times.

Rotation Program (1979-1993)

The first nationwide CLW resistance management program was adopted by Egypt in 1979 in cooperation with Dr. Sawiki to prevent or delay CLW resistance to pyrethroids as well as other insecticides. Dr. Sawiki began communications with managers of the Ministry of Agriculture and visited Egypt in 1975 and 1983. A program based on organophosphates (OP) + insect growth regulators (IGR) as the first spray for the first generation of CLW, OP as a second spray to face CLW, pyrethroids (PY) as a third spray to control cotton bollworm (CBW) and possibly CLW, OP for the fourth spray for CBW, and carbamates (CAR) as the final spray to control CBW, was initiated. During this period, the area treated for CLW larvae was minimal which achieved a good delay in resistance to most of the pyrethroids, in addition to a good crop yield. The CLW almost disappeared from a third of the total cotton area in the 5 governorates of south Egypt during 1989-1997 with very low infestation in the delta-north. This may be a result of two different modes of action of organophosphates and insect growth regulators in the first generation of CLW, accompanied by sterilization and reduction of fertility and fecundity of CLW moths due to the insect growth regulators (Radwan et al 1985).

Pyrethroids were introduced in 1975. Resistance of CLW to pyrethroids did not exceed 10-fold in 1980 (El-Dahan et al 1985). However, the resistance of CLW to pyrethroids ranged between 25.5- to 6667-fold by 1990 (El-Barmawy et al 1991-92). Resistance to pyrethroids was delayed for roughly 7-10 years by the efforts of Sawiki and the Egyptian government. Dr. Sawiki's approach to reducing CLW resistance required that pyrethroids be applied only once per season and solely on cotton.

The Ministry of Agriculture in Egypt maintained Sawiki's suggested policy on pyrethroids. In 1988, pirimiphos ethyl also was cancelled from the official spraying program due to increased resistance and low performance. In addition, cypermethrin, alphacypermethrin, and several other products were cancelled from Egypt's official resistance management program due to resistance problems and/or the increased occurrence of sucking insects. However, in 1999 cypermethrin was reintroduced because of its low cost and because resistance exists to virtually all pyrethroids and they are saved primarily for use on cotton for bollworm.

In 1991, ground motors replaced airplanes for spraying cotton. This last change was due to the start of the Improved Ground Application Techniques Project (IGATP).

Rotation Program with the Addition of some Alternatives (1993-95)

Beginning in 1993, several alternatives were used for CLW control: mineral oil, sulphur, B.t. products, irrigation with kerosene, and CLW-pheromones. Conventional insecticides were used below the recommended doses, especially in 1994. The rotation program was not stressed as strongly during this period. Disruption pheromones for pink bollworm (PBW) were used in small areas initially, reaching 50% of the total cotton area by 1995. In addition, conventional insecticides were applied in cases where infestations reached 3% in the bolls, and the CLW began to slowly rebuild its fecundity and fertility. This increase was due to the low use of conventional insecticides, including insect growth regulators for CLW or mixed CLW/CBW.

The addition of alternatives to the resistance management program resulted in a tremendous decrease in the cost of importation of conventional insecticides. In addition, cotton yields were still maintained at an acceptable level. During this time, cyanofos showed poor performance and therefore was cancelled from the official program around 1994.

Alternatives Period, Including Extensive use of PBW-Pheromones (1995-98)

By 1995, the rotation program had been discontinued. The resistance management program for CLW began to depend mostly on alternatives with the spray of conventional insecticides used only when infestation reached economic injury levels. In 1996, several conventional products were banned due to possible carcinogenesis (class B or C group carcinogens). Insect growth regulators were used alone without mixing with an organophosphate to conserve

natural enemies. In 1997, all conventional insecticides for the control of CLW were cancelled in vegetables and orchards and the resistance control program depended primarily on alternatives. Disruption pheromone for PBW was used extensively in all cotton areas.

CLW continued to rebuild its fecundity and fertility slowly as a result of the low pressure of conventional insecticides in cotton and outside cotton on vegetables and grapes. CLW returned to south Egypt after a disappearance of at least 8 years. Very high infestation rates in the delta-north area of Egypt also began to be recorded as early as 1998. By this time, spiny bollworm became established in new areas and occupied almost all governorates due to the commercial pheromone application that had targeted pink bollworm. This pheromone application saved the clean cotton bolls for the spiny bollworm. Cotton yield was significantly lower in 1998.

Rotation Program (1999-now)

Almost all slow-acting alternatives were cancelled, specifically from cotton uses, and a return to the normal rotation program (with the exception of agrin) occurred in 1999. Agrin (*Bacillus thuringiensis* subsp *aegypti*) had been used to control CLW during the egg-mass hatching period. Insect growth regulators alone were kept for the first spray in the control of newly hatched CLW larvae. Organophosphates were mixed with the growth regulators in the event of a high infestation. Cotton yields improved dramatically and CLW and CBW were better controlled. Natural enemies started to be seen on cotton leaves in considerable numbers.

OUTCOME of PREVIOUS PROGRAMS Egypt has suffered from cotton leafworm resistance for over 50 years. Initially, considerable tons active ingredient of conventional insecticides were used for CLW control, especially in the non-rotation program period (e.g. DDT as 13,500/1952-1971; Carbaryl as 21,000/1961-1987; Lindane as 11,300/1952-1987; and Endrin 10,500/1961-1981). During the rotation period and after, amounts of conventional insecticides has been decreased. Now, the Ministry of Agriculture allows only 4 insect growth regulators, 2 organophosphates (chlorpyrifos ethyl and profenfos), 1 pyrethroid (cypermethrin), and 1 carbamate (carbaryl) to combat CLW/CBW resistance. After being banned as carcinogens in 1996, these last two products, cypermethrin and carbaryl, were reintroduced because of national need and an absence of new chemicals. Some of the compounds now used show more than 10-fold resistance in CLW, specifically cypermethrin. Chlorpyrifos-ethyl (Dursban), which was introduced in Egypt in 1970, has had no record of field failure until now. Sebae (personal communication) believes that Dursban has more that one site of action.

Isolated Areas without any Insecticides (1996-now)

In 1996, the Ministry of Agriculture established several isolated areas as organic farms to produce vegetables and crops without any chemicals (5-6 governorates).

Future of the Cotton Resistance Program in Egypt

It is generally assumed that the rotation program will continue for significant period of time. The Ministry of Agriculture will likely continue to encourage the use of alternatives and natural products, especially if they have rapid kill rates. Different insect growth regulator groups will continue to be used during the egg-mass period to conserve the natural enemies and control CLW at the same time. Because these groups of regulators have different modes and sites of action from conventional insecticides, a few conventional insecticides will continue to be used until alternatives with quick kill rates can be developed.

Egypt is looking forward to the development of new products with new/unique modes of action, like spinosad (Thompson et al 1997). Temerak (under publication) indicates that this product is not easily affected by existing resistance mechanisms to conventional insecticides. He also adds that field populations of CLW with high levels of resistance to conventional insecticides are more susceptible to this product. He expects that spinosad may have a great future in the integrated pest management (IPM) of CLW in Egypt. Currently, the Ministry of Agriculture is still testing this product.

Spinosad could play a significant role to combat conventionally resistant insects as a result of its novel mode of action (Salgado 1997). Some individuals have even indicated that the low toxicity of Spionosad to natural enemies should allow it to be easily incorporated into most integrated pest management programs (Bret et al 1997, Peterson et al 1997). Based on United States Environmental Protection Agency (USEPA) reports, this product won the Green Chemical Challenge Award from the White House in 1999.

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Pyrethroïd Resistance in *Helicoverpa armigera* (Hübner): Recent Developments and Prospects for its Management in Côte d'Ivoire, West Africa

ABSTRACT The susceptibility to pyrethroids in the cotton bollworm *Helicoverpa armigera* (Hübner) from Côte d'Ivoire, West Africa, decreased steadly for years to such an extent that field infestations became critical and culminated in 1998. Accordingly, a relevant resistance management strategy was developed. Innovative programmes were implemented with several non-pyrethroid insecticides to control the first generation of *H. armigera* at the cotton vegetative stage, as pyrethroid insecticide sprays were restricted to the cotton fruiting stage. Three-year data showed that pyrethroïd resistance management programmes based on endosulfan or profenofos sprays at the cotton vegetative stage were effective in controlling *H. armigera* infestations and ensured satisfactory seed cotton yields. Similar programmes performed with new insecticides (spinosad, indoxacarb) appeared to be at least equivalent to endosulfan or profenofos based programs. The wide adoption of the insect resistance management strategy at the national level contributed to significantly reduced field populations of *H. armigera* for the last two years and helped stabilize the resistance level.

KEY WORDS Cotton, *Helicoverpa armigera*, pyrethroid resistance management programs, non-pyrethroid alternates, Côte d'Ivoire.

INTRODUCTION Early pest control strategies adopted in Côte d'Ivoire have contributed to increased seed cotton yields (Vaissayre et al., 1984). Pest management recommendations, while including selections of tolerant varieties and cultural practices, rely heavily on chemical treatments for seeds and plants. Accordingly, arthropod pest management is currently achieved through the use of pyrethroid-organophophate insecticide mixtures in order to control the whole cotton pest complex. On one hand, organophosphate insecticides are used either at normal dosage rates against sucking pests such as the yellow mite *Polyphagotarsonemus latus* (Banks), the whitefly *Bemisia tabaci* (Gennadius), and the aphid *Aphis gossypii* (Glover), or at reduced dosage rates against some leaf pests such as the Egyptian leafworm, *Spodoptera littoralis* (Boisduval), and the Leaf roller, *Syllepte derogata* (Fabricius). On the other hand, pyrethroids target the bollworm complex, exocarpic species (*Helicoverpa armigera* (Hübner), *Earias insulana* (Boisduval), and *Diparopsis watersi* (Rotchilds)) as well as endocarpic species (*Cryptophlebia leucotreta* (Meyrick) and *Pectinophora gossypiella* (Saunders)).

Known as very effective in controlling most cotton bollworm pests, pyrethroids have been widely used for more than twenty years in Côte d'Ivoire. Recently

(since 1994), during exceptional pest outbreaks, farmers have complained about several supplied insecticides. Moreover, cases of ineffectiveness of the pest control programme against *H. armigera* have been reported in Côte d'Ivoire (Ochou, 1994; Ochou et al., 1998). At the same time, most countries in West Africa (Benin, Burkina Faso, Guinea, Mali, Senegal, and Togo) experienced serious similar problems (Anonymous, 1999). With this regard, the routine calendar-based programme of applying six fortnightly sprays of pyrethroid-organophosphate insecticide mixtures over the whole cotton season (from 45th to 115th DAE -Day After Emergence of cotton) has been questionned as a critical decrease in the pyrethroïd susceptibility in *H. armigera* was noticed in 1995 by Vassal et al. (1997), in the routine laboratory monitoring of LD50 at Bouaké, Côte d'Ivoire.

The pyrethroid resistance in *H. armigera* was confirmed in 1996 by Martin et al. (2000) and (Ochou et al., 1998). Similar cases of resistance were reported in *H. armigera* in Australia (Gunning et al.,1984), Thailand (Collins, 1986), India (McCaffery et al., 1989), Turkey (Riley, 1990), Indonesia (McCaffery and Walker, 1991), China (Shen et al., 1992), and India (Armes et al., 1994). Inspired by the "Australian" strategy (Sawicki and Denholm, 1987), an insect resistance magement (IRM) strategy was designed in Côte d'Ivoire. Accordingly, earlier recommendations were amended and innovative pyrethroid resistance management programmes have been implemented, developed, and adopted nationwide by cotton farmers since 1998.

The local IRM strategy aimed at preventing and managing mainly the pyrethroid resistance in *H. armigera* by lessening pyrethroid selection pressure by restricting their use while using non-pyrethroid alternates in a kind of "window" programme. The insect resistance management (IRM) plan divides the *H. armigera* damage season into the vegetative stage (30-66 DAE) and the fruiting stage (73-115 DAE) as only two generations of *H. armigera* are known to occur through the cotton season. From the early stage up to early flowering of cotton plants, the innovative strategy advises sprays of selected non-pyrethroid insecticides (Table 1) for the control of both *H. armigera* and other key pests such as mites. During the second stage, a period that coincides with maximum flowering and the largest numbers of the most damaging endocarpic bollworm species (*C. leucotreta* and *P. gossypiella*) and exocarpic bollworms *(H. armigera* and *D. watersi*), the strategy recommends maintaining pyrethroid-organophosphate spray mixtures.

The present paper addresses the historical profile in the development of *H. armigera* resistance to pyrethroid insecticides in Côte d'Ivoire and the actual impact of the three year nationwide implementation of the insect resistance management (IRM) strategy with respect to actual field infestation profiles, evolutions of earlier pyrethroid resistance levels, and the effectiveness of new alternate non pyrethroid insecticides.

Table 1: Pest control schemes recommended with regard to restriction on the use of pyrethroids and to major pests distribution within cotton areas of Côte d'Ivoire.

COTTON AREAS		VEGETATIVE STAGE 45-73 DAE	FRUITING STAGE 73-101 DAE	FRUITING STAGE 101-122 DAE
ZONE 1-1 Extreme North	Major pests	*H. armigera, Earias*, Leafworms	*H. armigera, Earias, Diparopsis, C. leucotreta, P. gossypiella*, Leafworms	*H. armigera, Earias, D. watersi, C. leucotreta, P. gossypiella, B. tabaci*
	Number of sprays	2	2	2
	Insecticides	Endosulfan 700, profenofos 750, Spinosad 48 Indoxacarb 25, Thiodicarb 750 g/ha	Pyrethroids+OPs (leafworms)	Pyrethroids + Aleurodicides
			Pyrethroids + non OPs (leafworms)	
	Pyrethroid use restriction	**Use only Non Pyrethroids**	**Use Pyrethroids only after August 10th.**	
ZONE 1-2 North	Major pests	*H. armigera, Earias,P. gossypiella,* Mites, Leafworms	*H. armigera, Earias, C. leucotreta, P. gossypiella, D. watersi*, Leafworms, Mites	*H armigera, Earias, C. leucotreta, P. gossypiella, D. watersi,* Leafworms
	Number of sprays	2	2	2
	Insecticides	Endosulfan 700, profenofos 750, Spinosad 48 Indoxacarb 25, Thiodicarb 750 g/ha	Pyrethroids + OPs (leafworms)	Pyrethroids + OPs (leafworms)
			Pyrethroids + non OPs (leafworms)	Pyrethroids + non OPs (leafworms)
	Pyrethroid use restriction	**Use only Non Pyrethroids**	**Use Pyrethroids only after August 10th.**	
ZONE 2 South	Major pests	*H. armigera, Earias, C. leucotreta, P. gossypiella,* Mites, Leafworms	*H. armigera, E. insulana,* Leafworms *C. leucotreta, P. gospiella,* Mites	*H. armigera, E. insulana,* Leafworms *C. leucotreta, P. gospiella*, Mites,
	Number of sprays	2	2	2
	Insecticides	Endosulfan 700, profenofos 750, Spinosad 48 Indoxacarb 25, Thiodicarb 750 g/ha	Pyrethroids + OPs acaricides	Pyrethroids + OPs (leafworms)
			Pyrethroids + non OPs acaricides	Pyrethroids + non OPs (leafworms)
	Pyrethroid use restriction	**Use only Non Pyrethroids**	**Use Pyrethroids only after August 20th.**	

· Pyrethroids: It is advised to diversify active ingredients of pyrethroid family. Their use is restricted to after August 10 and 20 respectively for the northern and southern areas

· Non Pyrethroids: It is advised to associate some non pyrethroids with OPs (especially spinosad 48g/ha, indoxacarb 25g/ha) in order to control the whole pest complex.

· OPs acaricides: Targeted against mites mainly, most used OPs are triazophos 250 g/ha, profenofos 300 g/ha, chlorpyriphos ethyl 300 g/ha. They are used in mixture with Pyrethroids.

· OPs (leafworms): Targeted against leafworms, most used OPs are triazophos 150 g/ha, profenofos 150 g/ha, chlorpyriphos ethyl 150 g/ha. They are used in mixture with Pyrethroids.

· Non OPs acaricides: It is advised to use for the fruiting stage Non OPs acaricides after the use of profenofos 750 at vegetative stage, in order to limit the selection pressure on mites; selected Non OPs acaricides are diafenthiuron 300 g/ha, pyrimidiphen 50g/ha, pyridaben 150 g/ha.

· Aleurodicides: Targeted against *Bemisia tabaci* in the extreme north, dimethoate 300 g/ha, acetamiprid 10g/ha or benfuracarb 250g/ha are used in mixture with Pyrethroids.

MATERIALS and METHODS

Laboratory monitoring of LD50:

The susceptibility of *H. armigera* to pyrethroids has been monitored in Côte d'Ivoire since 1985. A topical application method was performed on different strains collected and reared in the entomological laboratory of the cotton research station based at Bouaké (Martin et al., 2000). The reference strain was collected in 1977. This strain, which has never been exposed to pyrethroids, is being reared in the CIRAD Montpellier laboratory. LD50 (in µg ai/g larva) values were determined for two active ingredients: cypermethrin and deltamethrin. Statistical analyses of data were performed with the log-probit method (Finney, 1971).

Monitoring of field population dynamics:

Field infestation levels of *H. armigera* have been monitored since 1991 in Côte d'Ivoire through a multilocal network involving 250-500 cotton farms chosen at random in groups of 10 fields in every cotton zone across the whole cotton area. Fields were scouted once a week by extension service agents from 30th to 122nd DAE (Day After Emergence of cotton) on a sample of 30 plants per field. Plants were selected at random and examined in groups of 5 consecutive plants along a line across the diagonal of the field, avoiding the outer 10 m of the field. The whole plant (leaves, buds, flowers, bolls) was scouted for the *H. armigera*. Annual variations of field infestation levels were determined as well as their seasonal and spatial profiles.

Implementation of insect resistance management (IRM) programmes:

First of all, IRM programmes were implemented in 1997-1998 with two non-pyrethroid insecticides (endosulfan 750-700g/ha and profenofos 750 g/ha). Field assessment of innovative programmes was performed at several sites in a paired plot design with 10 replicates each. Individual homogenous ½ ha plots were divided into two subplots of ¼ ha each, representing the control and the experimental programme. Cotton fields planted between June 2nd and 10th were selected. Innovative programmes were assessed with regard to bollworm pest control effectiveness and to seed cotton yields as compared to the control.

Subsequently, the IRM programmes were adopted first in the northern part of the country in 1998 and then nationwide since 1999. In practice, the adoption of the IRM programmes led to the determination of a pyrethroid-free season nationwide. The pyrethroid-free season is established by calendar dates that are related to optimal cotton sowing dates with regard to determined cotton growing zones. Deadlines are set to August 10 and August 20 respectively for northern and southern regions.

Assessment of new alternate non-pyrethroid insecticides:

New alternate non-pyrethroid insecticides were investigated in order to replace eventually endosulfan and profenofos, which for many reasons were being questioned (high toxicity, resistance risks, etc.). Accordingly, studies were undertaken in 1999-2000 to compare biological effeciency of eight (8) insecticides: endosulfan 750 g/ha (Phaser 375 EC, AgrEvo), profenofos 750 g/ha (Curacron 500 EC, Novartis), spinosad 48g/ha (Laser 480SC, Dow AgroScience), indoxacarb 25g/ha (Avaunt 150 SC, Dupont), isoxathion 600 g/ha (Karphos 600 EC, Calliope), thiodicarb 750 g/ha (Larvin 375 EC, Rhône Poulenc), chlorpyrifos éthyl 720 g/ha (Dursban 480 EC, Dow AgroScience), and deltamethrin 12 g/ha (Decis 12 EC, AgrEvo) used here as reference.

Insecticides were assessed within a Complete Bloc Design with six replicates. Individual plots were of 10 rows x 12 meters. Sprays were performed with an adapted horizontal boom knapsack sprayer debiting 60 l/ha of product-water mixture. Plots were treated every 14 days with the same insecticide from 45th to 115th DAE. Sucking pests (*A. gossypii, J. fascialis, D. voelkeri*) and bollworms pests (*C. leucotreta, P. gossypiella, H. armigera, E. Insulana*) and *S. littoralis* were scouted directly on plants or on shed organs and green bolls.

RESULTS

The development of resistance in H. armigera*:*

Laboratory data obtained within 1996-1998 showed a significant increase in the LD50 for both cypermethrin (Figure 1) and deltamethrin (Figure 2). A clear tendency for pyrethroids resistance to develop in *H. armigera* appeared. Calculated resistance factors were about 10 and 20 for cypermethrin and deltamethrin respectively. This situation suggested that the bollworm populations subjected to successive tests were becoming heterogeneous with an important fraction of *H. armigera* populations escaping the effect of pyrethroids.

Field data recorded for eight consecutive years pointed out that the pest infestation profiles changed deeply from 1991 to 1998. Two main phases were distinguished in the pest annual, seasonal, and geographic variation patterns (Figures 3, 4 & 5).

In 1991-1994, variations in the pattern showed that average annual infestation levels remained low and fluctuated within 0.08-0.27 larvae/30 plants (Figure 3). Within this phase (1991-1994), larval populations generally appeared not earlier than July 15th, depending mainly on cotton phenological stage and usually at flowering within 44-51 DAE. Larval

infestations remained low until early October, and infestation peaks occurred within the period of mid-October and mid-November. Infestation levels depended on the period in which cotton was sown (Figure 4). There was a clear tendency that showed that late-sown cottons (July-August) were more infested than early-sown cottons (May-June). The geographic distribution of infestations showed that heavy infestations were confined to a few localities in the western part of the cotton area (Figure 5).

In 1995-1998, during the second phase, a totally different pattern was observed in the pest infestation profiles. Average annual infestation levels were higher than during the first phase, and increased from 0.23 to 0.87 larvae/30 plants. The most critical infestation level was recorded in 1998. Larval populations appeared at high levels earlier than before, within July 21st - August 11th. Then, their occurrence increased quickly to reach several peaks in different periods: mid August, early September and early October. The tendency that only late sown cottons were heavily infested was no longer available. Important infestations were noticed on early-sown cottons as well as on late-sown cottons. In contrast to its earlier geographic distribution, *H. armigera* infestation outbreaks occurred in almost all of the cotton area.

Effectiveness of IRM programmes:

Data presented in Table 2 indicated that innovative programmes based on endosulfan 750 g/ha appeared to be at least equivalent to routine-based programmes. Endosulfan sprays at the cotton vegetative growing stage were more effective in controlling *H. armigera* infestations and ensured satisfactory seed cotton yields. The innovative programme performed with profenofos did not show a significant difference as *H. armigera* infestations were relatively low throughout most test sites. However, overall data showed satisfactory results in controlling endocarpic bollworm complex and in ensuring satisfactory seed cotton yields.

Table 2: Comparison of the effectiveness of non-pyrethroids based programmes with regard to *H. armigera* (average number of larvae/30 plants), endocarpic bollworms (average number of larvae p. 100 green bolls) and seed cotton yield (kg/ha) .

Comparative programmes	Participants	*H. armigera* T1-T2	*H. armigera* T1-T6	Endocarpic bollworms	Seed cotton yield
Check (Pyrethroids-OPs)	45	1.13 b	3.71 b	1.5	1209 b
Endosulfan 750 g/ha		0.27 a	1.51 a	1.1	1402 a
Check (Pyrethroids-OPs)	30	0	0.73	1.30 b	2340
Profenofos 750 g/ha		0.03	1.23	0.80 a	2343

T1-T2: average data over the vegetative stage of cotton, comprising two applications of defined insecticides.

T1-T6: average data over the vegetative and fruiting stages of cotton.

Impact of nationwide adoption of the IRM strategy on field infestations of H. armigera*:*

The main picture that came out of the nationwide adoption of the insect resistance management strategy is the important decrease in the field populations of the *H. armigera* over the last two years. Sufficient elements highlighted by data described below revealed the impact of the IRM programmes.

Annual variations for the last two years (1999 and 2000) pointed out an important regression of *H. armigera* field infestation levels (Figure 3). The average annual infestation levels dropped significantly from 0.87 larvae p. 30 plants in 1998 to 0.02 larvae p. 30 plants in 1999 and 0.10 larvae p. 30 plants in 2000. Over the last two years, a new pattern emerged in the pest seasonal infestation profile (Figure 4). Indeed, field data showed that infestations were rare and remained very low throughout the whole cotton-growing season. The pest outbreaks noticed in overall cotton areas in years 1995-1998, particularly in 1998 when several localities experienced the highest levels of infestations ever reached, dropped significantly between 1999-2000 and important distribution limited to only a very few localities (Figure 5).

Impact of nationwide adoption of the IRM strategy on the evolution of H. armigera *resistance levels:*

H. armigera infestations were relatively low throughout the last two seasons in most test sites and it was very hard finding larvae to perform laboratory tests. Topical application tests were performed on strains collected at different periods of the year, on various crops, and in various cotton areas in 1999 and 2000 (Table 3). LD50 values were more or less equal to values recorded since 1996 for deltamethrin. High deltamethrin resistant levels were obtained for strains collected on cotton in October. It appeared clearly that LD50 levels obtained for cypermethrin (Figure 1) and deltamethrin (Figure 2) did not increase significantly from 1998 but remained constant at the same levels or decreased in 2000.

Effectiveness of newly alternate non-pyrethroid insecticides:

Data presented in Table 4 showed that new insecticides such as spinosad (48g/ha) and indoxacarb (25 g/ha) were at least equivalent to deltamethrin in effectiveness against

most main pest components, with respect to *A. gosypii* (number of infested plants on 3 rows), *D. voelkeri* (average number of the insect p. 30 plants), *S. littoralis* (average number of larvae p. 30 plants), *H. armigera* (average number of larvae/30 plants), *E. insulana* (average number of larvae p. 30 plants), endocarpic bollworms (average number of larvae p. 100 green bolls), and the percent bored organs in the shedding.

DISCUSSION The performance of the IRM strategy explains indeed the reason of its wide adoption by farmers. By using alternate insecticides at the vegetative stage and by limiting pyrethroid use to the fruiting period, sufficient control of exocarpic bollworms and sucking pests was achieved. The present study pointed out the strengths and weaknesses of non-pyrethroids insecticides. Results showed differential activities with respect to insect species. Some insecticides may need to be reinforced by other insecticides in such a way to control the whole arthropod pest complex. The spectrum activity of proposed alternate pyrethroid insecticides should be considered in order to justify their positioning with regard to cotton crop phenology and seasonal development of main pest species.

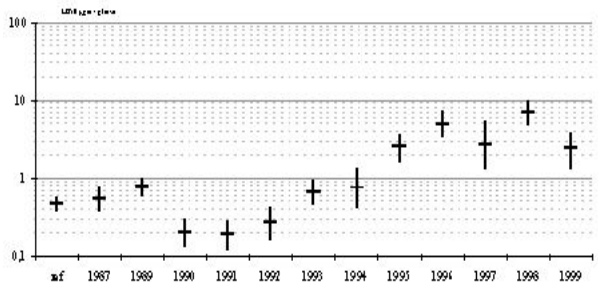

Figure 1: LD50 survey of cypermethrin from 1987 to 1999 with topical application tests on Helicoverpa armigera Bouaké strain.

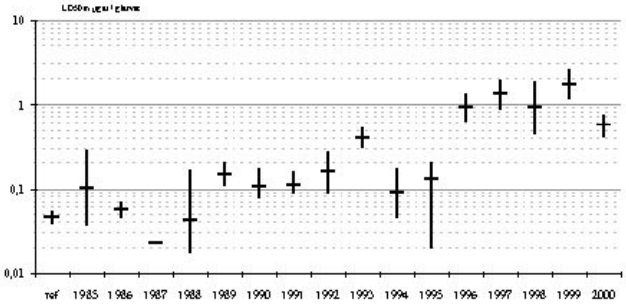

Figure 2: LD50 survey of deltamethrin from 1985 to 1998 with topical application tests on Helicoverpa armigera Bouaké strain

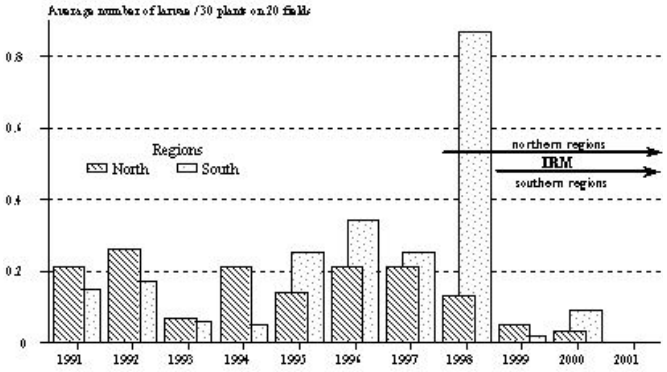

Figure 3: Annual variations of average field infestation levels of H. armigera in cotton areas of Côte d'Ivoire before and after IRM

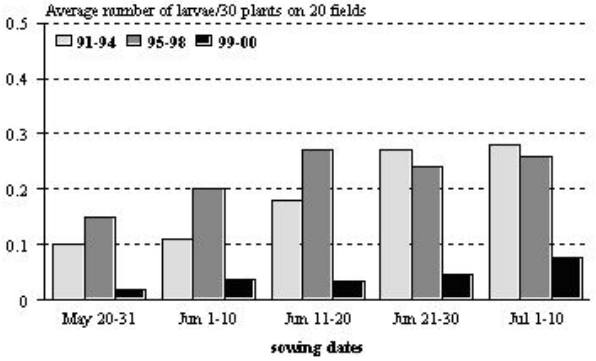

Figure 4: Comparison of average infestation levels of H. armigera according to sowing decades in northern cotton zones

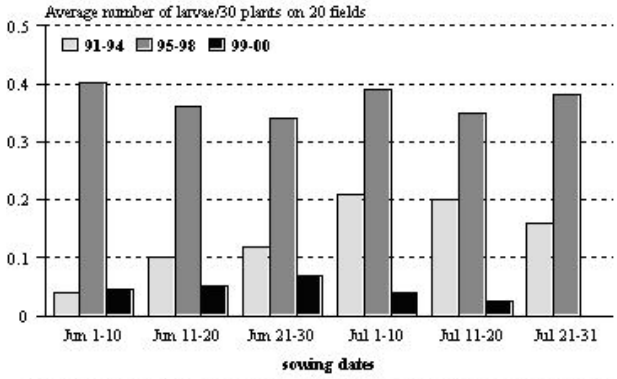

Figure 5: Comparison of average infestation levels of H. armigera according to sowing decades in southern cotton zones

Endosulfan is used in pyrethroid resistance management in Australia despite its resistance level in *H. armigera* (Sawicki and Denholm, 1987, Gunning and Easton, 1994). Actually no resistance to endosulfan, as for other pyrethroids alternatives, has been detected in Côte d'Ivoire. However, its recommendation is being questioned with regard to its toxicity. Some work is being done (Ochou & Martin, 2000) to revise the dosage in order to adapt relatively low doses of endosulfan or profenofos to the actual *H. armigera* infestation pressure in the fields. Future recommendations based on research data indicate effective minimal dosages at 525 g/ha and 500 g/ha for endosulfan and profenofos respectively. Furthermore, micro encapsulated formulations of endosulfan, assumed safer than the EC formulations, are being tested. They are to be recommended in the near future.

Table 3: LD_{50} and Resistance Factor (RF) for deltamethrin on field strain collected in various localities in 1998, 1999 et 2000.

Collection sites	Date	n	LD_{50} (µg /g)	Confidence intervals 95 %	slope ± es	R.F.
Bouaké	susceptible	-	0,046	0,039-0,055	2,10 ± 0,26	-
Bouaké	Juillet 98	180	0,910	0,448-1,849	1,02 ± 0,24	23
Bouaké	Mars 99	160	0,815	0,502-1,127	1,58 ± 0,33	18
	Octobre 99	120	1,740	1,187-2,649	1,52 ± 0,31	38
	Novembre 99	160	0,833	0,416-1,276	1,22 ± 0,28	18
Bouaké	Avril 00	120	0,418	0,136-0,814	0,95 ± 0,18	9
	Octobre 00	120	0,588	0,418-0,758	1,56 ± 0,22	13
	Novembre 00	120	0,320	0,115-0,658	1,58 ± 0,36	8
	Décembre 00	120	0,303	0,068-0,578	1,53 ± 0,37	7
Sarhala	Aug-98	180	0,453	0,185 - 1,112	0,71 ± 0,26	10
Ouangolo	Oct-98	180	0,777	0,491 - 1,228	1,60 ± 0,41	17
Niofoin	Oct-99	240	1,057	0,768 – 1,449	1,41 ± 0,21	23
Mankono	Oct-00	180	0.299	0,151 – 0,466	1,66 ± 0,25	7

Table 4: Comparison of effectiveness of alternate non pyrethroid insecticides

Active ingredients (g/ha)	*Aphis gossypii*	*Dysdercus völkeri*	*Spodoptera littoralis*	*H. armigera*	*Earias insulana*	% bored organs	Endocarpic species
Deltaméthrine 12	48.83 bcd	165.67 ab	1.50 ab	4.33 bc	1.00 c	32.35 ab	5.38 a
Indoxacarb 25	43.33 bc	181.67 ab	0.67 a	4.83 bc	0.67 abc	39.45 b	11.83 c
Spinozine 48	56.83 d	273.00 c	0.83 a	2.67 ab	0.17 ab	18.84 a	5.67 a
Profénofos 750	36.83 b	187.00 ab	1.00 a	2.67 ab	0.17 ab	36.84 b	9.41 abc
Endosulfan 750	36.83 b	222.67 bc	1.00 a	1.67 a	0.00 a	21.18 a	6.31 abc
Thiodicarb 750	51.50 cd	145.67 a	0.33 a	6.33 c	0.83 bc	30.07 ab	11.61 bc
Chlorpy. éthyl750	22.17 a	127.5 a	2.67 b	5.33 c	1.17 c	43.53 b	6.22 ab
Isoxathion 600	57.17 d	133.67 a	0.67 a	3.50 abc	0.17 ab	45.38 b	6.05 abc
Transformation			Sqr(x+1)	Sqr(x+1)	Sqr(x+1)	Sqr(x+1)	Sqr(x+1)
F	8.58	6.85	3	3.83	3.84	3.49	2.32
CV	22.58%	25.53%	23.71%	20.29%	18.11%	19.81%	23.83%
Signification (5%)	HS	HS	S	HS	HS	HS	S

IRM programmes with new insecticides such as spinosad (48 g/ha) and indoxacarb (25 g/ha) proved as effective as the earlier IRM programmes. Various benefits related to these new insecticides strongly advise their use as alternatives to endosulfan or profenofos by following a rational rotation plan. To be widely adopted, these insecticides should present more acttractive costs to farmers. In addition, actual interests are focusing on some insecticides which are able to control both exocarpic and endocarpic bollworm species, *H. armigera* as well as *P. gossypiella* and *C. leucotreta*. With this respect, novaluron (50-100 g/ha) and méthoxyfenozide (240 g/ha) are ending two-year trials. In the pyrethroid resistance prevention plan, these two insecticides could be used preferably at the fruiting stage in contrast to endosulfan, which is restricted to the vegetative stage.

Conjoining laboratory activities are being achieved to help set more reliable strategies and improve the whole pest management strategy. Bioassays performed with other classes of insecticides, especially alternate non-pyrethroïd insecticides such as DDT, endosulfan, profenofos, indoxacarb, and spinosad did not show any cross-resistance with pyrethroids in *H. armigera* (Martin, unpublished data). With regard to the pyrethroïd resistance mechanism there is evidence that resistance extended to all pyrethroids tested may be due to increased metabolic detoxification as resistance could be eliminated by treatment with pyrethroid and piperonyl butoxide (Martin et al. 2000). Further studies are now being performed to investigate the biochemical mechanisms of pyrethroïd resistance in *H. armigera* in Côte d'Ivoire.

CONCLUSION Laboratory data suggested that the strategically restricted use of pyrethroids on *H. armigera* has limited selection for resistance. On the whole, the wide use of alternate insecticides such as endosulfan or profenofos on cotton crops reduced the selection pressure of pyrethroids on *H. armigera*. Other pyrethroid alternatives such as spinosad, indoxacarb, and thiodicarb could be used in the first stage as well. The relatively low levels of field infestations due to *H. armigera* over the last two years confirmed the success of the adoption of the resistance management programmes evolved over the whole cotton zones in Côte d'Ivoire.

The overall positive results obtained from the nationwide development of "window" programmes supported the rational use of insecticides. For the resistance management to be sustainable, there is a clear need to educate cotton farmers in Côte d'Ivoire in order to allow them use rationally alternative non pyrethroid insecticides. Farmers need to perform spray thresholds during the cotton vegetative stage on the

basis of crop scouting in order to reduce costs and avoid unnecessary applications. To achieve this purpose, farmers need appropriate field diagnostic tools and techniques for monitoring, along with other facilities such as educational materials illustrated with simple texts and colour photographs of pests and their damage that will aid individual decision-making. It is recommended that the *H. armigera* resistance management strategy on cotton be applied to other crops, especially vegetable crops, where large amounts of pesticides are used, and alternatives to chemical control should focus on varietal resistance characters.

ACKNOWLEDGEMENTS We acknowledge the cotton development companies of Côte d'Ivoire (CIDT, IC, LCCI) for their financial support and the technical assistance of their research and development staff in collecting field data. Chemical samples were provided by Aventis, Dow AG, Syngenta, Dupont, Calliope, ALM, and STEPC.

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Positive and Negative Cross-resistance to Pyrethroids in *Helicoverpa armigera* from West Africa

ABSTRACT *Helicoverpa armigera* is the major insect pest of cotton in Africa, Asia, and Australia. Populations recently developed resistance to pyrethroids in West Africa via the overproduction of cytochrome P450 leading to treatment failures. One way to overcome this problem and to revert the resistance is the use of antiresistant compounds, more active against the resistant individual than against susceptible one. We found an organophosphate that has this antiresistant property, the triazophos. This insecticide is currently used to manage resistance in West African *H. armigera*.

KEY WORDS *Helicoverpa armigera*, resistance, insecticides, cotton, West Africa.

INTRODUCTION In West Africa, *Helicoverpa armigera* (Hübner) is an important pest of cotton and vegetable crops. Pyrethroids were used for control in the field for the beginning of 1980s. In 1996, following the failure of treatments to control *H. armigera*, pyrethroid resistance was diagnosed (Vassal et al. 1997). Cases of resistance have already been reported in *H. armigera* in Australia (Gunning et al., 1984), Thailand (Ahmad and McCaffery, 1988), India (McCaffery et al. 1989), Turkey (Ernst and Dittrich, 1992), Indonesia (McCaffery et al. 1991), and China (Shen et al. 1992). The two most frequently encountered mechanisms of resistance is nerve insensitivity (related to the presence of kdr gene) and metabolic detoxification involving oxidases or esterases (McCaffery, 1998). In all African

strains, resistance only originates from an increased metabolic detoxification due to oxidase overproduction (manuscript in preparation).

In response to this resistance problem, three strategies are currently used in cotton to manage the *H. armigera* resistance and to control the pest in West Africa.

1. Utilization of non-pyrethroid insecticides that did not show any cross-resistance. These insecticides are as active against the resistant individuals as susceptible ones. If resistance is linked to a decreased fitness, the proportion of resistant individuals in the population will decrease due to intraspecific competition
2. Utilization of synergists with pyrethroids. A synergist is a molecule that interferes with the enzyme responsible for the resistance. It is used in combination with the insecticide and it increases its toxicity in the resistant strain.
3. Utilization of anti-resistant compounds. An antiresistant molecule is a pesticide that is more active against the resistant individuals compared to the susceptible one.

In the present study, we screened several insecticides in order to find some that do not show any positive cross resistance (strategy 1), as well as those that show a negative cross resistance (strategy 3).

MATERIALS and METHODS

Insects. The susceptible *H. armigera* strain used (BK77) was originally collected in Côte d'Ivoire in 1977 and reared in CIRAD Entomological Laboratory in Montpellier, France. The deltamethrin resistant strain (BK99) was collected as larvae from cotton crops in Bouaké area, in 1999. This strain was homogenized for deltamethrin resistance (BK99R9) by retaining the survivors of discriminating doses (0.6 µg/g) applied topically on third instar larvae of nine generations. Larvae were reared on artificial diet at 25°C, 75% humidity and at photoperiod of 12h/12h in the laboratory as previously described (Martin et al. 2000).

Insecticides. Deltamethrin (99%), triazophos (70.6%), phosalone (93%), and thiodicarb (Larvin375SC) were obtained from Aventis CropScience. Etofenprox (99%) was obtained from Mitsui. Profenofos (91%) was obtained from Syngenta. Chlorpyriphos (99.7%), spinosad (Laser 480 SC), and methoxyfenozide (95%) were obtained from Dow Agroscience. Cyfluthrin (97.2%), betacyfluthrin (98%), and fenthion (96%) were obtained from Bayer. Fenvalerate (95%) was obtained from Sumitomo. Cypermethrin (93.2%), bifenthrin (93.5%), and ethion (96.2%) were obtained from FMC. Indoxacarb (Avaunt 150 SC) was obtained from Du Pont de Nemours. Acephate (97%), isoxathion (93%), and monocrotophos (55.2%) were from Calliope. Acetone was used for dilutions of technical grade materials.

Bioassays. For indoxacarb and spinozad, IRAC N°7 method was applied. Cotton leaves were dipped in the insecticide solution during 5 sec. They were drained and dried at room temperature. The turgenscence of the leaves was maintained by surrounding the petiole with a cotton wool saturated with water. Each leaf was put in a petri dish with 5 larvae at the second-instar. At least five replicates were done per dose. LD50 are expressed in mg a.i. / liter.

For thiodicarb and methoxyfenozide, an ingestion method was used. 1 µl of insecticide solution were put down the surface of 2.5mm^3 cubes of artifical diet. Third-instar larvae were allowed to feed on the artificial diet. 25 larvae were used per dose. LD50 was expressed in µg a.i. / g insect.

For all other insecticides, third-instar topical bioassays were used to determine insecticide toxicity (Martin et al. 2000). Five serially diluted concentrations were prepared. For each concentration, 24 third-instar larvae (35-45 mg) were treated with 1 µl of solution applied by a micro-applicator to the thorax. Each test was replicated three times and included acetone treated controls. LD50 are expressed in µg a.i. / g insect.

Mortality in all the controls was less than 10%. After dosage, the test larvae were held individually at 25°C, 75% humidity and at photoperiod of 12h/12h. Mortalities were assessed 72h after treatment. Larvae were considered dead if unable to move in a coordinated way when prodded with a needle.

Statistical analysis. In order to determine the LD50 (50% lethal dose) of an insecticide, the Finney (1971) method was used. Data from all bioassays were corrected for control mortality. The doses and mortality percentages were converted and the slopes of the response curves were estimated by probit analysis using a computer program developed by the CIRAD-CA, Montpellier, France. Log dose-probit mortality relationships were always consistent with straight lines. Resistance levels were determined by dividing the LD50 of each resistant strain by the LD50 for the susceptible strain. Differences among strains and insecticides were considered significant when the 95% confidence limits between LD50 non-overlapped.

RESULTS and DISCUSSION The response of the field BK99 strain to deltamethrin was significantly different from that of the susceptible BK77 strain. In order to analyze this resistance, we first homogenized this population by selecting for deltametrin during 9 generations by treatment at the LD50 at each generation. This led to a resistant strain BK99R9 with a 189-fold resistance factor. To select insecticides still

efficient against the resistant population, we screened for the resistance for several insecticides (Table 1).

Resistance was positively correlated to all other pyrethroids tested. Cross-resistance also applied to the non-ester molecule, etofenprox. Although all pyrethroids that could be used have not been assayed, these results suggested the limitation of pyrethroids on cotton crops of West Africa to avoid the expansion of *H. armigera* resistance.

DDT did not show any cross-resistance to deltamethrin. This molecule has been used during more than 20 years in West Africa as the only one insecticide to control *H. armigera*. With the discovery of pyrethroids, DDT, although still efficient, was replaced in early '80s and its utilization is now forbidden. Thus the absence of cross-resistance provides only the information that pyrethroid resistance does not originate from previous treatments with DDT and most probably from other organochlorines.

Endosulfan did not show any cross-resistance. This cyclodiene is used from the '70s in mixture with DDT and methyl-parathion. It was replaced by pyrethroids in 1984. But from the development of pyrethroid resistance and the first treatment failures, endosulfan was reused with success (Ochou and Martin, 2001). From this result, it appears that this success partially originated from the absence of cross-resistance with pyrethroids.

Methoxyfenozide, indoxacarb, and spinozad are new molecules that present no cross-resistance with deltamethrin. These three molecules are efficient to control *H. armigera* in the field. But they are more specific than pyrethroids. For example, indoxacarb was not found to be efficient on *Pectinophora gossypiella* Saunders and *Cryptophlebia leucotreta* Meyrick. However, these molecules appear to be good alternatives to endosulfan since they are less toxic for humans.

Thiodicarb is a carbamate that has the particularity to have ovicidal and larvicidal action on *H. armigera*. It has never been used in West Africa yet but, as we did not find any cross-resistance, this molecule can be an alternative to endosulfan.

Organophosphates (OPs) are used to control *Polyphagotarsonemus latus* Bank, *Aphis gossypii* Glover, *Bemisia tabaci* Gennadius, and all leafworms often in mixture with pyrethroids to increase their toxicities. Some of the OPs, such as isoxation, showed positive cross-resistance; these compounds should be avoided in future treatments. Some of the OPs, such as ethion, showed no cross-resistance; their utilization would not have any effect on the pyrethroid resistance. Interestingly, some of the OPs, such as acephate and triazophos, showed significant negative cross-resistance (Fig.1). Negative cross-resistance between pyrethroids and OPs has already been reported for Australian *H. armigera* (Forrester et al., 1993) and for the brown planthoppers (Miyata et al., 1983; Kassai and Ozaki, 1984) and may originate from the dual role of oxidase in OP metabolism. Phosphorothioate OPs are not toxic. They are bioactivated inside the insect by cytochrome P450 enzymes through oxidative desulphuration (the activation of P=S to P=O) to toxic oxon analogues (Kono et al., 1983). But P450 attacks could also lead to non-toxic metabolites via for example the degradation of oxidative ester cleavage. Depending on the OP, it seems that the overproduced P450 in the resistance strain either inactivates the insecticide leading to a positive cross-resistance or activates the insecticide leading to a negative cross-resistance.

Table 1. Toxicity of insecticides to susceptible (BK77), and resistant strain (BK99R9) of *H. armigera*.

Active ingredient	Strain	Slope ± se	LD_{50} [b]	(95% CL) [c]	RF_{50} [d]
Deltamethrin	S	2.16 ± 0.29	0.055	(0.043-0.066)	-
(Pyrethroid)	R	2.44 ± 0.37	10.4	(6.45-14.07)	189 s
Cypermethrin	S	3.56 ± 0.66	0.257	(0.206-0.299)	
(Pyrethroid)	R	1.69 ± 0.3	3.657	(1.413-6.195)	14 s
Cyfluthrin	S	2.95 ± 0.61	0.067	(0.037-0.091)	
(Pyrethroid)	R	1.86 ± 0.5	2.948	(0.945-4.691)	44 s
Betacyfluthrin	S	3.51 ± 0.69	0.099	(0.064-0.127)	
(Pyrethroid)	R	2.99 ± 0.59	1.946	(0.965-2.803)	20 s
Fenvalerate	S	1.62 ± 0.24	0.145	(0.108-0.176)	
(Pyrethroid)	R	1.34 ± 0.22	20.38	(0.729-57.5)	140 s
Bifenthrin	S	4.62 ± 1.16	0.129	(0.09-0.14)	
(pyrethroid)	R	2.10 ± 0.34	1.726	(0.17-3.49)	13 s
Etophenprox	S	2.86 ± 0.3	2.9	(1.9-3.8)	
(pyrethroid)	R	2.02 ± 0.4	15.8	(9.8-21.7)	5.4 s
Methoxyfenozide	S	0.9 ± 0.1	0.62	(0.27-1.15)	
(ecdysone agonist)	R	0.5 ± 0.1	0.75	(0.14-2.04)	1.2 ns
Indoxacarb	S	1.7 ± 0.3	1.1	(0.75-1.5)	
(sodium channel blocker)	R	1.2 ± 0.3	1.87	(1.1-3.19)	2 ns
Spinosad	S	1.8 ± 0.3	0.88	(0.64-1.15)	
(nicotinic receptor activator)	R	1.3 ± 0.3	0.39	(0.17-0.59)	0.4 ns
DDT	S	2.83 ± 0.35	59.8	(45.3-73.6)	
(organochlorine)	R	1.14 ± 0.41	41.2	(16.2-154)	0.68 ns
Endosulfan	S	1.9 ± 0.7	26.4	(19.3-39.6)	
(clyclodien)	R	2.68	16.34	(1.19-20.57)	0.6 ns
Thiodicarb	S	1.7 ± 0.3	3.06	(1.85-4.35)	
(carbamate)	R	1.1 ± 0.2	5.11	(3.05-7.91)	1.7 ns
Profenofos	S	5.1 ± 0.9	6.84	(6.11-7.83)	
(OP)	R	5.4 ± 0.8	9.9	(8.50-11.30)	1.4 s
Fenthion	S	1.4 ± 0.3	179	(59-327)	
(OP)	R	3.5 ± 0.5	67	(47-86)	0.4 ns
Ethion	S	2.4 ± 0.3	1062	(824-1285)	
(OP)	R	3.2 ± 0.6	781	(447-1056)	0.7 ns
Phosalone	S	2.1 ± 0.4	121	(78-185)	
(OP)	R	1.7 ± 0.5	77	(2.2-231)	0.5 ns
Acephate	S	10.4 ± 7.8	121	(78-185)	
(OP)	R	2.7 ± 0.7	36.8	(7.5-65)	0.3 s
Monocrotophos	S	4.5 ± 0.8	55	(47-63)	
(OP)	R	4.3 ± 1.1	233	(92-336)	4.2 s
Isoxathion	S	5.3 ± 0.7	11	(9.4-12.9)	
(OP)	R	1.9 ± 0.8	143	(35-307)	13 s
Chlorpyriphos ethyl	S	9.1 ± 5.1	3.42	(2.43-4.82)	
(OP)	R	4.6 ± 0.7	26	(21-30)	7.6 s
Triazophos	S	3.42 ± 0.41	104.9	(39.0-144.9)	
(OP)	R	1.96 ± 0.24	27.8	(18.8-37.7)	0.3 s

[b] LD_{50} is expressed in µg active ingredient per gramme of larvae; [c] 95% confidence limits shown beneath each LD_{50}; [d] RF_{50} : Resistance Factor 50 = LD_{50} resistant strain / LD_{50} susceptible strain;

Pyrethroid resistance of *H. armigera* in BK99R9 strain seems to be limited to pyrethroids. Assays with other strains originating from different countries gave similar results. This suggests that the specificity of the resistance is applied to the whole West African cotton area. This specificity allows the selection of insecticides that efficiently control *H. armigera* and can be used as antiresistant compounds to tentatively revert the resistance.

ACKNOWLEDGEMENTS The authors acknowledge the technical assistance of T. Konate, I. Ouattara and M.J. Mousso and thank Dr D. Russell (NRI), Dr R.V. Gunning (NSW Agriculture) for their corrections and comments.

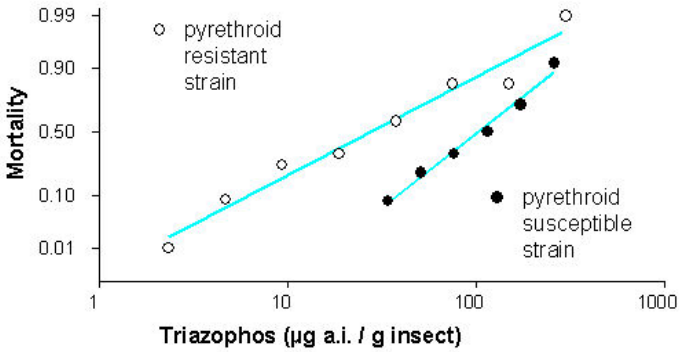

Figure 1. Toxicity of deltamethrin for *H. armigera* susceptible strain BK77 (•), and deltamethrin-selected strain BK99R9 (o).

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A Vial Test Method for the Survey of Pyrethroid Resistance in *Helicoverpa armigera* in West Africa

INTRODUCTION Insect resistance to pesticides is a natural phenomenon, resulting from mutations in the genome of some individuals, leading to a reduced susceptible to pesticides. It is therefore heritable from one generation to the next. The proportion of these resistant individuals in populations increases under the effect of the selection pressure exerted by the repeated application of insecticides until the treatments fail. Before addressing resistance, it is necessary to verify that the practices displaying today poor performance functioned satisfactorily in the past and that the problem does not reside in the conditions of application. Use is also made of bioassays (LD50) in the laboratory or in the field (vial tests) that demonstrate a real decrease in susceptibility in comparison with the past.

THE CASE of *Helicoverpa armigera* in WEST AFRICA The noctuid *Helicoverpa armigera* is a polyphagous insect with strong migratory habits. The annual number of generations in Africa is estimated at between 10 and 12. In most cotton-producing countries of West Africa, cotton pests are controlled with four to seven sprays applied on a calendar basis, the highest bollworm infestation being observed from September to October. Pyrethroid and OP mixtures are used from the mid seventies for pest control (Vaissayre, 1985).

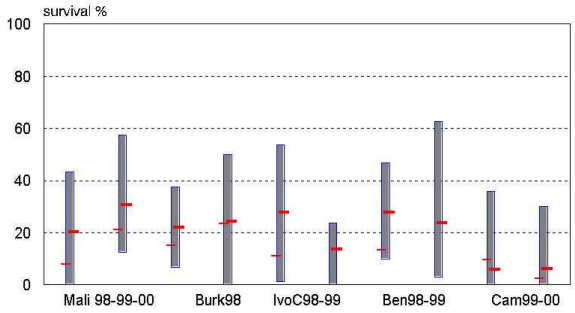
The first cases of bollworm resistance to pyrethroids appeared in Australia (1983) and then in South-East Asia (1984), India (1987), and China (1990) before the first failures in the field occurred in 1996 in Africa, in both the south and west of the continent (Martin et al. 2000). The west-african cotton companies and national agricultural research services reacted very rapidly, setting up a regional project (PR-PRAO), involving CIRAD and several agrochemical firms, represented through IRAC (Insecticide Resistance Action Committee). The project aimed at preventing the spread of resistance through both research operations and co-ordinated pest control practices.

ON-FARM SURVEYS During the three years of the project (1998-2000), CIRAD and IRAC proposed to the participating countries the performance of a survey using vial tests to determine the importance and geographic fluctuations of resistance throughout the cotton belt. The methodology is adapted from Kanga & Plapp (1995), but considers bollworm larvae instead of adults. The survey makes a distinction between two periods (beginning and end of the season) and different sampling locations in each country. Forty *H. armigera* larvae, between 10 and 15 mm long, are collected at the earliest five days after an insecticide application. They are laid individually in vials handled at the Du Pont de Nemours experimental station, Nambsheim (France), and mortality is recorded at 24 h. Technical cypermethrin (FMC), the dominant active ingredient in the West-African pyrethroid market, was used for the survey. Three batches of vials are supplied to the people entrusted with the survey:

- non-treated vials (control)
- vials treated with 5 µg (eliminates 100% of the susceptible laboratory population BK77)
- vials treated with 30 µg (considered to eliminate 60 to 80% of a resistant population collected in Benin in 1997).

Processing of the 1998, 1999 and 2000 data give the following overall results (fig. 1):

Fig. 1 : Percentage of larvae surviving a discriminating dose (30 µg cypermethrin per vial)



Cirad/Irac Survey 1998-2000

For each country and year, the vertical bar indicates the fluctuation of data resulting from the different samples. On each bar, the thin line on the left indicates the percent survival mean value obtained on the first bollworms sampled, and the thick line on the right the same result obtained on late infestations.

- The low level of infestation observed during the survey should not hide the fact that resistance is present in a number of countries, survival above 20% correponding with control failures in the field. The presence of resistant individuals is confirmed by LD50 and LD90 observed in the laboratory, the highest values being obtained in Benin.
- If we consider the results obtained inside Mali, the main cotton producing country in

West Africa, the cumulated mortality curves at the end of the first season of the Project (1998), for the beginning (July) and then for the end (October) of next one (fig 2) show that:

1. the resistance of H. armigera populations does not decrease significantly during the dry season,
2. in spite of the withdrawal of pyrethroids at the beginning of the season, selection pressure is obvious during the 1999 cotton growing period.

- The resistance management strategy implemented in West Africa seems however to give positive results for the last growing season (2000).

THE FUTURE of RESISTANCE MANAGMENT in WEST AFRICA

The success of such a project depends essentially on the setting up of a rational control programme aimed at reducing the selection pressure exerted by pyrethroids. The elimination of resistant individuals is obtaines by using active ingredients whose action and metabolic pathways are different to those of pyrethroid ones. This approach should allow a resumption of the use of pyrethroids at the end of the season, when they are not only the cheapest chemicals for the control of the american bollworm, but also the most suitable ones to control other bollworms (Red, spiny and pink bollworms and False Codling Moth). Weekly monitoring of *H. armigera* infestation levels makes it possible to appreciate the results achieved.

The procedure is illustrated in fig. 3, in which three key points are stressed:

1. a reduction of selection pressure by excluding pyrethroids at the beginning of the season,
2. a return to their use in the form of binary combinations during the fruiting phase,
3. monitoring of *H. armigera* populations with additional spraying if necessary.

Some points should be underlined in this approach:

- the exclusion period that must be respected in the whole cropping system in the region and not only for cotton;
- the maintaining of pyrethroid-organophosphorus combinations during the second phase for both economic reasons and in order to control a broad pest spectrum.
- if the attempt to break resistance to pyrethroids fails and *H. armigera* density reaches economic thresholds, new chemistry (indoxacarb, spinozad) has to be applied.

In the first phase of the Project (1999-2001), it was chosen to use endosulfan during the first part of the season. The choice of this active ingredient results from a number of considerations: WHO toxicological class, proven efficacy in the control of *H. armigera* and safety for some beneficials. Other active ingredients may be used during the first phase, but the prevention principle requires that they should be excluded from the subsequent phase. However, one can very well imagine a rotation in the future, with the use in one year of a first type of product and a product belonging to a different chemical family in the following year. Another key-point is that all the national development companies should follow the plan of utilization of insecticides in a concerted manner. In the light of the survival of integrated sectors in some countries, such a program can be set up for cotton growing with a good chance of success, but problems arise from bollworm populations developping on crops that are little or poorly supervised, such as market garden crops.

Fig 2 : Pattern of resistance in H. armigera populations from one season to the next

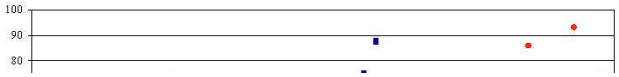
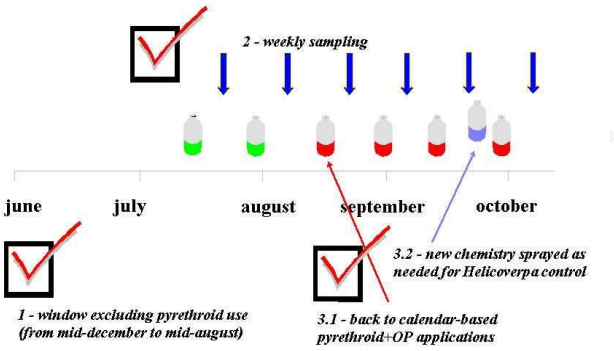


Fig 3 : Basic principles for resistance management of pyrethroid-resistant H. armigera adopted in West Africa



ACKNOWLEDGEMENTS The authors thank the NARS and cotton company personnel who helped in the vial test surveys.

NOTE. The views expressed in this paper are those of the CIRAD-IRAC working group. Although we hope that they are widely held, they in no way represent those of the PR-PRAO as a whole.

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Study on the Gossypol Sterilant to Control Resistant Cotton Bollworm (*Helicoverpa armigera*)

ABSTRACT So far cotton bollworm (*Helicoverpa armigera*) (CBW) has been resistant to all single chemical insecticides and most insecticide mixtures in the northern cotton areas of China. An effective method to control resistant CBW is the use of hereditary control tactics, like sterilants. Sterilant use can not only significantly decrease the density of field pest populations but also can enhance integrated pest management (IPM) strategies. During 1983-1997 we carried out the gossypol sterilant that has been used as a prophylactic in the 1970s in laboratory and field studies. The results showed that the sterility effect was rather good. Before CBW mating, we fed adults with over 800ppm gossypol in the laboratory. The efficiency was 100%. From 1995 to 1997, we applied a mixture of 3000ppm gossypol, male sex pheromone, and attractant to control 2nd, 3rd, and 4th generation CBW in a 333-1000 hectare cotton area. The results showed that gossypol, when applied from 2.25 to 6.75g per hectare, could decrease eggs 52.6-72.8% [mean 63.3%], decrease larvae 34.2-60.8% [mean 45.7%], and increase cotton yield 7.8-15.0% [mean 10.9%] as well as reduce by 7 times the chemical pesticide control per year. According to the index of the databank, this is the first use of a sterilant directly, eliminating the need to artificially rear sterile male insects in the control of field pests.

INTRODUCTION The resistance of cotton bollworm is a key factor that limits cotton production, especially in the northern cotton areas of China. In the 1990s, the cotton bollworm had high resistance to all single chemical insecticides and the control effect of mixture insecticides was gradually reduced. For instance, the 1993 resistance ratio of deltamethrin and monocrotophos were 5121.3-fold (Ld50: 11.6459:0.002274 mg/g) and 18.0- fold (Ld50: 409.6387:22.7513 mg/g) respectively compared with that of 1983. Their control efficacy was under 50% with 1000 times. Because of the integrated impacts of resistance, weather, and the plant system of agriculture, the CBW field population increased quickly. For example, from 1983 to 1993, the density of CBW adults, eggs, and larvae increased 47.0-fold (3666:78), 11.4-fold (3672:321), and 23.5-fold (73:3.1), respectively. As CBW increased, the chemical control accordingly increased from 7 to 24 chemicals per year between 1983 and in 1993. The quantity of the applied insecticides reached 14.4 kg per hectare in 1993. Despite presenting a huge pesticide selection pressure to field CBW, their population numbers also reached or surpassed the control threshold everyday (from June 18 to August 30, 1991-1995). Therefore, an important criterion for testing the control method will be based on how to decrease the field CBW population. Undoubtedly, the most suitable control method we select should not only reduce a great number of insects but also lessen their impact on human society.

It has been 50 years since people have made use of the hereditary control method to deal with pests. In 1954, A. H. Baumboves, etc. succeeded in using a radiation method to create male sterility in *Cochliomyia hominivorax* and flew them to control *Cochliomyia hominivorax*. Later there was a proposal to use chemical pesticides to form sterile male insects in the hope of controlling a pest in the field. Unfortunately most of these sterilants were mutagens for humans, relegating their use to research in a laboratory. Though some countries found non-mutagenicity action sterilants, they still had to artificially rear 20-100-fold sterile insects and fly them to compete in mating with field pests. Only by doing this could the sterilant reduce the density of the pest population. However, there are no facilities that have the capacity to rear sterile pests on a scale needed for this technique to be viable. At present, the field CBW quantities reach about 1,000 heads per hectare. 20,000-100,000 heads per hectare would have to be reared in a laboratory to control them with sterilants. Such mass production of sterile insects could not be efficiently acheived. The observation that gossypol can cause human male sterility inspired us to test male cotton bollworm. During 1983-1987, it was discovered that gossypol had better sterility action to male CBW adults in the laboratory - a success that was acheived by rearing sterile male adults and flying them in a 200hectare cotton field in Liaocheng in 1987.

However, we feel that it is very difficult to maintain populations of CBW in the laboratory and in the field simultaneously. After 1991 especially, the resistance numbers of CBW were increasing rapidly in cotton areas. After researching with the gossypol for many years, we proposed that a mixture of the gossypol sterilant, a CBW attractant, and male pheromone be used to directly control the resistance of CBW in the field. In combining the gossypol sterilant with other methods to control CBW, we acheived results that showed that the gossypol sterilant significantly decreased chemical control times, the quantity of pesticides applied, control costs, and cotton field pollution, as well as increased the control's effectiveness.

MATERIALS and METHODS

1. Monitoring the sterility effect of gossypol sterilant to CBW in laboratory.

   [1.1] We used 95% acetic acid gossypol sterilant.

   [1.2] The cotton bollworms were collected from Liaocheng cotton areas, Shandong province. The larvae were fed on artificial diet and the adults were put in the cages (30x30x30 cm). After dissolving gossypol in ethanol, we diluted 3000ppm gossypol sterilant with 8% sugar water then reared non-mating CBW adults to monitor sterility effect.

2. Monitoring the field demonstration sterility effect of gossypol to CBW.

   [2.1] From 1995 to 1997, we demonstrated the control of 2nd, 3rd, and 4th generation CBW in 333, 333, and 1000 hectare plots in the cotton area of Chiping County.

   [2.2] We made several holes in a container (A), and put CBW male sex attractant (D) in (A) to attract male CBW adults.

   [2.3] We put 10ml 3000 ppm gossypol solutions in a container (B) to make CBW suck in. (B) must be shaded from the sun to prevent photodecomposition of gossypol. We observed the solution daily and added more according to the quantity of the gossypol solution that had decomposed.

   [2.4] A bamboo (C) was put in the cotton field. During 1995-1996, we put 15 bamboos in a hectare. In 1997, we put 15, 30, 45 bamboos in a hectare respectively to observe the different sterility action. A, B and C are bound together and placed 10-20cm higher than the cotton plant (E). (Fig. 1)

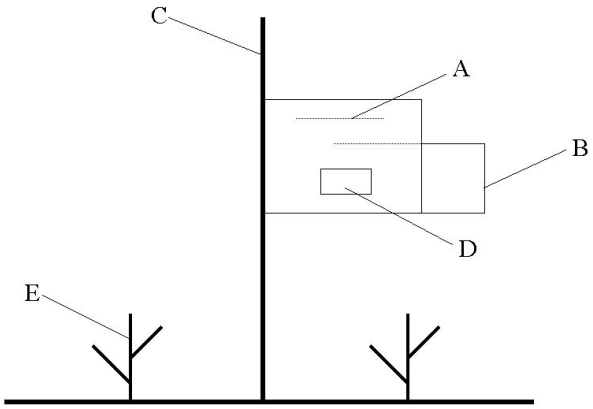

Fig.1: Gossypol sterilant as used in a cotton field: [A] container, [B] gossypol soution, [C] bamboo, [D] male sex attractant, [E] cotton.

   [2.5] We checked the eggs and larvae every three days. If their mounts reached the control threshold, we conducted a chemical control for CBW.

RESULTS and DISCUSSION The data (Table 1) indicated the sterility effect of gossypol sterilant to CBW in laboratory. When gossypol's concentration was 800 ppm, the eggs were reduced 97.6% more than CK, the hatch ratio was zero, and the sterility effect was 100%. Other of concentrations over 800 ppm resulted in sterility effects of 100%. Because of the many factors impacting field CBW, we tested with 3000ppm gossypol to monitor sterility effects in field demonstration.

Table 1. Monitoring the sterility Effect of Gossypol difference concentration to control CBW in laboratory

1983-1994

Gossypol Concentration (ppm)	Female: male	Eggs per Female (grain)	Effect (%)	Larvae (head)	Hatch (%)	Effect (%)
500	2:2	59	92.2	8	13.6	80.3
800	2:2	18	97.6	0	0	100
1000	2:2	0	100	0	0	100
3000	2:2	0	100	0	0	100
5000	2:2	0	100	0	0	100
CK	2:2	761	0	524	68.9	0

CK: 8% Sugar water.

The data from Tables 2 and 3 showed the control effect of gossypol sterilant to CBW in cotton fields. Both in 1995 and 1996, we controlled 2nd, 3rd, and 4th generation with 2.25g per hectare. The results indicated that gossypol sterilant treatment reduced eggs 63.1% and 58.5%; reduced larvae 54.0% and 46.8%; reduced chemical control by a factor of 7; and increased cotton yield 10.3% and 10.0% compared with chemical insecticides treatment.

In 1997, we designed three gossypol treatments (A: 2.25, B:4.5, C:6.75g per ha.) to control 2nd, 3rd, and 4th generation CBW in the field. Comparing the gossypol sterilant with the pesticide control treatment, the eggs were reduced 57.1%, 65.2%, and 72.8%, and

larvae were reduced 37.4%, 42.1%, and 48.7% respectively. The amount of chemical control required was decreased by a factor of 7 and the cotton yield was increased by 7.8%, 11.5%, and 15.0% respectively.

Significant analysis (Table 3) of the results showed that there were no significant differences between B and C in decreasing eggs, but there were highly significant differences among B compared to treatment A and C compared to treatment A. The decreasing larva of C was significant to both treatments A and B.

There were highly significant differences between cotton yield of treatments with a sterilant, versus treatments with a chemical pesticide. Beside the highly significant differences appearing between C and A, other treatments did not appear to have any significant difference.

From 1995 to 1997, the average control effects of gossypol sterilant to CBW were reducing eggs by 63.3%, larvae by 45.7%, chemical control by 7 times per year, and increasing cotton yield by 10.9%. To summarize, the results indicate that gossypol sterilant use is an important and effective method to control resistant CBW in field, while not destroying the ecological balance or polluting the environment. The new method is worth popularizing widely for resistance CBW and the others serious insects.

Table 3. Monitoring the Control Effect of Gossypol Difference Application Quantity to CBW in Cotton Field in 1997

Treatment	Application quantity	Eggs/100 plant		Larvae/100 plant		yield	
	(g/ha.)	(Eggs)	$P_{0.05}$ 0.01	(heads)	$P_{0.05}$ 0.01	(Kg/ha.)	$P_{0.05}$ 0.01
Gossypol	2.25	1242	B	170	B	1078.5	Bb
Sterilant	4.5	1008	Ca	157	B	1116	AaBb
	6.75	787	Ca	139	Ba	1150.5	Aa
Chemical Pesticide	14400	2894	A	271	A	1000.5	C

Table 2. Monitoring the control effect of gossypol to resistnat CBW in the field, 1995-1997, Chiping.

					Gossypol sterilant treatment					Chemical pesticide treatment					Gossypol than pesticide			
Year	CBW (Gene-ration)	Date	Apply Gossypol (g/ha.)	Bamboos Per ha.	Acreage (ha.)	Eggs/ 100 plant	Larvae/ 100 plant	Chemical control times per year	yield (kg/ha.)	Acreage (ha.)	Eggs/ 100 plant	Larvae/ 100 plant	Chemical control times per year	yield (Kg/ha.)	Eggs reduce (%)	Larvae reduce (%)	chemical control times reduce	yield Increas -e (%)
1995	2nd	9/6-9/7	2.25	15		2211	93	7			6303	236	9		64.9	60.6	2	
	3rd	11/7-13/8	2.25	15	333.3	548	63	7	1191	66.7	1430	153	9	1080	61.7	58.9	2	10.3
	4th	16/8-21/9	2.25	15		658	86	6			1521	137	9		56.7	37.2	3	
	Total		6.75	/		3417	242	20			9254	526	27		63.1	54	7	
1996	2nd	8/6-8/7	2.25	15		1087	62	6			2578	158	8		57.8	60.8	2	
	3rd	11/7-13/8	2.25	15	333.3	236	74	6	1072.5	66.7	498	110	7	976.5	52.6	32.7	1	10
	4th	16/8-21/9	2.25	15		230	63	4			662	103	8		65.3	38.8	4	
	Total		6.75	/		1553	199	16			3738	371	23		58.5	46.4	7	
1997	2nd	9/6-7-Sep	2.25	15		717	85	7							59	39.3	2	
			4.5	30	/	579	80	7	/		1748	140	9	/	66.9	42.9	2	/
			6.75	45		454	71	7							74	49.3	2	
	3rd	12/7-14/8	2.25	15		324	37	5							54.2	36.2	3	
			4.5	30	/	264	36	5	/		708	58	8	/	62.7	37.9	3	/
			6.75	45		198	34	5		66.7					72	41.4	3	
	4th	17/8-19/9	2.25	15		201	48	1							54.1	34.2	2	
			4.5	30	/	161	41	1	/		438	73	3	/	63.2	43.8	2	/
			6.75	45		135	38	1							69.2	47.9	2	
	Total		2.25	15	333.3	1242	170	13	1078.5						57.1	37.3	7	7.8
			4.5	30	333.3	1008	157	13	1116		2894	271	20	1000.5	65.2	42.1	7	11.5
			6.75	45	333.3	787	139	13	1150.5						72.8	48.7	7	15

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Negative Cross Insensitivity in a Dimethoate Resistant Strain of Cotton Aphid *Aphis gossypii* Glover in Northern Cameroon

ABSTRACT The sensitivity of an *Aphis gossypii* strain from Northern Cameroon to some organophosphates and carbamates was studied using the Potter tower bioassay technique. Comparison of this strain with a carbamate and organophosphate susceptible strain revealed a 36.7 fold resistance to dimethoate. No difference was noticed for methamidophos, monocrotophos and profenofos. On the other hand the Cameroon strain showed a 7.8 and 19.2 fold hypersensitivity respectively to carbosulfan and pirimicarb.

INTRODUCTION *Aphis gossypii* is a key cotton pest in Northern Cameroon. Damage occurs mainly at the beginning of the cropping season (trophic damage on seedlings) and at the end of the season (honeydew responsible for "sticky cotton"). Aphid management is mainly based on the use of chemicals. Organophosphates (mainly monocrotophos) are sprayed at the beginning of the cropping season, but also during the remaining part of the cropping season for bollworm control (mainly profenofos or chlorpyriphos, in addition to pyrethroids). Carbamates are becoming more widely used for seed treatment. Amiot (1993) studied the sensitivity of local *A. gossypii* strains in the laboratory and obtained results close to those from resistant strains published by Gubran et al. (1992). Deguine (1996) pointed apparent lack of efficiency of organophosphate sprays against *A. gossypii* in the field. On the other hand, the cotton extension agency does not mention any loss of efficiency in the field for organophosphate such as monocrotophos. A study was carried out for a better characterization of the sensitivity level of local *A. gossypii* strains to organophosphates and carbamates. Results presented here are a part of this study.

MATERIAL and METHODS

Bioassays

The Potter tower was used to quantify the sensitivity of aphids following Gubran et al. (1992). Cotton leaves are cut into discs of 4 cm diameter and are placed in a 5 cm Petri dish on a semi-solid agar gel (10 % agar). On each disc 10 aphids are placed. After anesthesia with carbon dioxide, aphids (still on the leaves) are sprayed with the Potter tower. The average volume of the solution sprayed is 3.6 mg / cm2. Petri dishes are stored at 25°C, LO 12:12. Mortality is checked 24 h after spraying. Aphids unable to move coordinately are considered dead.

Five doses were used, in geometric progression, plus an untreated check (sprayed with a 0.8% ethanol solution, see below). Three replicates of 10 aphids each were used for the five doses and for the untreated check. The whole experiment was repeated 3-5 times at different days.

Insecticides

Insecticides were diluted in a small quantity of ethanol before dilution in distilled water (resulting in a final 0.8 % ethanol concentration in the solution). Insecticides used were technical active ingredients:

- dimethoate 97% (Calliope)
- monocrotophos 72% (Calliope)
- methamidophos 73% (Calliope)
- profenofos 91% (Syngenta)
- carbosulfan 90% (Calliope)
- pirimicarb 99.9% (Syngenta).

Insects

Two aphid colonies were used for this study:

- the MR98 strain, collected from Maroua (Northern Cameroon) on September 16th, 1998 in an untreated cotton plot and then reared on cotton seedlings at the IRAD Maroua laboratory.
- the Navacelles strain from France. This strain is reared at INRA, Montpellier and was transferred to Maroua in October 2000. This strain is organophosphate and carbamate susceptible (Delorme et al., 1997).

Data analysis

Results of the replicated bioassays were pulled together before analysis. Data were stored and analyzed with the WIN DL 2.0. Software (Cirad, 1999), using Finney's log-probit method.

Sensitivity levels were estimated with the LC50 (concentration causing 50% mortality) expressed as mg of active ingredient per liter of solution. Resistance factors (RF) were computed by dividing the LC50 of the MR98 strain by the LC50 of the Navacelles strain. Where RFs were lower than unity (hyper- sensitivity) they were written as fraction. A RF equal to 1/n means that the MR98 strain is n fold more susceptible than the Navacelles strain.

RESULTS and DISCUSSION Results of bioassays are summarized in Table 1.

LC50 values obtained for the Navacelles strain for dimethoate and pirimicarb are close to those obtained by Delorme et al. (1997): 4.12 vs. 2.28 for dimethoate, and 2.56 vs 1.52 mg/l for pirimicarb. The LC50 value for dimethoate confirms the value obtained by Amiot (1993) in Maroua (123 mg/l). This result is of the same magnitude as the value given by Delorme et al. (1997)

for a resistant strain from the South of France, which was 180 mg/l. Gubran et al. (1992) reported values of the same order (200 to 423 mg/l) for resistant strains from Sudan.

The study of RF (Table 1) reveals three cases.

1. For dimethoate, a clear loss of sensitivity of the MR98 strain is observed. The existence of a dimethoate resistance in Northern Cameroon is questionable, as dimethoate has never been significantly used on cotton in the country. Nevertheless, dimethoate is widely used in cotton areas of Nigeria (Onu, 2000) and also in cotton areas of Chad and the Central African Republic (these three countries are bordering the cotton area of Cameroon) and in the vegetable cropping areas of South and West Cameroon.
2. For monocrotophos, methamidophos and profenofos, no strong differences could be noticed between both strains (RF between 5 and 1/5). This result is particularly noticeable for monocrotophos. This insecticide was indeed intensively used during the last decade in Northern Cameroon and a loss of sensitivity was to be feared.
3. For both carbamates, the MR98 strain is more susceptible than the Navacelles strain. This phenomenon is more obvious for pirimicarb than for carbosulfan. This result should be compared to the negative cross insensitivity described by Villate et al. (1999) in a *A. gossypii* strain from the South of France, resistant to pirimicarb and hypersensitive to bendiocarb.

ACKNOWLEDGEMENTS This study was funded by Cirad and carried out in collaboration with Irad (Institute of Agricultural Research for Development, Cameroon). The authors thank the societies Calliope and Syngenta for providing the active ingredients. The authors thank also G. Labonne, Inra Montpellier, for providing the Navacelles strain.

Table 1. LC50 (mg/l) and RF for Navacelles and MR98 strains.

Active ingredients	LC 50 (mg/l)		RF
	MR98	Navacelles	
dimethoate	151.2	4.12	36.7
monocrotophos	23.5	6.29	3.7
methamidophos	10.4	26.9	1 / 2.6
profenofos	21.6	39.9	1 / 1.8
carbosulfan	0.28	2.17	1 / 7.8
pirimicarb	0.13	2.56	1 / 19.2

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Insecticide Resistance in Populations of the Colorado Potato Beetle, *Leptinotarsa decemlineata* Spreads Westward in Canada

SUMMARY The susceptibility of Colorado potato beetles (*Leptinotarsa decemlineata*) from three provinces in western Canada was measured using a filter paper bioassay to substantiate the reported insecticide resistance by the beetle in Manitoba, and to compare the situation there to beetle populations from Saskatchewan and Alberta. Susceptibility of beetles was measured against five insecticides: the organophosphates, azinphos-methyl (Guthion), and methamidiphos (Monitor); the pyrethroid, permethrin (Ambush); the organochlorine, endosulfan (Thiodan); and the carbamate, carbaryl (Sevin). All 12 populations tested from Manitoba were found to have resistance to one or more of the insecticides. All populations were classified as either having resistance or intermediate resistance to permethrin; two of the populations were classified as having resistance to azinphos-methyl and three to methamidiphos. Two of four populations from

Saskatchewan were classified as having intermediate resistance to azinphos-methyl and methamidiphos. Intermediate resistance to permethrin was recorded in 12 of the13 populations from Alberta, with only one being highly susceptible. Two populations showed evidence of intermediate resistance to azinphos-methyl and three to methamidiphos. In all three provinces, the range of survival from different egg masses within the susceptible populations ranged from 0-100%, indicating the presence of individuals with either intermediate resistance, or high susceptibility within these populations. With the expanding potato acreage in western Canada and the detection of populations with resistance to insecticides, a resistance management program must be implemented to prevent the rapid selection of resistant populations.

INTRODUCTION The Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say) (Coleoptera, Chrysomelidae) is considered one of the most destructive foliage feeding pests of potatoes. The application of chemical insecticides has been the primary method used to control this pest in North America. However, widespread and repeated use of chemicals as a control method has resulted in the selection of insecticide resistant populations. In some potato producing areas in North America, nearly all the previously effective insecticides are no longer capable of reducing beetle populations and new insecticides lose their effectiveness within a few years because of cross resistance. Thus, the selection of insecticide resistant populations is a major threat to the potato industry and a continuing problem in Colorado potato beetle management.

In Canada, insecticide resistant populations have been reported from most of the eastern provinces where potatoes are grown (Boiteau 1988; Boiteau et al. 1987; Harris and Svec 1976, 1981; Stewart et al. 1997). The first reports of resistance were to organochlorine insecticides (Harris and Svec 1976; McDonald1976; McClanahan 1975). By 1981, populations showing resistance to organophosphates and carbamates were found in Quebec. In 1979, most populations tested in Ontario were susceptible to pyrethroids (permethrin, fenvalerate and cypermethrin), but by 1982, a 22-37-fold resistance was reported after just 2 years of their use (Harris and Turnbull 1986). In New Brunswick, there was a 70% increase in beetle populations between 1974-1980 that coincided with an increase in insecticide resistant populations (Boiteau et al. 1987). Some populations in these areas have developed resistance to insecticides in all classes, resulting in the emergency registration in 1995 of imidacloprid (Admire™), which belongs to the new chloronicotinyl class of insecticide.

In western Canada, there have been recent reports of insecticide resistance to three of the four classes of insecticides tested from Manitoba (Gavloski 1997). In Alberta, the last report of the presence of resistance was to DDT (McDonald 1976), but since then there have been no surveys conducted.

Over the last 10 years, Canadian potato production has increased by 55% and the area planted by 40% (Statistics Canada 1999). The future growth of the potato industry is expected to be in western Canada, including Alberta, as more potato processors establish there. This increasing demand for potatoes requires an increase in acreage, and may result in a decrease in rotation, factors that will favour Colorado potato beetle populations and the need for an increase in the use of insecticide treatments. This will consequently provide ideal conditions for the selection of insecticide resistant beetle populations.

The objective of this study was to measure the susceptibility to insecticides of beetles from three western Canadian provinces, to substantiate the reported occurrence of insecticide resistance to the beetle in Manitoba, to compare the situation there to beetle populations from Saskatchewan and Alberta, and to provide base-line data for future survey.

MATERIALS and METHODS Insecticide resistance in beetle populations from Manitoba, Saskatchewan, and Alberta was measured using a filter paper bioassay (French et al. 1992; Heim et al. 1990). Due to the low numbers of beetles found throughout parts of western Canada, we were unable to collect sufficient numbers of egg masses directly from the field. Consequently, during the summer of 1998, laboratory cultures of 35 egg-laying females and 15 males were established on potted "Russet Burbank" potato plants from field

Table 1. Insecticides tested for resistance in Colorado potato beetle populations from western Canada using a filter paper bioassay

Insecticide class	Insecticide	Diagnostic concentration[a] (µg ai/cm^2)	Mean mortality of susceptible strain[b] (%)	Mean mortality of resistant strain[b] (%)
Organophosphate	Azinphos-methyl (Guthion™) Bayer	1.05	95± 5	61±13
	Methamidiphos (Monitor™) Bayer	1.05	97± 9	72±10
Pyrethroid	Permethrin (Ambush™) Zeneca	0.16	93± 3	0
Organochlorine	Endosulfan (Endosulfan™) Aventis	0.11	100	0
Carbamate	Carbaryl (Sevin™) Aventis	30	100	92 ± 2

[a] As per French *et al.* 1992.

[b] Susceptible and resistant laboratory strains were obtained from S. Hilton, Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada, London, Ontario (Hilton *et al.* 1998). Ten egg masses of each strain were tested against each insecticide.

collected adults or fourth instar larvae from commercial fields in each province. Beetles from each commercial field were considered separate populations. The egg masses from each population were collected over a 1 to 3-month period and used in the assays. For the most part, eggs were collected from adults that arose directly from the field-collected larvae.

Each bioassay unit consisted of a filter paper that was pre-treated with a commercial insecticide dissolved in acetone using the diagnostic concentrations calculated by French et al. (1992) (Table 1). A single egg mass was placed on the insecticide treated paper in a Petri dish and incubated at 23EC and 16L:8D photoperiod. When at least 50% of the eggs hatched, the filter paper was moistened with about 0.3 ml water. The numbers of dead and living larvae were counted 24 h later. Egg masses for the controls were placed on acetone treated filter paper. Ten egg masses (> fifteen eggs per egg mass) per population per insecticide were tested. We followed the classification scheme of Kennedy and French (1994); egg masses showing <50% mortality were classified as resistant. The proportion of egg masses within each population showing resistance (i.e., <50% mortality) to the diagnostic concentrations of French et al. (1992) was used to classify the populations according to their susceptibility as follows: Resistant (>80% of the egg masses had <50% mortality); Intermediate (between 20 to 79% of the egg masses had <50% mortality); and Susceptible (between 0-19% of the egg masses had <50% mortality). Controls for all populations from the three provinces were run simultaneously. To verify the diagnostic concentrations against known resistant and susceptible populations of the beetle, we tested 10 egg masses per insecticide each from susceptible and resistant populations originating from populations from southern Ontario (Hilton et al. 1998). The resistant population was resistant to organochlorine, pyrethroid and organophosphate insecticides, with the exception that it had lost its resistance to carbofuran, and resistance to azinphos-methyl had decreased (S. Hilton, personal communication).

RESULTS Populations from all three western provinces demonstrated presence of individuals with resistance to one or more of the insecticides (Table 1). Of the 12 populations tested from Manitoba, 6 were classified as having resistance and 6 showed intermediate susceptibility to permethrin (Fig. 1; Table 2). Populations showed highest susceptibility to carbaryl with only two of the 12 populations classified as having resistance to this insecticide. Four of the 12 populations were classified as having resistance and 7 showed an intermediate level of susceptibility to endosulfan. Two populations showed resistance to azinphos-methyl and 3 populations to methamidiphos with 7 and 8 showing intermediate levels of susceptibility. One population (population 9) of the 12 tested was classified as highly susceptible to all three chemicals, azinphos-methyl, methamidiphos, and carbaryl; no populations were found to be highly susceptible to permethrin. Several populations demonstrated multiple resistance, with population 2

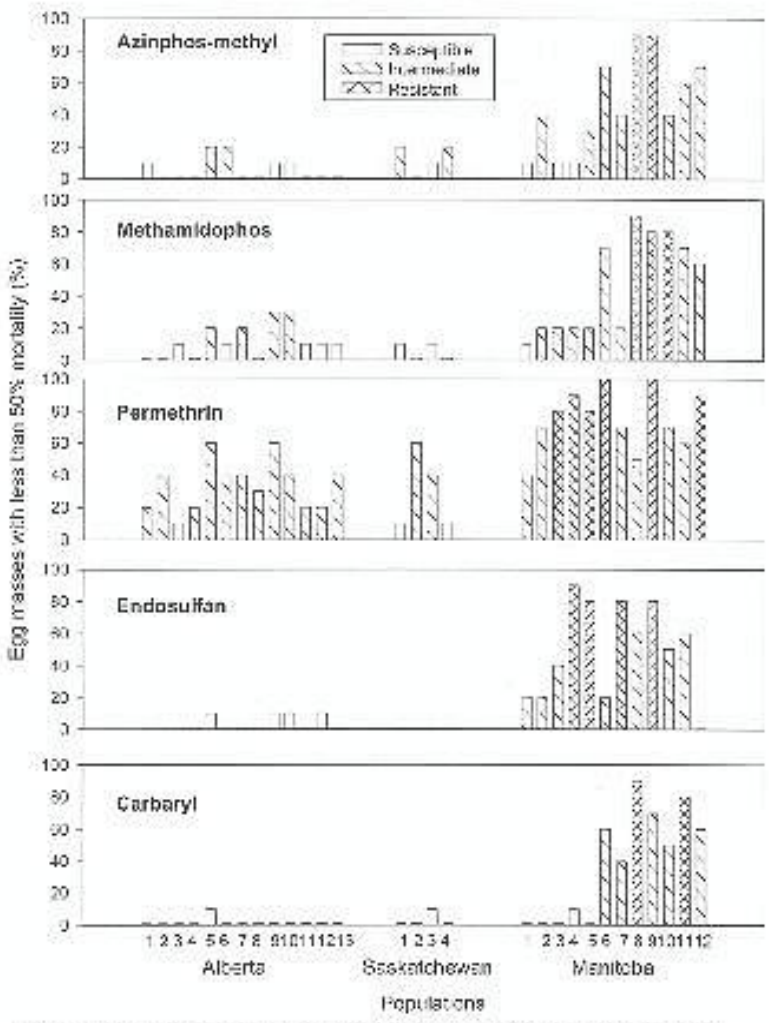

Figure 1. Proportion of egg masses of Leptinotarsa decemlineata from the Canadian prairie provinces showing different levels of susceptibility to the insecticides A) Azinphos-methyl; B) Methamidophos; C) Permethrin; D Endosulfan; and E) Carbaryl. Susceptibility categories are defined as follows: Resistant when ≥80% of the egg masses had < 50% mortality; Intermediate when between 20 to 79% of the egg masses had < 50% mortality; and Susceptible when between 0-19% of the egg masses had < 50% mortality (see Kennedy and French 1994). Insecticide concentrations used were those previously determined as diagnostic for resistant and susceptible populations from North Carolina (French et al. 1992).

demonstrating resistance to azinphos-methyl, methamidiphos, and carbaryl and population 11 to azinphos-methyl, methamidiphos, permethrin and endosulfan.

From Saskatchewan, only two populations of the four tested showed intermediate susceptibility to permethrin and azinphos-methyl (Fig. 1). All four populations were highly susceptible to the methamidophos, endosulfan and carbaryl.

In Alberta, an intermediate level of susceptibility to permethrin was recorded in 12 of the 13 populations tested (Fig. 1). Of the two organophosphates tested, two populations showed intermediate susceptibility to azinphos-methyl and four populations to methamidiphos. All 13 populations were classified as highly susceptible to endosulfan and carbaryl; 10 of the populations were highly susceptible to at least three insecticide classes.

Controls for all populations from the three provinces showed no mortality over the 24-hr assay period. The application of the French et al. (1992) diagnostic concentrations to laboratory-reared Canadian susceptible and resistant strains of potato beetles confirmed the suitability of these diagnostic concentrations used for this survey (Table 1). The intermediate resistance response of the resistant population to azinphos-methyl and methamidiphos was as expected for this colony (S. Hilton, personal communication).

Table 2. Mean percent mortality and range per egg mass of *Leptinotarsa decemlineata* for each insecticide and population tested. Numbers in bold represent resistant populations.

	Insecticide				
Population number	**Azinophos-methyl**	**Methamidiphos**	**Permethrin**	**Endosulfan**	**Carbaryl**
	Mean % (range)				
Manitoba					
1	57 (0-96)	69 (23-100)	39 (0-100)	85 (25-100)	95 (73-100)
2	**19 (0-100)**	**17 (0-100)**	48 (0-98)	50 (19- 86)	**29 (0-100)**
3	40 (0-81)	47(8-92)	**12 (0-67)**	77 (53-100)	46 (6-100)
4	78 (4-100)	78 (9-100)	**30 (0-69)**	56 (9-100)	99 (91-100)
5	78 (39-100)	69 (6-100)	**26 (0-52)**	**17 (0-93)**	83 (23-100)
6	52 (0-100)	**36 (0-93)**	38 (0-100)	57 (13-100)	57 (8-100)
7	39 (0-80)	34 (0-100)	45 (4-100)	37 (0-100)	**13 (0-71)**
8	57 (21-92)	73 (0-100)	32 (5-67)	**35 (0-100)**	57 (0-95)
9	87 (29-100)	92 (29-100)	64 (0-100)	82 (17-100)	99 (95-100)
10	74 (7-100)	82 (13-100)	**14 (0- 67)**	**28 (0- 94)**	97 (79-100)
11	**28 (0-71)**	**30 (0- 67)**	**1 (0-7)**	**39 (6- 82)**	40 (0-89)
12	39 (0-100)	43 (15-90)	**<1 (0-3)**	88 (39-100)	43 (0-89)
Saskatchewan					
1	74 (41-100)	84 (30-100)	87 (38-100)	100	99 (97-100)
2	82 (40-100)	98 (92-100)	76 (46-100)	100	99 (94-100)
3	87 (60-100)	88 (66-100)	46 (0-100)	97 (83-100)	99 (96-100)
4	77 (47- 97)	88 (45-100)	48 (0-95)	95 (80-100)	94 (48-100)
Alberta					
1	87 (33-100)	67 (28-100)	42 (0-95)	85 (11-100)	99 (95-100)
2	80 (38-100)	90 (18-100)	69 (14-100)	99 (96-100)	100
3	92 (75-100)	82 (3-100)	73 (23-100)	98 (90-100)	97 (83-100)
4	90 (72-100)	71 (19-100)	63 (9-100)	95 (50-100)	99 (87-100)
5	83 (32-100)	74 (23-100)	54 (5-88)	93 (79-100)	97 (79-100)
6	86 (67-100)	93 (65-100)	68 (16-100)	99 (96-100)	100
7	88 (66-100)	88 (45-100)	62 (15-100)	97 (85-100)	99 (96-100)
8	81 (5-100)	99 (93-100)	77 (11-100)	100	100
9	97 (74-100)	99 (95-100)	82 (27-100)	100	100
10	92 (77-100)	71 (3-100)	80 (40-95)	99 (96-100)	99 (92-100)
11	78 (24-100)	74 (3-100)	27 (0-84)	80 (0-100)	92 (24-100)
12	83 (17-100)	70 (26-100)	49 (0-86)	76 (22-100)	98 (75-100)
13	84 (60-100)	86 (54-100)	55 (14-100)	100	100

DISCUSSION Populations from all three western provinces demonstrated some level of resistance to one or more of the insecticides tested. Resistance was most prevalent in populations from Manitoba, which is consistent with the results of a previous survey, where populations from 21 of 55 potato fields in Manitoba were found to be resistant to at least one of the nine insecticides tested (Gavloski 1997). Although the diagnostic concentrations used in this study were calculated for resistant and susceptible populations from North Carolina, the concentrations were verified, by Stewart et al (1997), for populations from Prince Edward Island and Ontario and a resistant and susceptible population from Ontario in this study. However, we have chosen to report our results according to level of susceptibility to French et al.'s (1992) diagnostic concentrations rather than to probability of field control (Kennedy and French 1994), because base-line data on the susceptibility of beetle populations from western Canada had not yet been determined and consequently, local resistant and susceptible populations were not available for us to verify these diagnostic concentrations. The results of the present study can now be used as a base-line to establish local dose-response regressions in order to fine tune, if necessary, the diagnostic concentrations we used for further monitoring of the evolution of resistance in the prairie provinces. These results can also be used as a base to measure the future development of resistance to insecticides by Colorado potato beetles in the Canadian prairie provinces.

In our study, populations most commonly showed some level of resistance to the pyrethroid, permethrin. The prevalence of populations with low susceptibility to permethrin throughout all three western provinces and especially in Manitoba should be a warning signal to producers that pyrethroids should be used cautiously.

Our data also shows that the development of resistance to organophosphate insecticides may soon occur in western Canada. In Manitoba, 17% of the populations were classified as having resistance to azinphos-methyl and 25% to methamidiphos. However, 58% and 67% of the populations were classified as having intermediate levels of susceptibility to these two insecticides in Manitoba, and 15% and 23% had intermediate susceptibility in Alberta. This is an indication that the selection of resistant beetles has already started and these populations typify a "mixed" population.

In all three provinces there were populations classified as highly susceptible to at least one or more insecticides. The numbers of highly susceptible populations were much greater in Alberta and Saskatchewan than in Manitoba. However, even within

these susceptible populations, the range of survival within the bioassay units varied considerably, ranging from 0-100% for some insecticides. Although such natural variations are common, it underscores that there is always the potential for the rapid development of resistance, even in a highly susceptible population and that the development of resistance can be very localized. A similar variation was observed in the populations classified as having intermediate levels of susceptibility. Continuous pressure by the use of insecticides within the same class, especially on these intermediate populations, would result in the eventual selection of a resistant population.

The presence of larger numbers of populations with low susceptibility in Manitoba as compared to Alberta and Saskatchewan may be attributed to the recent rapid increase in potato acreage in Manitoba (62% since 1992) (Statistics Canada 1999) resulting in an increase in Colorado potato beetle populations, and the need to control these populations using insecticides (J. Gavloski, personal communication). In Saskatchewan and Alberta, Colorado potato beetle populations have been traditionally low, but with the expanding potato industry, an increase in the Colorado potato beetle population is expected, which in turn could result in increased insecticide applications and the eventual rapid development of resistance. Thus caution must be exercised and a resistance management program should be implemented immediately in order to prevent, or at least delay, further selection of insecticide resistant populations.

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Response of Whitefly *Bemisia tabaci* to Selection by Different Insecticides and Genetic Analysis of Attained Resistance

ABSTRACT Adults of whitefly, *Bemisia tabaci* (Gennadius) were collected from different crops in Punjab, India during 2001 and a mixed population raised on cotton plants in screen house. The subsets of this population were selected for resistance against imidacloprid, bifenthrin and fenvalerate up to 8th generation. Selection pressure was given by exposing the adults to insecticides using the treated leaf discs in the Petri dishes at the dosages sufficient to give 60 - 80 per cent mortality. After 8th generation, populations selected with these three insecticides exhibited 21.90, 7.12 and 4.13-fold increase in tolerance, respectively. Estimates of realized heritability of insecticide resistance in *B. tabaci* were 0.16, 0.07 and 0.02 in first three generations against imidacloprid, bifenthrin and fenvalerate, respectively. The mode of inheritance of insecticide resistance in *B. tabaci* was found to be controlled by nearly completely recessive, more than one gene having additive effects and segregating in the base population under continuos selection.

KEYWORDS *Bemisia tabaci*, bifenthrin, fenvalerate, heritability, imidacloprid, insecticidal resistance, selection, whitefly.

INTRODUCTION The sweet potato whitefly, *Bemisia tabaci* (Gennadius) is an important worldwide pest of cotton, vegetables and ornamentals. It was known as secondary pest since 1920's (Azab et al., 1971, Byrne et al., 1990). In the last two decades, the species has increased its host and geographical range and attained the status of primary pest (Dittrich et al., 1985). Direct feeding on the plant, contamination cause damage to the crop with sticky moulds on honeydew excretion and transmission of plant diseases (Byrne et al., 1992).

Resurgence of *B. tabaci* population has been reported from many countries (Dittrich et al., 1985, 1990, Prabhaker et al., 1985, Horowitz 1986, Jayaraj et al., 1986). Among the suggested causes of whitefly resurgence were climatic factors (Jayaraj et al., 1986, Byrne et al., 1992), cropping practices (Byrne et al., 1992), and the use of insecticides. Sublethal doses of insecticides were reported to increase reproduction in many insect species (Ball & Su 1979, Chelliah & Heinrich, 1980) including *B. tabaci* (Dittrich et al., 1985, Byrne et al., 1992).

The evolutionary adaptation of insects to their chemical environment eliminates genetically susceptible individuals resulting in increased number of resistant individuals in a population (Roush & McKenzie, 1987). Resistance in several insects to commonly used insecticides is widespread (Georghiou, 1990) and continued use of conventional insecticides is likely to intensify selection for resistance in the field thereby aggravating the problem of resistance. For resistance management tactics to be effective resistance must be detected in its early stages (Roush & Miller, 1986) and early detection necessitates testing of large number of individuals at each location where resistance is suspected.

The purpose of our study was to investigate status of resistance in *B. tabaci* to insecticides viz. imidacloprid, bifenthrin and fenvalerate and to elucidate the dynamics of resistance in natural populations from Punjab. Information about the genetic basis of resistance can facilitate efforts to detect and monitor resistance, to assess the risk of resistance, to model the evolution of resistance and to delay resistance development in pests.

MATERIALS and METHODS Commercial formulations of test insecticides: Confidor 200 SL (imidacloprid), Bifenthrin 10 WP (bifenthrin) and Fenval 20 EC (fenvalerate) were used in these studies. Serial dilutions of the insecticides were prepared in distilled water.

Collection Sites and Rearing Conditions:

Potted cotton plants of *Gossypium hirsutum* var. F846 were raised (free of insecticides) in large insect proof cages inside the screen houses. Wooden cages (45 cmx45 cmx60 cm) lined with insect-proof mesh on two sides and sliding glass pans to facilitate operations on other two sides were used to raise whiteflies within screen houses. The top of cage was also lined with fixed glass pan for ambient lighting and temperature conditions in the screen house. Five to six weeks old cotton plants were used for rearing whiteflies. A large number of genetically diverse populations of whitefly (> 10, 000 adults) was collected in 2001 from different crops in Punjab, India. A mixed colony was established before initiation of selection. Subsets of the colony were used to establish four strains in the screen houses, selected with (1) imidacloprid, (2) bifenthrin, (3) fenvalerate and (4) the unselected founder strain, which was not exposed to any insecticide. Each strain was reared in two to three similar cages. Care was taken to minimise the risk of contamination by hanging yellow sticky traps.

Bioassay Procedures and Resistance Estimates:

Assays were performed with adults of each strain. The bioassay method was adapted from Dittrich et al. (1985) with slight modifications to determine the tolerance levels of adults of each strain to imidacloprid, bifenthrin and fenvalerate. Cotton leaf discs were dipped in aqueous solution of formulated materials for 10 seconds and allowed to dry for 1 h. To maintain the turgidity after drying, the treated leaf disc was placed in a plastic Petri dish lid (4.0 cm dia) containing a thin layer of agar. Twenty to thirty (unsexed) whitefly adults were transferred to leaf disc in the Petri dish lid and the lid was inverted over the other half of the dish. The insects attached themselves to the underside of the leaf disc in their regular feeding position. Ventilation was provided by mesh-covered openings drilled on the sides of the Petri dish. These Petri dishes were held at 27.0±2.0 oC with a photoperiod of 12:12 (L:D) and observations for mortality recorded after 24 h. The symmetric design recommended by Finney (1971) for precise estimation of LC50 was used in all studies. A series of five doses was used for each insecticide tested. The entire procedure was replicated five times. Control tests were conducted with cotton leaf discs without any insecticide treatment in Petri dishes.

Selection Procedure:

Three subsets of the field-collected *B. tabaci* strains were selected separately with imidacloprid, bifenthrin and fenvalerate by following the above mentioned bioassay procedure. Concentration-mortality regression was worked out using probit analysis package POLO-PC (Le-Ora Software-1987 based on Finney, 1971)). The selection pressure

equivalent to 60-80 per cent mortality was applied through 9 generations.

Data Analyses:

Slope and LC50 values were based on concentration. Mortality regression was compared between generations to monitor the development of resistance. Two LC50 values were considered significantly different if their 95 % fiducial limits did not overlap. Tolerance ratios were computed as a ratio between LC50 of unselected strain population and LC50 of the insecticide-selected strain. Resistance risk assessment was made by calculating realized heritability values (h^2) as described by Tabashnik (1992):

$$h^2 = R/S$$

where R is the response to selection and S is the selection differential (Hartl, 1988, Falconer, 1989).

Response to selection (R), the difference in mean phenotype between offspring of the selected parents, and the parent generation before selection (Falconer, 1989) was estimated as

$$R = \frac{\log(\text{final } LC_{50}) - \log(\text{initial } LC_{50})}{n}$$

where final LC50 is the LC50 of the offspring after 'n' generations of selection and initial LC50 is the LC50 of the parental generation before 'n' generations of selection. The difference between LC50s was calculated on a logarithmic scale because the logarithm of tolerance was assumed to be normally distributed while the numerator of above equation for R estimates the cumulative responses to selection over 'n' generations.

The selection differential (S), the difference in mean phenotype between the selected parents and the entire parental generation (Hartl, 1988) was estimated as

$$S = i\sigma_p$$

where 'i' is the intensity of selection and σ_p is the phenotypic standard deviation. Intensity of selection (i) was estimated from p, which is the percentage of the population with values above the selection threshold (i.e. the percentage surviving selection) using Appendix of Falconer (1989), which is based on the properties of normal distribution.

The phenotypic standard deviation (σ_p) was estimated as the reciprocal of the mean of the estimated slopes of probit regression lines (Finney, 1971) from the parental selection before insecticidal selection (initial slope) and the offspring after 'n' generations of selection (final slope).

$$\sigma_p = [1/2 (\text{initial slope} + \text{final slope})]^{-1}$$

The number of generations (G) required for a 10-fold increase in LC50, is the reciprocal of R.

$$G = R^{-1}$$

The degree of dominance (of resistant component) was estimated separately for the three insecticide-selected strains with the following formula (Stone 1968):

$$D = \frac{(2Y_3 - Y_2 - Y_1)}{(Y_2 - Y_1)}$$

where:

- D - Dominance of the examined character (resistance)
- Y1 - Log10 of the LC50 of the F0 generation of the unselected check strain
- Y2 - Log10 of the LC50 of the F8 generation of the insecticide-selected strain
- Y3 - Log10 of the LC50 of the F1 generation of the insecticide-selected strain

This formula will result in a value of -1 if the resistance is completely recessive, a value of 0 if there is no dominance, and a value of +1 if the resistance is completely dominant.

The number of independent genes with additive effects that contribute to the expression of a trait (such as insecticide resistance) was estimated from the mortality data obtained in successive generations selected with three insecticides as per Raymond et al. (1987).

$$n_E = \log_{10}(\%\ \text{survivors}) / \log_{10}(1/2)$$

Another independent method given by Lande (1981) to estimate number of genes with additive effects contributing to the expression of a quantitative character (insecticide resistance) was

$$n_E = [\sum_{i=1}^{N} \sigma_i^2] / \sum_{i=1}^{N} (\sigma_i)^2$$

where σ^2 is the genetic variance of the insecticide-selected strain, estimated as $(\text{slope}^{-1})^2$ and N is the number of generations.

RESULTS

Response of Whitefly to Imidacloprid, Bifenthrin, and Fenvalerate:

The LC50s of the control strain to imidacloprid did not show any significant change during the period of selection (Table 1), where as selection of whiteflies with imidacloprid at LC60-80 concentration for 8 generations produced an increase in resistance (Table 2). Whiteflies did not show any appreciable change in

the tolerance to imidacloprid during the first two generations (LC50 ranged from 50 to 90 ppm).

Tolerance ratio during this period was 1.73 fold (Table 3). Minimal increase in LC50s was seen between generations 7 (890 ppm) and 8 (920 ppm, 1.03-fold). Beginning in the generation F2, an upward trend continued, culminating in 21.90-fold (TR) resistance in the F8 generation, with LC50 of 920 ppm. The results did not show appreciable increase in the values of slope in the successive generations in spite of continued selection pressure. The slope values related to imidacloprid ranged from 0.62 to 1.63, indicating considerable heterogeneity in the response of these whiteflies to Imidacloprid, suggesting a greater potential for the development of higher levels of resistance.

Table 1. Susceptibility of unselected population of cotton whitefly to different insecticides

Generation	Imidacloprid		Bifenthrin		Fenvalerate	
	Slope (±SE)	LC_{50} (95 % CL, ppm)	Slope (±SE)	LC_{50} (95 % CL, ppm)	Slope (±SE)	LC_{50} (95 % CL, ppm)
F_0	0.99 (0.12)	50 (39-64)	0.69 (0.12)	110 (60-160)	0.46 (0.18)	2320 (1980-42600)
F_1	0.70 (0.12)	52 (40-68)	0.52 (0.13)	95 (56-147)	0.39 (0.16)	2060 (1870-41700)
F_2	2.2 (0.26)	39 (29-51)	0.91 (0.17)	100 (63 –152)	1.12 (0.23)	2030 (1820-40500)
F_6	1.61 (0.21)	52 (43-62)	1.27 (0.24)	103 (66-158)	0.69 (0.20)	2490 (2130-53400)
F_7	0.59 (0.11)	45 (30-66)	0.57 (0.12)	86 (51-137)	0.53 (0.14)	1870 (1510-3550)
F_8	1.21 (0.18)	42 (28-61)	0.60 (0.11)	93 (54-145)	0.50 (0.16)	1968 (1627-39681)

Table 2. Susceptibility of the insecticide–selected strain of the whitefly to different insecticides

Generation	Imidacloprid		Bifenthrin		Fenvalerate	
	Slope (±SE)	LC_{50} (95 % CL, ppm)	Slope (±SE)	LC_{50} (95 % CL, ppm)	Slope (±SE)	LC_{50} (95 % CL, ppm)
F_0	0.99 (0.12)	50 (39-64)	0.69 (0.12)	110 (60-160)	0.46 (0.18)	2320 (1980-42600)
F_1	0.76 (0.10)	90 (60-140)	0.71 (0.13)	180 (70-294)	0.47 (0.10)	2860 (2056-62460)
F_2	0.62 (0.13)	230 (164-560)	0.78 (0.11)	220 (90-496)	0.56 (0.12)	3170 (2190-73900)
F_6	0.85 (0.10)	740 (712-1579)	4.59 (0.18)	530 (321-960)	5.90 (0.15)	6680 (3570-189600)
F_7	0.89 (0.11)	890 (490-2940)	4.73 (0.13)	590 (332-1050)	7.93 (0.24)	7400 (3920-275460)
F_8	1.63 (0.14)	920 (480-3370)	5.98 (0.15)	663 (372-1964)	9.54 (0.37)	8130 (4570-314670)

LC50 values of the unselected population to bifenthrin and fenvalerate also showed no perceptible change during the course of eight generations. The LC50s values for bifenthrin and fenvalerate of unselected population were ranged invariably between 93-110 and 1870-2490 ppm, respectively in the successive generations (Table 1). Whitefly populations through eight generations of selection achieved only moderate levels of increased tolerance (Tables 2 and 3). The LC50 of bifenthrin and fenvalerate-selected strains continued to increase over the generations and peaking in F8 (LC50 = 663 and 8130 ppm, TR = 7.12 and 4.13-fold respectively). With the development of resistance over the generations, these strains showed a correspondingly increasing slope values when selected with Bifenthrin (0.69 - 5.98) and Fenvalerate (0.46 - 9.54). The high slope values point to the establishment of highly homozygous populations with regard to the resistance trait.

Resistance Risk Assessment:

Estimated h2 to imidacloprid was 0.16 and 0.02 in the F0 to F2 and F6 to F8 (Table 4). The values of h2 obtained at the end of three generations of selection were very high (8 times) and could indicate a high level of risk in the field populations for development of resistance to imidacloprid. The results also suggest that brief selection period (three generations) may be sufficient to detect the potential for the development of resistance.

On the other hand, estimated h2 was 0.07 and 0.21 for the F0 to F2 and F6 to F8, respectively in case of bifenthrin and 0.02 and 0.38 for the F0 to F2 and F6 to F8, respectively in case of fenvalerate. The h2 values estimated at the end of three generations of selection (F1 to F2) were 3 times less than those for F6 to F8 in case of bifenthrin, 19 times in case fenvalerate. This reflects that high levels of resistance to bifenthrin and fenvalerate can only be realized after long periods of selections (eight generations) in the field populations of whitefly.

Table 3. Tolerance ratio of the whitefly strains selected with different insecticides in successive generations

Filial generation	Imidacloprid	Bifenthrin	Fenvalerate
F_0	1	1	1
F_1	1.73 (1.5-2.06)	1.89 (1.25-2.00)	1.39 (1.10-1.50)
F_2	5.90 (5.65-10.98)	2.20 (1.42-3.26)	1.56 (1.20-1.82)
F_6	14.23 (11.39-25.47)	5.14 (4.86-6.07)	2.68 (1.68-3.55)
F_7	19.78 (16.33-44.54)	6.86 (6.51-7.66)	3.96 (2.60-77.60)
F_8	21.90 (17.14-55.24)	7.12 (6.89-13.54)	4.13 (2.81-7.93)

Projected Rates of Resistance Development:

The number of generations (G) required for 10-fold increase in LC50 was estimated to be 5, 10 and 25 with mean response of first three generations (F0 to F2) in case of imidacloprid, bifenthrin and fenvalerate. On the other hand, 33 generations were calculated to be needed for the development of 10-fold resistance against all the three insecticides with the mean response of last three generations (F6 to F8).

Degree of Dominance:

The degree of dominance of the resistant trait in imidacloprid, bifenthrin and fenvalerate-selected strains of whitefly were - 0.60, - 0.43 and -0.52

respectively. This indicates that insecticidal resistance against above three insecticides in whitefly is nearly completely recessive.

Estimation of the Number of Genes Involved in Insecticide Resistance:

The number of genes involved in insecticide resistance against imidacloprid, bifenthrin and fenvalerate as per Raymond et al. (1987) was found to be more than 1 up to F2 generation and ultimately resistance was controlled by single gene. But the mean calculated number genes are 1.24, 1.13 and 1.01 for three insecticides respectively.

With the use of Lande's (1981) formula for effective number of factors (genes) segregating across eight generations was estimated to be 4.85, 3.09 and 2.95 for the insecticide resistance against imidacloprid, bifenthrin and fenvalerate, respectively.

Table 4. Realized heritability (h^2) of insecticide resistance in whitefly

No. of generations selected (n)	Estimate of mean response per generation			Estimate of mean selection differential per generation						h^2
	Initial LC_{50} (log)	Final LC_{50} (log)	R	p	i	Initial slope	Final slope	σ^2	S	
Imidacloprid										
$F_0 - F_2$	1.7	2.36	0.22	32.1	1.12	0.99	0.62	1.24	1.39	0.16
$F_6 - F_8$	2.87	2.96	0.03	53	0.78	0.85	1.63	0.81	0.63	0.02
Bifenthrin										
$F_0 - F_2$	2.04	2.34	0.1	36.7	1.08	0.69	0.78	1.36	1.47	0.07
$F_6 - F_8$	2.72	2.82	0.03	55.2	0.71	4.59	5.98	0.19	0.14	0.21
Fenvalerate										
$F_0 - F_2$	3.36	3.5	0.04	38.5	1.02	0.46	0.56	1.96	2	0.02
$F_6 - F_8$	3.82	3.91	0.03	61.8	0.6	5.9	9.54	0.13	0.08	0.38

Table 5. Estimation of number of genes contributing to insecticide resistance in whitefly against different insecticides

Generation	Imidacloprid		Bifenthrin		Fenvalerate	
	Survival (%)	Number of genes (n)	Survival (%)	Number of genes (n)	Survival (%)	Number of genes (n)
F_0	22.3	2.2	30	1.7	32.4	1.6
F_1	34	1.6	36	1.5	40	1.3
F_2	40	1	44	1.2	43	1.2
F_6	50.5	0.9	52	1	58	0.7
F_7	53.5	0.9	56.3	0.8	62	0.7
F_8	55	0.9	57.3	0.8	63.3	0.7
Mean	42.55	1.24	45.93	1.13	49.78	1.01

DISCUSSION The increase in resistance in all the three selected lines indicates that imidacloprid, bifenthrin or fenvalerate resistance in B. tabaci is at least in part genetically determined (Bloch and Wool, 1994) and that a part of the variation in resistance is additive (Falconer, 1989).

One of the important uses of heritability estimates is prediction of future response (Hartl 1988, Falconer, 1989). The purpose of pesticide resistance studies is to predict the rate of development of resistance in response to pesticide application (Via 1986, Firko and Hyes, 1991). Heritability estimate after one generation of selection is often a reliable approximation of the heritability of the trait in the parental population because laboratory environment has minimal effects (Tabashnik, 1991). As the mean response for the first three generations is high in case of imidacloprid, therefore risk for development of resistance is higher as compared to bifenthrin and fenvalerate. Almost more than 25 generations are required to develop 10-fold increase in LC50 both in case of bifenthrin and fenvalerate against only 5 generations in case of imidacloprid.

The inheritance of resistance according to Raymond et al. (1987) formula is found to be a multifactor phenomenon in the early generations under continuos selection pressure while single factor in the later generations.

Calculations based on Lande's (1981) formula suggest that insecticide resistance against imidacloprid, bifenthrin and fenvalerate is controlled by more than 1 gene. This can further be substantiated by the significant increase in tolerance ratios in response to the selection over the generations. Apparently, combinations of alleles responsible for tolerance not present in parental generations were produced in the succeeding generations (under continuos selection pressure) as a result of inbreeding among tolerant individuals, as suggested by Bloch and Wool (1994). Roush and McKenzie (1987) noted that most significant cases of resistance are caused by allelic variants at one or two loci. They reasoned that polygenic resistance is favoured by laboratory regimes that select at moderate doses from small samples, where as field applications that select at high doses from large populations favour monogene resistance by rare alleles.

Estimates of dominance in this study indicated that insecticide resistance was nearly completely recessive. This pattern could result from the additive inheritance of multiple genes (Keena and Granett, 1990).

Without suitable genetic markers, use of bioassays to discriminate between inheritance mediated by a single gene with modifiers and a more complex mode of inheritance is generally difficult and is almost impossible with overlapping concentration-response lines (Tsukamoto, 1963).

In conclusion, although extremely high levels of resistance were attained by these laboratory-selected populations, the differences may not necessarily translate to the reduction or loss of field performance of these insecticides (Denholm et al., 1984). We must

exercise caution in directly extrapolating results from laboratory experiments to the field situations. Populations found in the fields are usually more heterogenous and their responses to insecticides pressures are more complex and diverse. Field responses would be the result of the interactions of environment, population structure and selection intensity (Bloch and Wool, 1994). The alteration of insecticides and immigration of susceptible populations from other crops could delay the evolution of resistance in the field. Many of the insecticides used to control whiteflies are rendered ineffective more easily because of the occurrence of cross and multiple resistance.

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Resistance to Azoxystrobin in the Gummy Stem Blight Pathogen in Georgia

INTRODUCTION Gummy stem blight, caused by the fungus *Didymella bryoniae*, is the most widespread and destructive disease of watermelon in Georgia and in many other watermelon-producing areas of the U.S. (Fig. 1). Azoxystrobin (Quadris, Syngenta Crop Protection, Greensboro, NC) was shown to have excellent efficacy on gummy stem blight by several researchers in the early 1990s and was granted Section 18 emergency exemption status in Georgia in 1997 and 1998 specifically for gummy stem blight control. A full Section 3 national label was granted for azoxystrobin use on cucurbit crops in March of 1999 that led to widespread and routine use of the fungicide to control a broad spectrum of foliar cucurbit diseases. However,

compared to previous reports of disease control with azoxystrobin (Sumner and Hall 1997), reduced efficacy of azoxystrobin on gummy stem blight was first observed in Georgia as early as 1999 in watermelon field trials (Langston et al. 2000) and commercial watermelon fields treated with Quadris. Isolates of the pathogen collected in 2000 from watermelon fields in Delaware, Maryland, South Carolina, and Georgia, where disease control was unsatisfactory, were confirmed to be resistant to azoxystrobin in in vitro laboratory assays (Olaya and Holm 2001). In 2001, an extensive survey was conducted to determine the frequency of azoxystrobin-resistant isolates in commercial watermelon fields in Georgia.

MATERIALS and METHODS Isolates of the fungus were obtained from samples of infected watermelon collected in 2001 from 25 commercial watermelon fields and research sites in Georgia (Fig. 2). Sensitivity of each isolate to azoxystrobin was determined using a spore germination assay on water agar (WA) medium amended with azoxystrobin (0, 0.0001, 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, or 10 µg a.i./ml) and 100 µg/ml salicylhydroxamic acid (SHAM) to inhibit an alternative respiratory pathway in the fungus that can interfere with the activity of the fungicide. Technical grade azoxystrobin was dissolved in acetone and serially diluted to the appropriate concentration, and added to autoclaved WA cooled to 60 C, such that the concentration of acetone was 0.1% vol/vol in all treatments. Conidial suspensions of each isolate were prepared and transferred to fungicide-amended or nonamended medium in small petri dishes (60 H 15 mm). Two replicate dishes of each fungicide concentration and isolate combination were prepared. After 48 h of incubation at 23-25°C, 50 conidia per dish were examined microscopically and the percentage of germinated conidia was recorded. Fungicide sensitivity was expressed as the EC50 value (the fungicide concentration that inhibits spore germination by 50% relative to the control). As reported in the previous study (Olaya and Holm 2001), an isolate was considered resistant to azoxystrobin if the EC50 value was >10 µg ml-1.

RESULTS and DISCUSSION Results of in vitro sensitivity assays provided evidence of widespread resistance in the gummy stem blight pathogen to azoxystrobin in Georgia. Of the 272 isolates from 27 fields in 13 counties, 247 (91%) were found to be resistant to azoxystrobin based on the spore germination assay. Sampling for sensitivity monitoring was continued in 2002. It is very difficult to determine at this time exactly where the azoxystrobin-resistant isolates originated. However, overuse of the product both in the greenhouse and in the field is the suspected cause. When a fungicide of this type is used repeatedly,

Figure 1. Symptoms of gummy stem blight, caused by Didymella bryoniae on a watermelon leaf.

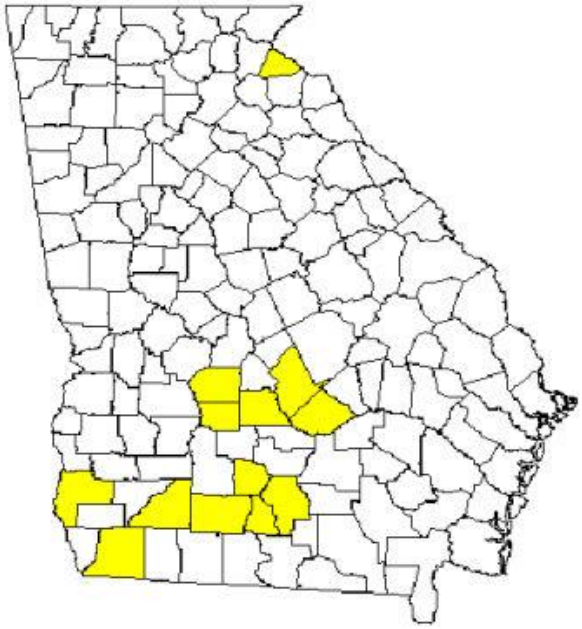

Figure 2. Counties in Georgia where sampled fields in 2001 were located are shown in yellow.

without rotating to fungicides with a different mode of action, the chance of selecting for a fungicide-resistant population of the target fungus is very great. Rotating to different fungicide chemistries will hopefully control the resistant populations before they can reproduce and spread. Growers are encouraged to use the more traditional fungicides for protecting their watermelon crops. Mancozeb and chlorothalonil products both suppress gummy stem blight to some degree. Mancozeb products alone are usually marginally effective at best and chlorothalonil products have been implicated with a rind burn when applied within two

Table 1. Frequencies of azoxystrobin-resistant isolates of the gummy stem blight pathogen, *Didymella bryoniae*, in watermelon fields in Georgia in 2001

County	Field	Azoxystrobin use in 2001	Total # isolates	# Resistant isolates[y]	% Resistant isolates
Berrien	1	Yes	19	19	100
	2	Yes	12	10	83
Colquitt	1	NA[z]	6	6	100
Cook	1	Yes	6	6	100
	2	Yes	15	15	100
Crisp	1	No	6	4	67
	2	NA	13	10	77
Decatur	1	Yes	8	8	100
	2	No	17	15	88
	3	Yes	14	14	100
Dodge	1	No	8	8	100
Dooly	1	No	7	7	100
	2	NA	6	4	67
Early	1	Yes	6	6	100
Mitchell	1	Yes	14	14	100
Stephens	1	Yes	14	13	93
Telfair	1	Yes	11	11	100
Tift	1	Yes	6	5	83
	2	NA	8	8	100
	3	NA	6	5	83
	4	NA	9	7	78
Wilcox	1	NA	14	14	100
	2	NA	10	10	100
	3	NA	9	7	78
	4	NA	16	9	56
TOTAL			**272**	**247**	**91**

[y] Isolate considered resistant to azoxystrobin if EC_{50} value was >10 $\mu g\ ml^{-1}$, based on a conidial germination assay.

[z] No information available on fungicide use.

weeks of harvest. However, chlorothalonil remains our most effective labeled material for gummy stem blight suppression where azoxystrobin resistance has been detected. Table 1.

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Resistance of Little Seed Canary-grass *Phalaris minor* Retz. and Hood Canary-grass *Phalaris paradoxa* L. to commercial herbicides in the Yaqui Valley of Sonora, México

ABSTRACT Wheat is the most important crop grown in northwestern México. In this crop, key narrow leaf weeds are *Avena fatua* L. and *Phalaris* spp. These weeds have been traditionally controlled with herbicides possessing the same mode of action, which has resulted in a high selection pressure. Reports indicate that some populations of *Phalaris* spp. have not been controlled by commonly used herbicides in the Yaqui Valley of Sonora, México. These reports were important in order to establish a study to determine the possibility that these populations were tolerant or resistant to the herbicides used for its control. During the fall and winter seasons of 1997 and 1998, seeds of Little Seed Canary-grass and Hood Canary-grass were collected from commercial crops that were sprayed with herbicides and still had spots with these weeds. Collections of seeds from these spots, were grown in bio-climatic chambers, and later sprayed with the herbicides fenoxaprop-p-ethyl (Puma S 75 WE), tralkoxydim (Grasp 25 SC), dichlofop-methyl (Iloxan 28 CE), and clodinafop (Topik) at commercial dosages (1X) and twice this dosage (2X). Data (% control) were obtained at 7, 15, 30 and 60 days after herbicide application to determine the effect of treatments. Results indicated that some populations were resistant to fenoxaprop-p-ethyl since there was no control in any one of the two species evaluated at the commercial (1X) and twice (2X) dosages. However these populations were efficiently controlled with the commercial dosage of diclofop-methyl and clodinafop. The herbicide tralkoxydim had control only with the 2X dosage. The commercial dosage (1X) had less than 10% control of both species indicating resistance problems.

INTRODUCTION Wheat is the most important crop grown in northwestern México. Key narrow leaf weeds for this crop are *Avena fatua* L. and *Phalaris* spp. These weeds have been traditionally controlled with herbicides possessing the same mode of action, which has resulted in a high selection pressure. Reports indicate that some populations of *Phalaris* spp. have not been controlled by commonly used herbicides in the Yaqui Valley of Sonora, México.

Little Seed Canary Grass, *Phalaris minor* L., is a weed in the Poaceae family. In México, this weed first

evolved resistance to Group A/1 herbicides in 1996 infested wheat. Group A/1 herbicides are known as ACCase inhibitors (Inhibition of acetyl CoA carboxylase). Research has shown that these particular biotypes are resistant to fenoxaprop-p-ethyl and that they may be cross-resistant to other Group A/1 herbicides (Sayre, K. 1996).

Local weed scientists estimated that Group A/1 resistant Little Seed Canary grass infests between 501-1000 sites in irrigated wheat production areas of central highlands in the states of Guanajuato and Michoacan, México, and that the number of sites is increasing. They also estimate that there are 1001-10000 acres infested with Group A/1 resistant weeds and that the infested area is increasing (Sayre, K. 1996).

The objective of this work, was to determine the response to commercial herbicides of selected populations of Little Seed Canary-grass *Phalaris minor* Retz. and Hood Canary-grass *Phalaris paradoxa* L. in the Yaqui Valley, Sonora, México.

MATERIALS and METHODS During the fall and winter seasons of 1997 and 1998, seeds of Little Seed Canary-grass and Hood Canary-grass were collected from commercial crops that were sprayed with herbicides but still presented spots with these weeds. Collections of seeds from these spots were grown in bio-climatic chambers, and later sprayed with the herbicides fenoxaprop-p-ethyl (Puma S 75 WE), tralkoxydim (Grasp 25 SC), dichlofop methyl (Iloxan 28 CE) and clodinafop (Topik) at commercial dosages (1X) and twice this dosage (2X). Data (% control) were obtained at 7, 15, 30 and 60 days after herbicide application to determine the effect of treatments.

RESULTS and DISCUSSION Results indicated that some populations of Little Seed Canary-grass and Hood Canary-grass were resistant to fenoxaprop-p-ethyl since there was not efficient control in any one of the two species evaluated at the commercial (1X) and twice (2X) dosage. Control of these populations was 5-10% with the commercial dosage and 10-15% in the 2X dosage.

The same populations were controlled efficiently with the commercial dosage of diclofop-methyl, which resulted in 95% control of Little Seed Canary-grass 15 days after treatment (dat) (Figure 1), and 100% control 30 dat for Hood Canary-grass populations (Figure 2). The 2X dosage had 100% control of both species 30 day after treatment.

Clodinafop controlled efficiently these populations at the commercial dosage. This herbicide showed 95% control of Little Seed Canary-grass (Figure 1) at 15 dat, and 95% control of Hood Canary-grass populations at 30 dat (Figure 2). The 2X dosage presented 100% control of both species at 60 dat. Tralkoxydim at the 2X dosage showed 100% control of both species at 15 dat. The commercial dosage presented less than 10% control of both species indicating resistance problems.

CONCLUSION

1. Selected populations of Little Seed and Hood Canary-grass, from the Yaqui Valley of Sonora Mexico, showed resistance to the herbicide fenoxaprp-p-ethyl.
2. These populations were not resistant to clodinafop and diclofop-methyl.
3. Both species presented resistance (commercial dosage do not had control) to the herbicide tralkoxidim.

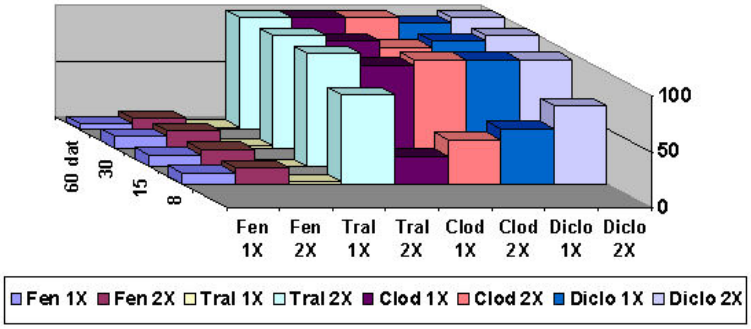

Figure1. Herbicide efficacy tests on Little Seed Canary-grass *Phalaris minor* Retz. at the commercial (1X) and at 2X dosage. Yaqui, Valley, Sonora, México.

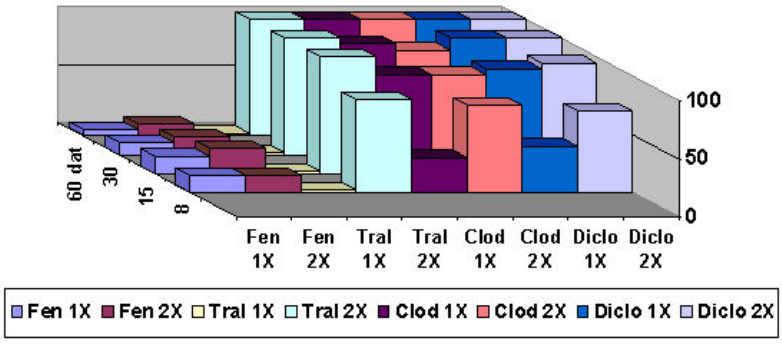

Figure2. Herbicide efficacy tests on Hood Canary-grass *Phalaris paradoxa* L. at the commercial dosages (1X) and at the 2X dosages. Yaqui Valley, Sonora, México.

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Research in Resistance Management

Quantifying the Incidence of Herbicide Resistance in the Winter Rainfall Regions of South Africa

ABSTRACT Resistance of weeds to herbicides has developed in most countries in the world and South Africa is no exception. The incidence of herbicide resistance in South Africa is increasing, but little data is available to quantify the increase in occurrence of resistance. In this study the incidence of suspected and confirmed herbicide resistance is investigated by making use of literature reports, surveys and databases of relevant research institutions. Apart from confirmed cases of resistance reported on in the literature, more information on resistance was obtained by making use of questionnaires and by greenhouse testing of suspected resistant weed populations. The questionnaires were distributed in 1999, 2000 and 2001 to obtain a clearer picture of the incidence of suspected herbicide resistance in the winter rainfall area of the Western Cape Province. Seed samples obtained from suspected resistant weed populations were germinated and the resulting seedlings were subjected to spraying with three different herbicides at four different doses. The treatments were replicated three times and the dry mass production and mortality of the seedlings were evaluated four weeks after date of application. Results from the questionnaires indicated that there was a steady increase in the number of suspected resistance cases in the area surveyed and that more than 230 suspected cases occurred in 2000. Results from the greenhouse tests indicated that, from a total of 49 weed populations tested up to now, 8 populations exhibited no resistance, 12 exhibited single resistance, 14 exhibited cross resistance and 15 populations exhibited multiple resistance. These results are a clear indication that herbicide resistance are present and spreading like wildfire in this particular area and that everybody in the industry should implement strategies aimed at curbing the rate of spread of herbicide resistance.

KEYWORDS herbicide resistance, South Africa, winter rainfall area

INTRODUCTION Towards the end of this century the International Survey of Herbicide Resistant Weeds recorded 235 herbicide resistant weed biotypes of which 150 unique species can be found in 43 countries (http://www.weedscience.com). In the winter rainfall region of South Africa, the first case of resistance was recorded in 1985 involving *Avena fatua* and diclofop-methyl (Cairns & Laubscher, 1985). Subsequently resistance was recorded in *Lolium* spp. (Smit & de Villiers, 1998; Smit, Smit & de Villiers, 1999), *Phalaris minor* (Smit & Cairns, 2000), and *Raphanus raphanistrum* (Smit & Cairns, 2001).

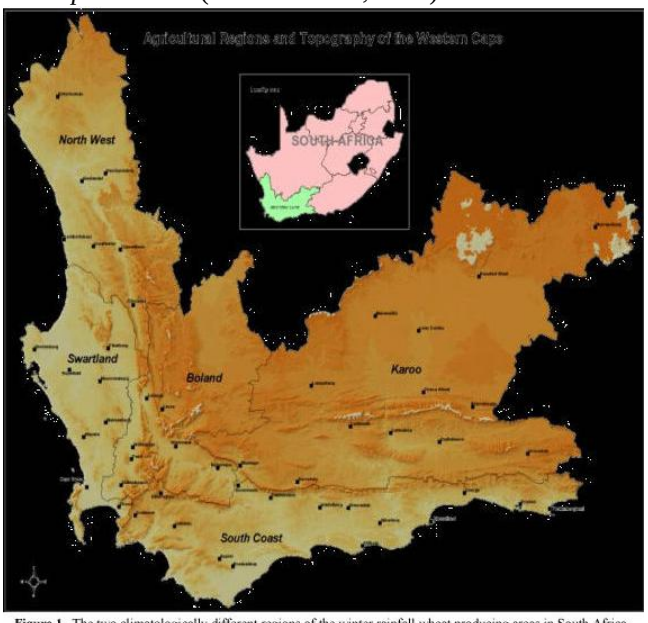

Figure 1. The two climatologically different regions of the winter rainfall wheat producing areas in South Africa viz. the Swartland and South Coast regions.

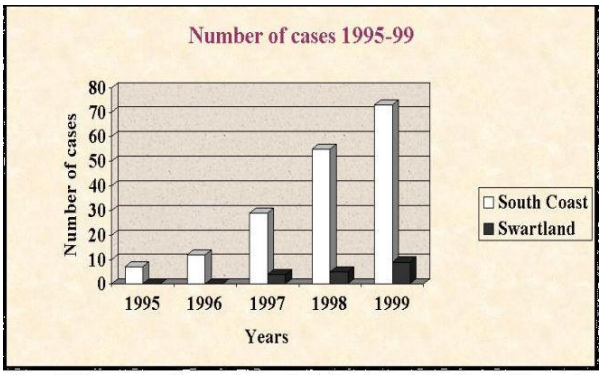

Figure 2. The occurrance of suspected cases of herbicide resistance in the winter rainfall areas of South Africa from 1995 to 1999.

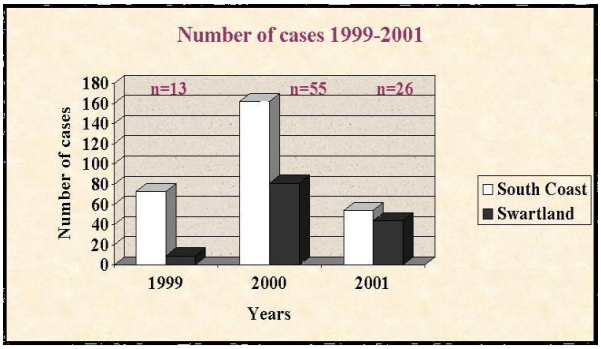

Figure 3. The occurrence of expected cases of herbicide resistance during the winter rainfall areas of South Africa from 1999 to 2001.

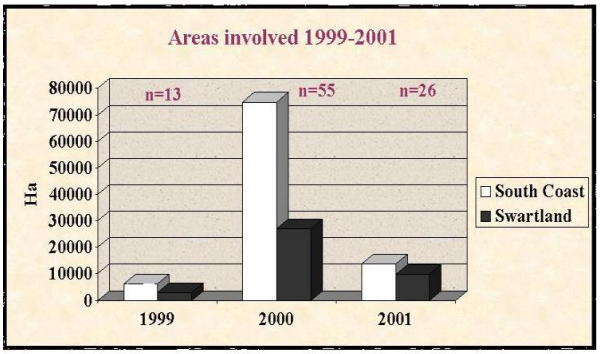

Figure 4. The areas involved in suspected in cases of herbicide resistance in the winter rainfall areas of South Africa from 1999 to 2001.

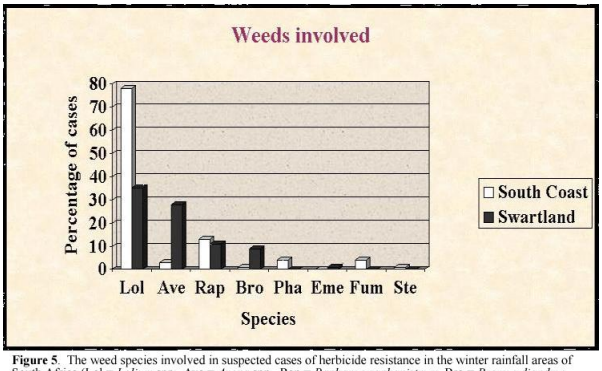

Figure 5. The weed species involved in suspected cases of herbicide resistance in the winter rainfall areas of South Africa (Lol = *Lolium* spp., Ave = *Avena* spp., Rap = *Raphanus raphanistrum*, Bro = *Bromus diandrus*, Pha = *Phalaris minor*, Eme = *Emex australis*, Fum = *Fumaria officinalis*, Ste = *Stellaria media*).

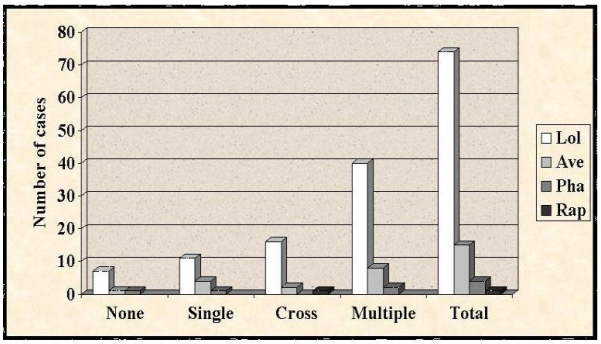

Figure 6. Occurrence of single-, cross-, and multiple resistance in weed populations tested during the 2000 and 2001 seasons (Lol = *Lolium* spp., Ave = *Avena* spp., Rap = *Raphanus raphanistrum*, Pha = *Phalaris minor*).

Although these weed species were proven to be resistant and various warnings about the threat of herbicide resistance have been issued (Lochner, 1999; Khorombi, 2000; de Villiers, 2001) the extent of the problem has not been quantified. This project attempts to quantify the incidence of suspected herbicide resistance in the winter rainfall wheat producing area of South Africa. Furthermore, samples from suspected herbicide resistant weed populations are tested under controlled conditions to verify resistance if present.

MATERIALS and METHODS

Suspected herbicide resistance

Questionnaires were distributed to agriculturalists in the winter rainfall wheat-producing region of South Africa. The questionnaires requested information on the occurrence of suspected herbicide resistance cases, the area involved, crop species and weed species involved. The area was divided into two climatologically distinct areas, viz. the Swartland, a strict winter rainfall area and the South Coast, where precipitation is distributed more evenly throughout the year (Figure 1). The data was arranged accordingly.

Confirmed cases of resistance

Investigations to confirm resistance in suspected resistant weed populations have been carried out at the Department of Agronomy at the University of Stellenbosch since 1999. In most cases, if enough material was available, three herbicides from two different modes of action were applied to each suspected resistant weed accession. Data obtained from the tests gave an indication of the type of resistance present, i.e. simple-, cross- or multiple resistance.

RESULTS and DISCUSSION

Suspected cases of resistance

In 1999 only 13 questionnaires were returned. According to these questionnaires, the number of suspected herbicide cases has increased from less than 10 in 1995 to about 80 in 1999, with most of these

Table 1. Weed species and herbicides involved in confirmed cases of resistance in the winter rainfall area of South Africa

Herbicides	Weed species			
	Lolium spp.	*Avena spp.*	*Phalaris minor*	*Raphanus raphanistrum*
ACC-ase inhibitors				
Codinafop-propargyl	X	X	X	
Diclofop-methyl	X	X	X	
Fenoxaprop-P-ethyl		X	X	
Fluazifop-P-butyl	X			
Haloxyfop-R-methyl	X		X	
Propaquizafop	X	X		
Quizalofop-P-tefuryl	X		X	
ALS inhibitors				
Chlorsulfuron				X
Imazamox	X			
Iodosulfuron	X	X	X	
Iodosulfuron + mesosulfuron	X	X	X	
Sulfosulfuron		X		
Triasulfuron				X
Prosulfuron				X

cases in the South coast region (Figure 2). In 2000 the number of questionnaires returned was 55, and the number of cases was about 240. In 2001 only 26 questionnaires were returned and they reported about 100 cases of suspected resistance (Figure 3). This indicates that resistance is quite common in these regions. It is difficult to make an accurate estimation of the areas involved in resistance but Figure 4 indicates that the areas involved may well be in the excess of 100 000 ha. The weed species involved in these cases of suspected resistance are given in Figure 5. It is obvious that *Lolium* spp. and *Avena* spp. are the most important species involved in resistance. *Lolium* spp. is more important in the South Coast than in the Swartland, probably because there is more pastures crops in that area.

Confirmed cases of resistance

Lolium spp. has developed resistance to about every ACC-ase herbicide that are registered on it, as well as all the ALS inhibitors (Table 1). Although no cases has been observed yet, it is highly probable that some *Lolium* populations could have developed resistance to most or all herbicides registered against it. *Avena* spp. and *Phalaris minor* also developed

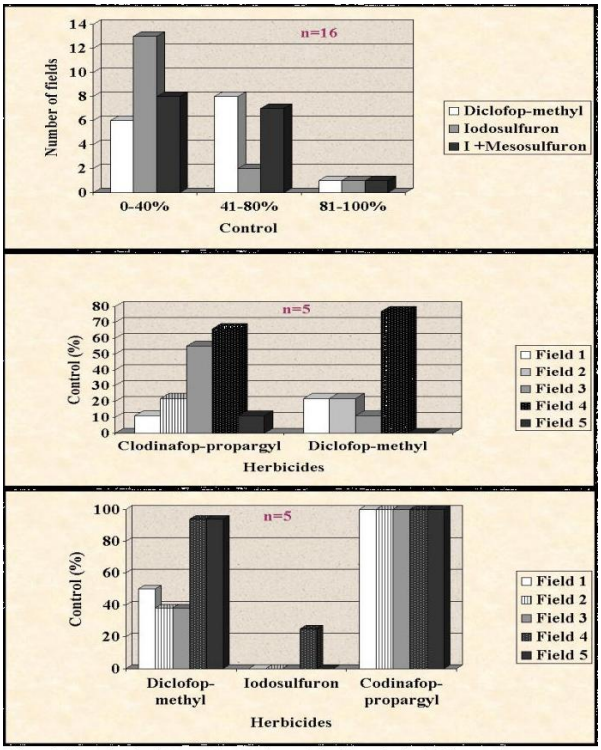

Figure 7. Examples of resistance patterns on farms: a) Resistance levels of *Lolium* spp. to diclofop-methyl, iodosulfuron, and iodosulfuron + mesosulfuron in 16 fields on one farm; b) Resistance levels of *Lolium* spp. to clodinafop-propargyl and diclofop-methyl in 5 fields on one farm; c) Resistance levels of *Avena* spp. to diclofop-methyl, iododsulfuron and clodinafop-propargyl in 5 fields on one farm.

resistance against a few ACC-ase inhibitors and ALS inhibitors (Table 1). From Figure 6 it is obvious that more than 80% of suspected resistant weed populations were tested positive for resistance. It is alarming that such a high percentage of populations exhibit multiple resistance.

Resistance is not a simple problem, as can be seen from Figure 6. If resistance has been confirmed on a farm, it does not necessarily mean that all the fields are infested with resistant biotypes. From Figure 7 it is clear that resistance to different herbicides can differ from field to field.

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Resistance Management News

Briefings: Grant Announcement ~ August 30, 2002

The IR-4 Biopesticide Research Program announces a request for grant proposals for funding in 2003

Biopesticide proposals are due on November 15, 2002. Researchers will be informed about funding status by early March 2003. Instructions for proposal content, format, and submission are attached. With newer targeted conventional chemicals there is interest in resistance management to maintain the utility of those products. Therefore, IR-4 is especially interested in proposals containing biopesticides as resistance management tools, rotated with conventional products. While resistance management is an important interest, the proposal must still have a **majority focus on biopesticides**. Selection of treatments and experimental design should be considered to elucidate the contribution of each component to the pest control system. In addition the proposal should focus on biopesticide uses that are not currently registered. Electronic submissions are encouraged. The amount of funding available in 2003 will be $ 400,000.

USDA/IR-4 Biopesticides Research Program Guidelines

Background

The IR-4 Project (IR-4) is a federally funded agricultural program whose mission is to assist specialty or minor crop producers by facilitating the availability of safe and effective pest control products. The program was initiated in 1963 by the directors of the state agricultural experiment stations and historically has focused on registration and reregistration of crop protection chemicals for use on minor crops or for minor uses on major crops.

Since 1982, IR-4 included research and support leading to registration of a wide range of biopesticides including microbials, such as fungi, bacteria, and viruses, low toxicity biochemicals, pheromones, insect and plant growth regulators, and plant incorporated protectants. In general, the number and type of studies required to register these products are different from the studies required to register conventional crop protection chemicals. Biochemicals must have an indirect mode of action and either be naturally occurring or a synthetic analog. IR-4 will consider biochemicals that meet the EPA definition as well as other low exposure, naturally occurring biochemicals which have pest control activity, provided they are considered safe and do not have significant toxicity to man, mammals, fish or birds.

The program is committed to developing pest control products on minor food and ornamental crops by working cooperatively with public and private sector individuals and organizations. IR-4 interacts with the USDA, EPA, Food and Drug Administration (FDA) and product registrants to determine the requirements for registration of proposed uses. The program has the resources to develop research protocols, assist with Experimental Use Permits, coordinate and fund field and laboratory research, assist in the development of Tier I toxicology and non-target organism data, and prepare data packages for submission to the EPA. IR-4 research is conducted according to EPA Good Laboratory Practice regulations utilizing the IR-4 Quality Assurance network.

Biologicals such as arthropod (insect) parasites and predators or predacious nematodes are not regulated under FIFRA and do not fall under the IR-4 program.

IR-4 Assistance for Biopesticide Programs

The primary objective of the IR-4 Biopesticides Research Program is to further the development and registration of biopesticides for use in pest management systems for specialty crops or for minor uses on major crops. Areas of IR-4 assistance include:

1. Develop an approved research protocol.
2. Assist in complying with EPA Good Laboratory Practice regulations.
3. Fund small and large-scale field efficacy trials.
4. Fund magnitude of residue trials, if needed.
5. Assist in obtaining Experimental Use Permits from EPA.
6. Prepare and submit petitions to the EPA to support clearances.
7. Develop data to expand registration to include additional crops and uses.
8. Prepare registration documents for submission to EPA.

IR-4 Biopesticide Clearance Program

General guidelines and submission of biopesticide clearance request forms:

The general guidelines that will be used to initially review a proposed biopesticide clearance request are shown in Appendix I. A proposal for assistance in clearing a minor use biopesticide should include a biopesticide clearance request (BPCR) form. A BPCR

form is shown in Appendix II. This form asks for information that is pertinent to the intended use of the biopesticide and serves as a pre-proposal to determine whether it is a project that is within the scope of IR-4. Blank forms are available from IR-4 national or regional offices or the IR-4 website www.cook.rutgers.edu/~ir4. Each BPCR form should be filled out as completely as possible and submitted to an IR-4 Regional Field Coordinator (see last page) who will forward it to IR-4 Headquarters.

Submission of research proposals:

Proposals are invited for early stage as well as advanced stage biopesticides. Potential registrants are strongly encouraged to cooperate with public institutions in proposal submission; however proposals submitted soley from a company will not be considered. Early stage biopesticides are biopesticides for which EPA subpart M Tier I data requirements are not completed or satisfied by appropriate waivers. Early stage biopesticide research proposals will include the plan of work, budget, and timetable for completion. The guidelines for submitting an early stage research proposal are attached (Appendix III). For advanced stage biopesticide proposals requesting funding of efficacy research, the following items need to be submitted: 1) a biopesticide clearance request (BPCR) form (see Appendix II). Preliminary efficacy data should be attached to the request form if available. 2) a research proposal, which includes details such as treatments, rates, number of applications, spray intervals (if appropriate), data to be collected and amount of funding requested. The proposal should include a breakdown of budget (e.g. supplies, equipment, wages, etc.). Grant requesters are encouraged to interact with their Regional Field Coordinator and the potential registrant prior to developing and submitting a proposal. All completed proposals should be submitted to the Manager of the IR-4 Biopesticide Program at IR-4 Headquarters. Proposals will then be reviewed for merit by IR-4 internal and external reviewers based on the criteria shown in Appendix IV (early stage proposals) or Appendix V (advanced stage proposals).

Selection of projects for funding:

Comments from the internal and external reviewers will be summarized and a recommendation for funding will be made by the IR-4 New Technology Team to the IR-4 Project Management Committee (PMC). The PMC will authorize all funding decisions.

Notification of Project Funding:

The IR-4 Biopesticide Program Coordinator will notify the requestor of the funding decision of the IR-4 PMC.

Progress reports:

Annual progress reports are required if the research is not completed within one year. Otherwise, a final report is required. All reports should be sent to the IR-4 Biopesticide Program Coordinator.

Continuation Grants/Renewal Grants:

In a majority of cases, IR-4 will commit research funds for only one year at a time. In order to receive funding beyond the first year, the grantee must submit a request for continuation of funding, a progress report on research conducted under the existing grant, justification for continued funding, and a plan of work to be carried out under the continued grant. Decisions regarding continued support and the actual funding levels are made by the IR-4 New Technology Team and PMC after consideration of such factors as grantee's progress, availability of funds and chance of commercial success.

Data for an experimental use permit (EUP) or full registration of a biopesticide:

Data for biopesticide registration under the IR-4 Project are different than that for chemical registrations. The IR-4 Biopesticide Program can assist in the development of efficacy and crop safety data. This may require interfacing with EPA, USDA, FDA and State Regulatory Agency such as the California Department of Pesticide Regulation depending on the specific project. Projects considered for support must be carefully reviewed to insure that adequate data are available or can be obtained in a reasonable period of time to be able to support a new registration.

Appendix I

General Guidelines

- The biopesticide must be subject to registration under the Federal Insecticide, Fungicide, and Rodenticide Act as Amended. Biopesticides include microbials, nonviable microbials, and biochemical pesticides including pheromones, attractants, insect growth regulators, plant growth regulators, and other compounds such as natural products, and plant incorporated protectants.
- IR-4 will support the development of data for the registration of a biopesticide where the need is in the public interest and there is reasonable potential for commercial production and the use involves a minor crop or a minor use on a major crop.
- In efficacy studies, an integrated approach looking at the role of biopesticides as resistance management tools in rotation with conventional chemical products is strongly encouraged. The experimental design should

enable the evaluation of the individual products in addition to rotational treatments.
- The support by IR-4 for the registration of biopesticides will not exceed that needed for EPA Tier I studies under Subpart M and efficacy data.
- Preliminary data are available supporting efficacy against target pest(s).
- A production method is feasible and there is potential for a commercially formulated product.
- Practical application technology exists.
- The use pattern is compatible with other agricultural practices.
- The host range and pathogenicity are known and safety data to protect the researcher exists.
- The potential researcher can meet GLPs when they are required.

Appendix II

An electronic copy of the Biopesticide Clearance Request Form is available at the following site:

http://www.cook.rutgers.edu/~ir4

Appendix III

Format for Biopesticide Proposal

1. **Cover page** - See Proposal Cover Page in Appendix VI
2. **Summary page**
   Common name of biopesticide. (If applicable.). Please provide a copy of the current or proposed label. Genus and species or chemical name. Commodities or sites protected. Target pests (identify by common name and scientific name). **If known**: For early stage proposals; disclosure of the formulation, indicate active and inert ingredients where applicable and indicate potential registrant.
3. **Background**. The background page should include a brief summary of the current literature pertaining to the project. Also include laboratory, greenhouse and/or field data supporting the project. Justification for product use should be included. Alternative controls and potential hazards should be addressed as well as how the proposed work fits into the overall strategy for registration. For early stage proposals, production method for organism and level of commercial interest should be noted and list the type of toxicology studies completed. Note any licensing or patent rights related to this biopesticide.
4. **Plan of work**.
   1. For those studies where the intent is to develop performance data the following should be addressed:
      - The objectives of the research.
      - Experimental design including statistical tests.
      - The treatments to be applied.
      - Research plan and chronology of the experiment (including plot size, replicates. dosage rates, sampling times, methods of sampling, and other applicable information.).
      - For efficacy studies, an integrated approach looking at the role of biopesticides as resistance management tools in rotation with conventional chemical products is strongly encouraged.
      - The experimental design should enable the evaluation of the individual products in addition to rotational treatments for California registration requirements.
      - All studies should include the "typical" commercial standard as a treated control.
5. **Budget**. The proposal should include a breakdown of budget (e.g., supplies, equipment, wages, etc.). No overhead is allowed. Matching funding from the registrant/potential registrant and commodity groups is strongly encouraged. List other public or private support related to this proposal and the amount of support.
6. **Equipment and Facilities Available**. A description of essential equipment and facilities available should be included.
7. **Potential benefits**. Economic and environmental, include pest importance and distribution, probability of user acceptance. Indicate how the biopesticide could be used in integrated resistance management programs.
8. **Commercialization**. How does this research proposal support registration or other commercial aspects of this product. A letter of support from the registrant or potential registrant should be included.
9. **Curriculum vitae** of the principal investigator and cooperators.
10. **Literature cited**.

Appendix IV

Criteria for Evaluation of Formal Proposals For Early Stage Biopesticides*

The following criteria were established to assist the reviewers in selecting biopesticide projects for funding that: (1) have a high probability of being registered in a reasonable period of time, and (2) will be useful in meeting pest control needs involving minor crops (uses), including minor uses on major crops.

1. Adequacy of investigators, facilities, experimental design, work plan and background research.
2. Evaluation of budget: amount requested from IR-4 and other support.
3. Time to completion and probability of attaining objectives in the proposed time frame.
4. Relevance of the proposal toward the development of data for registration.
5. Evidence of efficacy. Provide information on performance relative to conventional control practices and how the biopesticide might fit into Integrated Resistance Management programs.
6. Availability of a potential registrant. Likelihood of developing a formulated commercial product.

* Early stage biopesticides are biopesticides for which EPA subpart M Tier I data requirements are not completed or satisfied by appropriate waivers.

Appendix V

Criteria for Evaluation of Advanced Stage Biopesticide Proposals

The following criteria were established to assist the reviewers in selecting biopesticide projects for funding that: (1) are either in a more advanced stage of development (as opposed to exploratory or early stage of development) or involve expansion of the label, (2) have a high probability of being registered/marketed in a reasonable period of time, and (3) will be useful in meeting pest control needs involving minor crops (uses), including minor uses on major crops.

1. Adequacy of investigators, facilities, experimental design, work plan and background research.
2. Evaluation of budget, including matching funding from registrant and/or commodity group.
3. Relevance of the proposal toward the development of data for registration or label expansion of the biopesticide (field or greenhouse testing has priority over lab testing).
4. Probability of biopesticide being used by growers (factors such as commitment of registrant, time to registration, availability of commercial formulations, effectiveness and economics of use rates should be considered). In addition, the potential for integration of the biopesticide into a rotation with conventional products will also be considered.

Appendix VI

Please be sure that these items are included with your proposal.

Early Stage Proposal

- Biopesticide Proposal Cover Page
- Clearly defined product (chemical name, organism genus, species, isolate)
- Status of toxicology work/data waivers
- Body: Clearly list of treatments, crops, data collection, experimental design
- Well defined budget
- Copy of proposed label (if applicable)
- Letter of support from registrant/potential registrant
- Supporting information/previous efficacy data or literature
- Completed PCR form(s)

Advanced Stage Proposal

- Biopesticide Proposal Cover Page
- Product labels or proposed labels
- Body: Clear list of treatments, crops, data collection, experimental design
- Well defined budget
- Letter of support from registrant
- Supporting information /previous efficacy data or literature
- Completed PCR form(s)

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Industry News: **Center to Combat Herbicide Resistance Established in Australia**
Contributed by: Steve Powles

In response to massive levels of herbicide resistance in Australia's largest grain-producing state of Western Australia, a major research center focussed on herbicide resistance has been created at the University of Western Australia. The Western Australian Herbicide Resistance Initiative (WAHRI) receives major farmer and government funding (GRDC) to tackle all aspects of the herbicide resistance problem.

With Stephen Powles as Director, the center comprises five postdoctoral fellows, five PhD students, three research assistants and other students and support staff.

WAHRI activites are divided into four programs: Resistance population genetics, Resistance mechanisms, Resistance management and Extension/economics.

For details on the people in WAHRI, a description of all the projects underway and other information please examine the website http//wahri.agric.uwa.edu.au.

Industry News: ***The Handbook of Pest Management in Agriculture***

The Commonwealth Education Foundation, London is making the "The Handbook of Pest Management in Agriculture"in two volumes by David Pimentel and published by the C.R.C.Press International available to Pesticide Manufacturers / Researchers at a substantially subsidized price.

The contents include (1,098 pages):

- Introduction
- Estimated Losses of Crops and Livestock to Pests
- Estimated Losses without Pesticides and substituting only readily available non chemical controls
- Environmental control of pests on crops
- Environmental control of pests on livestock
- Extent and quantities of pesticide used
- Utilization of biological, cultural and quarantine control in crops
- Methods of pesticide application
- Biological pest contro
- Insect pests
- Plant pathogens
- Livestock pests
- Bee pollinators and their problems
- Human pesticide poisoning

These are latest editions in mint condition. Although the original price is $184 (Rs.8000), the Commonwealth Education Foundation, London, has made these two volumes available to you for Rs.4400 only ($99), including freight packing and registered postage to your door. This means a saving of Rs.3600 ($85). To take advantage of this offer, kindly fill out the acquisition form below and send a draft in favor of World Book Centre to the address below. Be sure to let us know your delivery address. The books will reach you within three weeks.

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Abstracts

Citrus Rust Mite Develops Resistance to Mancozeb

In response to an increasing number of treatment failures with mancozeb for the control of citrus rust mite (CRM), *Phyllocoptruta oleivora*, and the need for alternatives to Acarol (bromopropylate) and Mitac (amitraz) whose permitted residues on export citrus are under threat, the efficacies of several acaricides were tested. Experiments were conducted in lemon and Valencia orchards near Nelspruit in Mpumalanga, South Africa. The registered dosage rate of mancozeb (Sancozeb) for CRM of 0.06% a.i. and the higher rate (0.16% a.i.) registered for the control of black spot disease *Guignardia citricarpa* were used. Results confirmed that CRM in the Nelspruit area is resistant to mancozeb, even when used at the higher dosage rate. Tedion (tetradifon) at 0.0162% a.i. was ineffective against CRM. The new acaricide Envidor (spirodiclofen) at 0.0036% a.i. and Agrimec (abamectin) at 0.00027% a.i. plus oil at 0.3% were both as effective as the standard Acarol at 0.0125% a.i. in controlling CRM, and would be considered suitable for use in Integrated Pest Management (IPM) orchards. Hunter (chlorfenapyr) at 0.0108% a.i. also gave excellent control of CRM but is not considered as IPM-compatible as the previous two products. Usage of mancozeb as a fungicide will continue so resistance of CRM to this product is likely to increase and become more widespread.

This article was recently published in S A Fruit Journal 1(2): 40, 43, 51. Aug/Sep 2002.

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Symposia

International Symposium on Molecular Genetics of Pesticide Resistance and Integrated Pest Management

March 26 (Wednesday) - 30 (Sunday), 2003
Beijing Yiquan Shanzhang, Beijing, China

Scientific Program

The scientific program will include opening and closing lectures, plenary lectures, and symposia and poster sessions on the following topics:

1. Strategies of Integrated Pest Management (IPM) in the 21th Century
2. Chemical Pesticides (including application techniques, resistance, pesticide management, etc.)
3. Biochemisrty and Ecology of IPM
4. Toxicology and Physiology of Pesticides
5. Molecular Genetics of Pesticide Resistance
6. Control of Medical Pests
7. Environmental Indentification of Pesticides

8. Toxicology of Environmental Pesticides
9. Ecotoxicology and Risk Assessment
10. Environmental Chemistry Actions of Pesticides

Commercial Advertisements

A 'Commercial Advertisements' page will be included in the program. For further information about including an advertisement, please contact with the Conference Secretariat.

Suggested Social and Tour Program

1. There will be an Opening Reception and a Banquet.
2. Local tours will be offered during the Symposium: the Great Wall and Ming Tombs, the Forbidden City and the Temple of Heaven, Beijing Zoo and the Summer Palace, etc.
3. Pre-and Post-symposium tours: Xi An (Bing Ma Yong), West Lake of Hangzhou, etc.

Language The official language of the symposium is English.

Reply Card
Symposium on Molecular Genetics of Pesticide Resistance and Integrated Pest Management, 26-30 March 2003

Title: Prof. ____ Dr. ____ Mr. ____ Ms. ____

First Name:_____ Family Name_____

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If you are interested in this Symposium, please reture this form by fax or by mail to:

Qiao Chuanling
E-mail: qiaocl@panda.ioz.ac.cn

Registration of Interest If you are interested in attending MGPR IPM 2003, please fill in the **Reply Card** and send back the enclosed Reply Card by post.

International Symposium on Pesticides and Environmental Safety (ISPES) - Beijing, China

The International Symposium on Pesticides and Environmental Safety (ISPES) will be held from **April 27-29, 2003** in the city of Beijing, China

Theme: Pesticides and Environmental Safety

Objective: To enhance the progress of pesticides and environmental safety and to promote the global exchange and collaboration of Chinese and international scientists worldwide for the improvement of life in general.

The scientific program will include opening and closing lectures, plenary lectures, and symposia and poster sessions on the following topics:

1. Current Safety Problems of Pesticides in China
2. Strategies of Integrated Pest Management in the 21st Century
3. Safety Problems of Pesticide Applications in Taiwan
4. Evaluation and Management of Pesticide Environmental Toxicology
5. Identification of Pesticide Environment Pollutions
6. Perspectives of Pesticide Development
7. IPM: Molecular Biology and Ecology, Applications of Insecticides in IPM
8. Insecticides from Natural Products, Plant Pesticides, and Biological Pesticides

Scientific Review: Environmental reviews of Kun canal

Language: The official languages of the Symposium are English and Chinese.

Call for Papers: All speakers and all authors of posters are requested to prepare a manuscript in English language (or Chinese) to be submitted electronically via the ISPES scientific committee to the following email address: qiaocl@panda.ioz.ac.cn

Reply Card for the International Symposium on Pesticides and Environmental Safety (ISPES), 27-29 April 2003

Sponsors: Beijing Pesticides Society

Co-Sponsors: China Agriculture University Institute of Zoology, Chinese Academy of Sciences Institute for the Control of Agrochemicals, Ministry of Agriculture, PRC

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Scientific Committee: Chuanling Qiao, Fuheng Chen, Yijun Wu, Feineng Zheng, Xinling Yang, Yongquan Zheng, Zongxing Si, Zhongning Zhang Jinshen Che, Nannan Liu, Zhisheng Hu

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Local Organizing Committee: Zhengrong Yuan, Yan Lin, Li Hong, Shoushan Wang, Ling Zhang, Guibiao Ye, Yong Gong, Chuan Jiang Tao, Guoqiang Li

Announcements and Submission Deadlines

As you are probably aware, the server for the newsletter has changed. This means that the Internet address has also changed. The new address is:

http://whalonlab.msu.edu/rpmnews

If you visit the old site you will be redirected to our current location. We apologise for any inconvenience this may have caused. Don't forget to change your bookmark to the newsletter!

On another note, the newsletter thrives significantly on the support of our subscribers via their contributions. To ensure that the newsletter remains available to our readers, it is imperative that we continue to receive these submissions on a regular basis.

Our past call for articles did not produce many submissions to share with our readers. This is likely due to the new format for the newsletter and the fact that many of our past subscribers have not heard of the new electronic venue. Please make an effort to inform your colleagues of this resource.

We encourage you to submit articles, abstracts, opinions, etc. The Newsletter is a resource to many around the globe. It is also a wonderful and effective way to enhance the flow of ideas and stimulate communication among global colleagues. We appreciate your efforts to support the newsletter and we look forward to your continued contributions.

The next two **submission deadlines** are:

Monday, March 17th, 2003
Monday, September 15th, 2003

We hope you consider the newsletter as a forum for displaying your ideas and research.

Also, if you haven't already, visit our web page and submit your e-mail address to receive updates about the newsletter!

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