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. 11, No. 2 (Spring 2002)

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Letter from the Editors

We are proud to introduce the electronic version of the Resistance Pest Management Newsletter (RPM News). This is the 10th year of publication and our 1st electronic version. We certainly invite any suggestions or constructive alternatives to our new format.

As you can see the newsletter is supported by the Center for Integrated Plant Systems at Michigan State University, the International Resistance Action Committee (IRAC) and the US Department of Agriculture. We are grateful for their support and resources that make the Newsletter and Resistant Arthropod Database (http://www.cips.msu.edu/whalonlab) possible.

Our electronic format allows us greater facility to serve you through rapid publication of breaking events in resistance development and management. Our hope is that the decreased time between volumes and increased access will lead to a more informative and complete reporting process for resistance pest management globally. In addition, we are intending to publish updated lists and other relevant information from the Resistant Arthropod Database in the RPM News. We trust that you will find many of the articles, abstracts and other information in this issue useful and informative.

In the future, we may be able to publish the RPM News more frequently, and our goal is to increase our volumes to three or four Newsletters this year. Ultimately this goal depends on two key issues; 1) your submission of resistance information to the RPM News and Database and 2) our continued support base.

Together with the posting of this web-based newsletter we will be sending over 2300 postcards out to our mailing list. These postcards will inform our historical subscribers of the change in our publication format and the location on the web where they can successfully access the RPM News. In addition, we are systematically placing all of the twenty-two previous volumes of the newsletter on site.

The editorial and support staff of RPM News is excited about our new web venue and hope that it will serve your resistance information needs effectively.

Robert Hollingworth &
Mark E. Whalon
Center for Integrated Plant Systems
Michigan State University
East Lansing, MI 48824-1311
United States

Resistance Management Reviews

Fire Ant Behavior - A Guide for Control
By Dr. Terry Mabbett

Research at Imperial College, University of London is helping to tame one of North America's most feared and loathsome public health pests. Red imported fire ant (IFA) (*Solenopsis invicta* Buren), introduced from South America 70 years ago and currently infesting 250-300 million acres in eleven southern states are fierce, territorial, and persistent. Each fire ant nest may contain up to 3000 'queens' that offer enormous reproductive potential. Throughout spring and summer, active mounds send out 'swarmer' ants (wingless males and females) that start new colonies. These are established in a wide variety of environments including picnic area, playgrounds, pool areas, and around buildings at home and at work. Fire ants deliver a nasty sting - capable of killing hypersensitive people - and as such are a priority target for North American pest control operators (PCOs).

The red IFA also disrupts a wide range of agricultural operations. Reports of attacks on livestock including piglets, new-born calves, and chicks are widespread as is crop damage from ants gnawing holes in roots, stems and buds of a wide range of crops. Red IFA mounds disrupt crop harvesting leaving farmers with two unsatisfactory options: to push the mounds over with plowing equipment, reducing yield and risking equipment damage, or to avoid the mounds, reducing the area of the harvested crop.

Control by insecticide baiting of mounds relies on worker ants bringing back active material into the nest to ensure that the queen(s) die. Many factors including heavy rain, poor bait placement, and bait shyness may conspire to cause control failure. Gordon Daly (postgraduate student on the MSc Pest Management Course at Imperial College, Silwood Park, England) looked at red imported fire ants management from an altogether different angle. Using four different synthetic pyrethroid insecticides - lambda cyhalothrin, deltamethrin, bifenthrin, and betacyfluthrin - he evaluated the magnitude and consequences of perceived fire ant repulsion and activated behaviorial responses following contact with these insecticides.

Fire ants react with a 'hot coals' response, which involves the ants walking up to the deposit of insecticide and promptly retiring. Research is underway to determine the nature of this behavior and to evaluate whether positive results, such as keeping fire ants away from buildings, are outweighed by potentially negative effects. It is suspected that a reduced exposure time of worker ants to insecticide deposit means they will fail to pick up a lethal dose of insecticide and carry it back to the nest. To examine the pesticide exposure of ants within the colony, Daly looked at the role of other ants in the nest. The necrophytic/necrophagic ants remove those that have died of insecticide poisoning and place them outside the nest. If insecticide is intended to reach ants resident in the nest, including nurse ants, larvae, and queens, it is crucial for the insecticide deposit to be acquired from the cadavers and passed up the 'chain of command' to the queen.

The sequence of events, observed and recorded by Gordon Daly, which describe the response of red IFA workers to residual deposits to synthetic pyrethroid insecticides are as followes:

- When individual ants approach insecticide-treated surfaces they are not repelled and subsequently move onto the surface where they acquired a lethal dose, providing that the time of exposure is sufficiently long.
- If the ant remains on the deposit for a period of 30 seconds or more then knockdown will occur between 13 and 16 minutes later, depending on the active ingredient. Longer exposure periods hasten knockdown. Exposure to insecticide may induce activation

behavior or inhibition behavior in the ant, depending on the formulation encountered.

- During post-exposure periods the ant may act as a vector of the insecticide by transferring active ingredient to the other individuals of the colony via physical contact. This will continue even after the ant has died because nest mates, exhibiting necrophoric* behavior, will remove the corpse from the nest. (*This term is used to distinguish the disposal of corpses from other sanitation tasks, which serve the hygiene of the colony as a while.) The individuals involved in the corpse removal will then pick up a lethal dose and distribute it further within the colony.
- Corpses will initially transfer toxicity more rapidly to other individuals than do contaminated, live ants. However, the end result in terms of maximum knockdown and mortality will be the same. In addition, toxicity may be transferred from a live individual to another via behavioral activities such as nest mate recognition or food begging.

Control of most public health and agricultural pests with synthetic pyrethroid insecticides is straightforward. Individuals pick up lethal doses of the contact and stomach-acting neurotoxins and die. These individual events contribute directly to insect pest control through the erosion of the reproductive potential of the population. The control situation with colony-forming insects such as fire ants is more complex and difficult. Control relies on an understanding of the social behavior patterns of these insects to ensure that insecticide reaches the 'nerve centre' of the colony and kills the queen(s). The work reported here takes a significant step forward in understanding fire ant behavior in relation to the use of synthetic pyrethroid 'chemistry' and in how it can be harnessed for control of this fierce insect pest.

For further information and details, contact:

Dr. Terry Mabbett
Dr. Terry Mabbett Consultants
2 Albemarle Avenue
Potters Bar, Herts EN6 1TD
United Kingdom Tel/Fax: +44 (0) 1707 644953
E-mail: DrTerryMabbett@btinternet.com

OR

Dr. Denis Wright
Department of Biology Imperial College at Silwood Park
Ascot, Berkshire SL5 7PY
United Kingdom
Tel/Fax: +44 (0) 1344 294339
E-mail: dwright@ic.ac.uk

Resistance Management from Around the Globe

Generating Base Line Data for Insecticide Resistance Monitoring in Cotton Leafhopper, *Amrasca devastans* (Distant)

ABSTRACT The base line susceptibility response to five commonly used insecticides viz., dimethoate, methyl demeton, acephate and imidacloprid in *Amrasca devastans* (Distant) field population collected from the college farm of Agricultural college and Research Institute, Tamil Nadu agricultural University, Madurai, Tamil Nadu, was determined by IRAC method No.8 developed and recommended by Insecticide Resistance Action Committee with the slight modification. The LC50 (ppm) values varied from 41.03 to 153.90 for dimethoate (seven generations); 50.32 to 205.92 for methyl demeton and 46.02 to 114.79 for acephate (six generations). The LC50 value of imidacloprid was 0.00056 (one generation).

KEY WORDS: *Amrasca devastans*, insecticide resistance, discriminating dose

INTRODUCTION Cotton leafhopper *Amrasca devastans* (Dist) is the most important cotton pest and accounts for 35 per cent reduction in the Cambodias (Neelakantan 1957) and 11.6 per cent reduction in seed cotton yield (Dhawn et al. 1988). Though it is an early phase pest, it occurs all through the season serving as one of the limiting factors in economic productivity of the crop. *A. devastans* is a serious pest of cotton and okra in India, Pakistan, Thailand, and other South East Asian countries. In addition to cotton and okra, it is found to live and breed on holly hock, brinjal, potato, kapos, maize, sorghum, pigeonpea, lobia, groundnut, sunflower, jute, beetroot, amaranthus, rhodes grass, mulberry, and marigold.

The pest problem is aggravated more rapidly due to control failures in many areas. Though control failure may be due to many factors, one of the major factors is the development of resistance to insecticides.

Due to the extensive use of pyrethroids, resistance develops in cotton leafhoppers. The chief objective in resistance monitoring is to exaggerate the differences between susceptible and resistant individuals such that the frequency of misclassification is greatly reduced (Ffrench-constant and Roush 1990). This is fulfilled by fixing discriminating doses.

Resistance to *A. devastans* is in the initial stages of development and no systematic work on monitoring of insecticide resistance is done in Tamil Nadu as it is done in *Helicoverpa armigera* (Hub), *Plutella xylostella* (Linn.), and *Spodoptera litura* (Niranjankumar and Regupathy 2001). Keeping the above in view, the present study was undertaken.

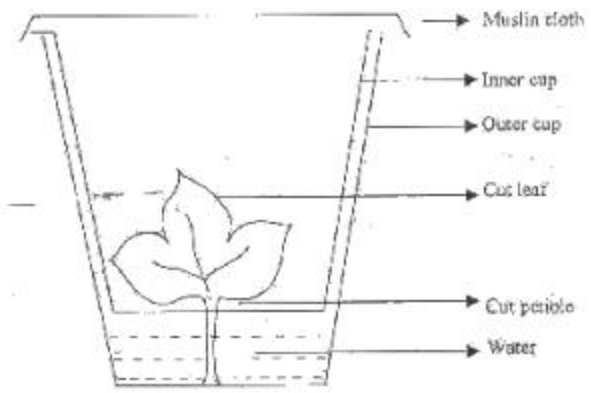
MATERIALS and METHODS The test insects were collected from the college farm of Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai, Tamil Nadu. The population was maintained for seven continuous generations without exposure to pesticides for generating baseline data i.e., for fixing discriminating doses.

The dilutions required were prepared from the formulated products of the insecticide using distilled water. The dosages were attained after preliminary range finding studies for constructing log-concentration-probit-mortality (lcpm) lines (Regupathy and Dhamu, 2001).

The nymphs of third instar of ca 1.3mm size and weighing ca 0.14mg were taken from the culture maintained for the treatment. Each replication consisted of 10 nymphs and there were three replications. Bioassays were conducted following the procedure based on the standard *B. tabaci* susceptibility test IRAC method No.8 developed and recommended by the Insecticide Resistance Action Committee with slight modification.

Two cups were taken, one as inner test chamber and the other as outer water reservoir. The cup, which served as the inner test chamber, was taken and a hole was pierced in the centre of the bottom side of the cup (Fig.1). Then uncontaminated cotton leaves were selected and the petiole was cut to a length of approximately 4-cm. The test concentration of insecticides were prepared and the leaves were dipped in them for 5 seconds holding the leaf by the petiole. Then the leaves were left for drying in the open air (approximately 5 min). The petiole of the test leaf was passed through the inner cup at 10 nymphs/cup and the cup was covered with muslin cloth tightened with rubber band. A small amount of water was placed in a second cup and the test cup was placed inside that, so that it was supported by the protruding petiole. Observations on mortality of leafhoppers were recorded after 48 h. The results were expressed as

Fig. 1. IRAC NO.8 Bioassay setup



percentage mortality.

RESULTS and DISCUSSION (Table 1) The LC50 and LC95 (ppm) of insecticides for F1 and F7 generations varied from 153.90 to 41.03 and 2722.70 to 812.83 to dimethoate. F1 to F6 varied from 205.92 to 50.32 and 4466.86 to 416.87 to methyl demeton. 114.79 to 46.02 and 3981.10 to 831.76 to acephate and 0.000457 and 0.00575 being the LC50 and LC95 for F1 generation to imidacloprid respectively.

Imidacloprid was the most toxic pesticide. The acute toxicity of the other insecticides based on LC50 was in the order of acephate >/= (greater than or equal to) dimethoate >/= methyl demeton for first, second, and third generations and acephate >/= methyl demeton >/= dimethoate for fourth, fifth, and sixth generations. The order of toxicity based on LC95 was dimethoate > acephate >/= methyl demeton for first and third generation, dimethoate > methyl demeton >/= acephate for second generation and methyl demeton > dimethoate >/= acephate for second generation and

Table 1. Susceptibility of *Amrasca devastans* different insecticides

Insecticides	First generation					Seventh/Sixth generation				
	LC50 (ppm)	LC95 (ppm)	a	B	χ^2 (n-2)	LC50 (ppm)	LC95 (ppm)	a	b	χ^2 (n-2)
Dimethoate	153.9	2722.7	2.181	1.288	-2.50(4)	41.03	812.83	2.993	1.244	1.21(4)
Methyl demeton	205.92	4466.86	1.05	1.192	-0.22(3)	50.32	416.87	1.12	1.435	4.31(3)
Acephate	114.79	3981.1	2.765	1.085	0.046(4)	46.02	831.76	2.908	1.258	0.129(4)

methyl demeton > dimethoate >/= acephate for the subsequent generations.

Imidacloprid was found to be the highest toxic chemical. The toxicity was in the order Imidacloprid > dimethoate (Gul and Gul 1998) and Imidacloprid > methyl demeton > dimethoate (Patil et al. 1999) for *A. devastans* under field condition. The toxicity order reported earlier was dimethoate = methyl demeton > disulfoton > phorate (Regupathy and Jayaraj 1973) and aldicarb > dimethoate > monocrotophos > carbaryl > phosphamidon (Patel et al. 1980) for *Amrasca devastans* under field condition, and Dimethoate > malathion > formothion by dryfilm bioassay (Talpur et al. 1994).

Methyl demeton was proved to be more toxic for the fourth, fifth, and sixth generations than dimethoate and other chemicals (Manisegaran and Kumaraswami 1994). The position of dimethoate, methyl demeton, and acephate interchanged among themselves often when checked for their acute toxicity tests. This might due to the excessive use and subsequent resistance development in different populations of *A. devastans* in various locations.

The susceptibility index based on LC50 varied between 0.74 to 0.92 for dimethoate, 0.63 to 0.84 for methyl demeton and 0.76 to 0.97 for acephate. The susceptibility index based on LC95 varied between 0.71 to 1.10 for for dimethoate, 0.21 to 0.99 for methyl demeton and 0.50 to 1.00 for acephate respectively.

The susceptibility gradually increased with the succeeding generations which is evident from the decline in LC50 and LC95 values to all the insecticides tested. The extent of decline was more in methyl demeton and acephate respectively. The susceptibility base-line data are not available for insecticides taken for this study. Hence the LC95's of the insecticides are considered as discriminating doses for monitoring the field populations for their resistance to these insecticides. The discriminating dose (ppm) fixed were 800 for dimethoate, 400 for methyl demeton and 850 for acephate.

Based on the slope function and increased susceptibility, the discriminating dose screen was fixed for monitoring the level of insecticide resistance in future. Use of discriminating dose though not used for *A. devastans* in India, has been successfully used in monitoring programmes involving *H. armigera*, *P. xylostella*, and *C. medinalis*.

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S. Jeya Pradeepa and A. Regupathy
Agricultural College and Research Institute
Madurai - 625 104, Tamil Nadu
India.

Mailing Adress:
Dr. A. Regupathy Ph.D., Professor
Department of Entomology
Tamil Nadu Agricultural University
Coimbatore - 641003, Tamil Nadu
Email : cfc_nri@vsnl.in; regupathya@yahoo.com
Phone : 091-422-431222 Ext. 297, 091-422-451840
Fax : 091-422-43167

Generating a Resistance Database in *Heliothis virescens* Populations from Areas Planted with Bt-Transgenic Cotton in Mexico

Since 1996, genetically modified cotton that expresses the Cry IA (c) toxin of *Bacillus thuringiensis* has been planted in Mexico. The area has increased from 900 hectares in 1996 up to 36,430 in 1998 which were reduced to 24,763 in 1999 and 24,650 in the year 2000. This reduction was general for cotton production

in Mexico, due to different situations including low fiber price and high production costs. The transgenic cotton used up to now in Mexico is known as the Bt or BOLLGARD(R) cotton and it used against pink bollworm, tobacco budworm, and bollworm. Due to the high selection pressure that these plants may have on insect populations affected by the toxin, it is a concern that resistance could be developed to this toxin. As a preventative measure for resistance management, agricultural authorities in Mexico require the development of data on the response of populations under selection pressure from Bt cotton in order to detect any change that could indicate resistance problems. In this regard MONSANTO (line owner of this technology) has established a resistance management strategy based on leaving refuges of conventional cotton close to areas planted with Bt cotton. In order to evaluate the success of the strategy and detect any shift in response to the Cry IA(c) toxin base line information and technology for monitoring resistance was developed. This paper reports data obtained up to now in the monitoring program for tobacco budworm (TBW) that has taken place in populations collected from different cotton growing areas in Mexico.

TBW larvae were collected from commercial cotton fields from several agricultural areas in Mexico. This material was sent to the INIFAP entomology facility located in Cd. Obregón, Sonora, were they were reared on artificial diet and maintained in walk-in chambers at 27° C, 70% R,H and 14:10 (L:D) Photoperiod until used for bioassays. A colony maintained in the laboratory since 1982 has been used as a reference strain for susceptibility. Lyophilized MVPII toxin (Mycogen Corp.), was used as the standard for the Cry IA(c) toxin. Different concentrations of the toxin suspended on agar, were applied over 1.0 ml artificial diet placed on each of the 2.0 ml wells of a 64 well assay tray. One neonate TBW larvae was placed in each well, then the assay trays were covered with a plastic ventilated cover and incubated for five days in the walk-in chambers. Around 500 larvae were used for each colony and bioassay. Mortality, body weight, and number of larvae reaching the 3rd instar were recorded. Percent inhibition (stunting) was estimated by dividing the larval weight of treated individuals by the larval weight of a control individuals multiplied by 100.

Results of the Probit analysis data for dosage-response to CryIA(c) in a susceptible and two field *H. virescens* populations, from northwestern and northeastern Mexico are shown in Table 1. According to this data there were not differences among populations. It was observed that this type of data do not represent the effect of the toxin in the larvae, since mortality was not clear. A better criteria was stunting and prevention of larvae reaching third instar.

Data for the 2000 year are presented in Table 2. More than 500 larvae were evaluated for each site of collection and for the susceptible colony, there were no third instars observed and the percent inhibition was 98% for all the field colonies and 99% for the susceptible. This indicates that the populations were susceptible to the toxin.

Table 1 Probit Analysis data for *CryIA(c)* in tobacco budworm populations from Mexico.

Colony	LC50 µg/ml	Fiducial Limits 95%	LC95 µg/ml	Slope
Susceptible	0.072	0.055 - 0.092	1.7	1.2
S. Tamps	0.061	0.042 - 0.084	1.71	1.13
Yaqui, V.	0.063	0.044 - 0.086	2.13	1.08

Based on these results it could be concluded that larval mortality was not a good parameter for discrimination of resistant genotypes. The 0.05 µg/ml concentration, prevented larvae from reaching the 3rd instar, and is a concentration that has been used as diagnostic dose for discrimination of resistant genotypes (Sims et al 1996, Martínez and Berdegue, 1999). This concentration was used for resistance monitoring in TBW populations from Mexico, considering as criterion that larvae did not reached 3rd instar and percent weight inhibition. The results of this process since 1997, indicate that TBW populations are still susceptible to this toxin, since there has not been a change in response of the field populations as compared to the susceptible colony as demonstrated with the 2000 year data presented.

Tabla 2. Data from the 2000 year of Monitoring Resistanse to the Cry IA (c) of *Bacillus thringiensis* on tobacco budworm populations from northwestern Mexico

Colony	No. Treated	3rd instar	Treated Median Weight	Untreated Median Weight	% Inhibition
Gua-2000	560	0	0.6	24.69	98
VY-2000-1	544	0	0.41	25.54	98
VY-2000-2	576	0	0.47	26.84	98
VY-2000-3	560	0	0.54	26.63	98
Cab-2000	544	0	0.63	25.92	98
Mex-2000	544	0	0.61	27.43	98
Susc.	576	0	0.25	41.32	99

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José L. Martínez-Carrillo
Instituto Nacional de Investigaciones Forestales y Agropecuarias
Mexico

Urbano Nava-Camberos
Instituto Nacional de Investigaciones Forestales y Agropecuarias
Mexico

J. Concepción Rodríguez Maciel
Colegio de Posgraduados
México

Resurgence of spider mite, *Tetranychus urticae* Koch on okra

ABSTRACT In recent years, spider mite, *Tetranychus urticae* Koch, has assumed a major pest status on okra, Abelmoschus esculentus L. Moench., during summer months. Therefore, a neem formulation (Azadirachtin 0.03 %) and some conventional acaricide were evaluated against spider mite for studies of resurgence to determine the most environmentally friendly pesticide for effective integrated pest/crop management (IPM/ICM). In this study, ten pesticides were used at the recommended doses. These pesticides were observed at four different intervals: at 1, 3, 7, and 14 days. The results indicate that phosphamidon, fluvalinate, fenvalerate, dimethoate, and monocrotophos show resurgence of spider mite (SM). Malathion and the neem formulation gave a more encouraging performance but showed some resurgence. Resurgence was not noticed in dicofol, sulphur, and phosalone.

Key words: Spider mite, resurgence, neem formulation, conventional acaricides.

INTRODUCTION Spider mite (SM), *Tetranychus urticae* Koch, has become a destructive pest of okra (Singh, 1995). It has caused considerable damage in the eastern regions of Uttar Pradesh particularly when high temperatures prevail during the months of May and June. An increase in mite population has also resulted from indiscriminate use of insecticides / acaricides in vegetable ecosystems and there have been several reports available against insecticides / acaricides enhancing the mite population (Singh and Mukherjee 1989). Hence, a study was undertaken to determine the extent of mite population build-up on okra, when insecticides / acaricides are used continuously.

MATERIALS AND METHODS A field trial on okra was conducted during 1997 - 1998 in a randomized block design with four replications to determine the influence of different insecticides / acaricides against spider mite, *Tetranychus urticae* Koch. Six insecticides, three acaricides, and a botanical formulation [Neem, (Azadirachtin 0.03 %)] were included in this study and compared with an untreated control (control = water spray) (Table 1 and 2). The insecticides / acaricides were sprayed two times with the recommended concentrations when the mite population surged. A second spraying was conducted following a final data collection on the 14th day of the first spray. These data were treated as the pre-spray data of the second spray. The populations of mites were recorded at 24 hrs before spraying (pre-spray data) and at 1, 3, 7, and 14 days after spraying (rest data). Five leaves per plot were selected randomly from the top, middle, and bottom of the plants, and the population was recorded in 2cm2 areas at four locations on each leaf. The data was transformed and analyzed statistically.

RESULTS AND DISCUSSION A critical analysis of the data (Table 1) shows that the population level in the untreated control had been on the rise until the second spray. The population in the treated plots with dimethoate, neem formulation, fenvalerate, fluvalinate, malathion, monocrotophos, and phosphamidon had gradually increased following the second spray. However, the population trends in dicofol, phosalone, and sulphur were quite different from the others, with a decrease in the wake of each spray followed by a moderate increase thereafter. Because the mean population levels in the plots with insecticides viz., malathion, neem formulation, monocrotophos, dimethoate, fenvalerate, fluvalinate, and phosphamidon, were much greater than that of the untreated control, that their application was not effective and caused spider mite resurgence. This might be due to destruction of natural enemies, stimulated reproduction rates of the mite, or poor efficacy as in the case of phosphamidon. Resurgence in insect pests after pesticide application is a well

Table 1 : Influence of insecticide / acaricide on the population of spider mite, *T. urticae* Koch on Okra in first spray. (No. of mites in 2 cm 2 on 20 leaves – mean of 80 observations)

Insecticides/Acaricides	Concentration (%)	Spider mite population (Days After) 1	3	7	14	Mean	% Increase or decrease over control
Dicofol 18.5. EC	0.5	1	26.2	43.2	15	21.35	-6.78
		-1.22	-5.16	-6.61	-3.93	-4.67	
Dimethoate 30 EC	0.03	8.4	26.98	78.48	86	49.96	118.18
		-2.98	-5.24	-8.88	-9.3	-7.06	
Fenvalerate 20 EC	0.005	6.86	29.82	68/92	94.8	50.1	118.77
		-2.71	-5.5	-8.33	((9.76)	-7.11	
Fluvalinate 25 EC	0.005	7.82	33.68	67.26	92.4	50.29	119.6
		-2.88	-5.84	-8.23	-9.63	-7.12	
Malathion 50 EC	o.1	5.3	16.4	44.9	29.8	24.1	5.24
		-2.4	-4.11	-6.73	-5.5	-4.95	
Monocrotophos 36 SL	0.036	8.06	32.8	64.6	86.8	48.56	112.05
		-2.92	-5.77	-8.06	-9.34	-7	
Neem formulation Azadirachin (0.03 %)	5	6.85	22.6	46.9	28.9	26.31	14.89
		-2.71	-4.8	-6.88	-5.42	-5.17	
Phosalone 35 EC	0.07	0.8	28.8	40.08	7.2	19.22	-16.06
		-1.14	-5.41	-6.37	-2.77	-4.44	
Phosphamidon 85 SL	0.025	11.62	28.5	74.8	94.4	52.33	128.51
		-3.48	-5.38	-8.67	-9.74	-7.26	
Sulphur 80 WP	0.1	1.2	32.8	37.36	5.62	19.24	-15.98
		-1.3	-5.77	-6.15	-2.47	-4.44	
Untreated (Control)	-	4.68	15.46	44.8	26.66	22.9	
		-2.27	-3.99	-6.73	-5.21	-4.83	
Mean	-	5.69	26.73	55.57	51.59		
		-2.48	-5.2	-7.48	-7.21		

Figures in parentheses are mean of Ö x + 0.5 transformed values, C.D. (P = 0.05)
Between insecticide / acaricide : 0.77** , Between periods : 0.46**
Interaction between insecticide / acaricide and periods : 1.97**
** Significant at one per cent level.

documented phenomenon (Bartlett, 1968; Reynolds, 1971; Eveleens et. al., 1973; and McClure, 1977).Spider mite outbreaks on crop plants following the use of broad-spectrum insecticides have been reported by Huffaker et. al. (1970). There have been instances where resurgence of mites was met with a consequent application of synthetic pyrethroids which were extremely toxic to the predatory mites (Hoyt et. al., 1978; Hall, 1979; Sellammal and Balasubramanian, 1980). Dittrich et. al. (1974) discussed the effect of insecticides on mite resurgence and found that sublethal rates directly affected the oviposition rate of *Tetranychus urticae* Koch. There is now overwhelming evidence that the use of broad-spectrum pesticides is responsible for the enormous outbreak of red spider mites that have occurred worldwide since the introduction of DDT (Ripper, 1956 and McMurtry et. al., 1970).

The trend in the reduction of the mite population over the control by dicofol (6.78 %), sulphur (15.98 %), and phosalone (16.06 %) in the first spray changes in second spray: phosalone (40.25 %), dicofol (54.89 %) and sulphur (55.87 %) (Table 1 and 2). The data indicates that although they did not induce any mite outbreak, they did not control them to a certain extent. The effectiveness of dicofol (Jeppson et. al., 1975; Mote, 1976; Karuppuchamy and Mohanasundaram, 1986 and David, 1986) and of phosalone (Abdul Kareem et. al., 1977 and Dhandapani and Kumaraswami, 1983) against mites has earlier been reported. However, data from an earlier publication on monocrotiphos at 0.05 and 0.1 per cent effectively controlling the mite on vegetables (Mote, 1976; Dhandapani and Abdul Kareem, 1983; Dhandapani and Kumaraswami, 1983 and Singh and Singh, 1992) are contrary to the present study where monocrotophos at

Table 2 : Influence of insecticide / acaricide on the population of spider mite, *T. urticae* Koch on Okra in second spray. (No of mites in 2 cm 2 on 20 leaves – mean of 80 observations)

Insecticides/Acaricides	Concentration (%)	Spider mite population (Days After)				Mean	% increase or Decrease
		1	3	7	14		
Dicofol 18.5. EC	0.5	0.94	17.6	95	5.28	8.33	-54.89
		-1.2	-4.25	-3.16	-2.4	-2.97	
Dimethoate 30 EC	0.03	4.66	40.28	52.4	77.8	43.78	137.06
		-2.27	-6.38	-7.27	-8.84	-6.65	
Fenvalerate 20 EC	0.005	6.86	26.68	48.6	98.4	44.34	140.06
		-2.71	-5.21	-8.33	((9.76)	-6.69	
Fluvalinate 25 EC	0.005	4.28	28.98	50.66	97.8	45.43	145.96
		-2.18	-5.42	-7.15	-9.91	-6.67	
Malathion 50 EC	0.1	6.89	38.8	58.6	66.8	42.77	131.56
		-2.71	-6.26	-7.68	-8.2	-6.57	
Monocrotophos 36 SL	0.036	5.86	42.6	56.4	69.2	43.51	135.56
		-2.52	-6.56	-7.54	-8.32	-6.63	
Neem formulation, Azadirachin (0.03 %)	5	7.02	39.08	58.8	67	42.97	132.67
		-2.74	-6.29	-7.7	-8.21	-6.59	
Phosalone 35 EC	0.07	1.89	18.8	10.6	6.2	9.37	-40.25
		-1.54	-4.39	-3.33	-2.58	-3.14	
Phosphamidon 85 SL	0.025	3.84	29.8	48.89	99.82	45.58	146.81
		-2.08	-5.5	-7.02	-10.01	-6.78	
Sulphur 80 WP	0.1	0.86	16.6	9.56	5.6	8.15	-55.87
		-1.16	-4.13	-3.17	-2.46	-2.94	
Untreated (Check)	-	2.68	24.6	34	12.62	18.47	
		-1.78	-5	-5.87	-3.62	-4.35	
Mean	-	3.87	29.43	39.81	55.13		
		-2.09	-5.41	-6.34	-7.45		

Figures in parentheses are mean of Ö x + 0.5 transformed values, C.D. (P = 0.05)
Between insecticide / acaricide : 0.55**, Between periods : 0.33**
Interaction between insecticide / acaricide and periods : 1.11**
** Significant at one per cent level.

0.04 per cent caused resurgence of the pest. In this study, a similar trend in mite population was observed in first and second sprays from maximum to minimum in phosphamidon, fluvalinate, fenvalerate, dimethoate, monocrotophos, neem formulation, and malathion. This data indicates these insecticides cause the mite resurgence.

Furthermore, it is apparently evident that dicofol, sulphur, and phosalone lost their effectiveness against the mites upon second application. Phosalone, dicofol, and sulphur reduced the mite incidence over the control by 40.25, 54.89, and 55.87% respectively.

Dicofol, sulphur, and phosalone were statistically on par with the untreated control but superior to phosphamidon, fluvalinate, fenvalerate, dimethoate, monocrotophos, neem formulation, and malathion, indicating that they did not induce any mite resurgence but became ineffective against the mites with the second spray. This might have led to the development of resistance by mites to the acaricides since resistance is defined as a decreased response of a population of animal or plant species to a pesticide on control measure as a result of their application (Winteringham, 1969). Brader, (1977) has reviewed the occurrence of resistance to dicofol in European red mite, *Panonychus ulmi* Koch, red spider mite, *Tetranychus urticae* Koch, and Daniel mite, *Tetranychus mcdemieli* McGregor, in apple, pear, and peach orchards.

ACKNOWLEDEMENT The authors are grateful to Prof. Janardan Singh, Head of Entomology & Agricultural Zoology for providing necessary facilities and suggesting valuable advice.

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Sunil Kumar and R.N. Singh
Department of Entomology and Agricultural Zoology
Institute of Agricultural Sciences
Banaras Hindu University
Varanasi - 221 005, India

Profenofos: Response of Field-Collected Strains of Bollworm and Tobacco Budworm in South Texas, USA and Mexico

Profenofos and methyl parathion were equally toxic to a 1990 field collected and a laboratory reference strain of bollworms (Wolfenbarger et al 1998).

Profenofos and methyl parathion were equally toxic to a field collected and a laboratory referenced strain of the tobacco budworm (Nosky et al 1980). In 1990 and 1991, Elzen et al (1992) showed no significant difference in LD50s between a laboratory reference strain and six field collected strains; LD50s were <2 µg/larva and they were all susceptible. Susceptibility to profenofos of field collected strains of tobacco budworm across the United States was also shown by Ray et al (1996).

It was hypothesized that profenofos could be used against field collected bollworm and tobacco budworm populations where resistance to methyl parathion was shown. We wanted to determine comparative toxicity of the two insecticides to field collected strains of the bollworm and tobacco budworm from Mexico and Weslaco in the Lower Rio Grande Valley of Texas. For this reason field collected strains of both pest species were tested against both insecticides in 1981 to 1983, 1989 to 1991, and 1993. Toxicity of both insecticides was determined to support or reject the hypothesis that profenofos could be used to replace methyl parathion should it no longer be effective.

Technical profenofos at 98% was obtained from Novartis, Inc., Greensboro, NC. Three insect collections of bollworm were tested as described by Wolfenbarger et al (1998). Six collections of tobacco budworm were tested as described by Wolfenbarger and Vargas-Camplis (1997). Methods of handling the pupae, adults, and larvae prior to treatment was the same for both species.

Profenofos was diluted in acetone and one microliter of solution was applied to 20±6 mg larvae of both species. Dilutions of 50% from 50 to 0.04875 µg/larva were made. Mortalities were determined after 48 h. Larvae were classed as dead when they did not move following gentle probing.

LD50 values as µg profenofos/larva, 95% confidence interval values (C.I.), and slope±standard errors (SE) were determined by SAS probit analysis (SAS Institute 1985). The LD50s whose C.I. values did not overlap were considered to be significantly

different. LD50s with overlapping C.I. were considered equal.

All field collected strains of bollworm were susceptible to profenofos (Table 1). LD50s were <2 µg/larva for strains from southwestern Mexico and Weslaco, TX in 1982, 1983, and 1990. Profenofos was significantly more toxic than methyl parathion in Tapachula, Mexico in 1982 and 1983. LD50s of methyl parathion were <31 µg/larva for both years. These results suggest that resistance mechanisms of bollworm against methyl parathion do not affect profenofos. It is possible that several mechanisms for resistance by the two insecticides are involved by varying degrees.

Tobacco budworm strains from western and northwestern Mexico were susceptible to profenofos in 1981 (Table 1). LD50s were <2 µg/larva. Both strains from Mexico were resistant to methyl parathion because LD50s were significantly greater than profenofos at >14 µg/larva. In 1989 an LD50 <4 was determined for profenofos from a field collected strain from Weslaco, TX strain. There was no significant difference in this LD50 and the one for methyl parathion; the strain was susceptible to both insecticides. In 1992 (Vargas-Camplis et al 1993) showed that both strains from northern and southern Tamaulipas were susceptible to profenofos. However, the LD50 for profenofos for both strains from Tamaulipas was equal to the LD50s for methyl parathion. LD50s of both insecticides were <4 µg/larva.

In 1993, LD50s of both profenofos (Table 1) and methyl parathion (Vargas-Camplis and Wolfenbarger 1994) were >12 µg/larva. They were equal and indicated resistance. This is the first report of resistance by the same strain of tobacco budworm to both profenofos and methyl parathion. It is also a single determining factor indicating other collections from the same areas need to be made to confirm this finding.

Response to profenofos is shown by topical application bioassay in this study and in Elzen et al (1992), while Kanaga et al (1994) showed response to adults of the same pest by vial bioassay. In addition to the toxicity data by topical applications Elzen et al (1992) showed a co-dominance and no definitive inheritance pattern for profenofos with crosses between the two field collected strains and the reference strain by bioassay of larvae on treated plants. Results indicate that both methods of bioassay were effective in determining responses by field collected larval populations.

Table 1. Toxicity of profenofos to bollworm and tobacco budworm strains from Mexico and Weslaco, TX. 1982-1993.

Insect	Year	Location	Larvae tested	Slope±SE	LD50 (µg/larva)	95% Confidence Interval
Bollworm	1982	Tapachula, Chiapas	409	1.35±0.85	0.93	0.73-1.16
	1983	Tapachula, Chiapas	3853	1.15±0.21	1.2	0.59-2.37
	1990	Weslaco, TX	280	0.73±0.2	0.24	0.0012-0.72
Tobacco Budworm	1981	Caborca, Sonora	2981	1.5±0.81	0.78	0.61-1.64
		Felepe Carrillo Puerto, Michoacan	293	1.53±0.18	0.77	0.61-1.04
	1989	Weslaco, TX	50	0.93±0.46	3.57	1.42-7.7x10 108
	1992	Estacian Cuauhtemoc, Tamaulipas	270	1.51±0.81	0.61	0.22-1.68
		Rio Bravo, Tamaulipas	214	2.34±0.28	0.92	0.74-1.18
	1993	Matamoros, Tamaulipas	178	0.66±0.18	21.51	6.76-715.69`

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D. A. Wolfenbarger
55 Calle Cenizo
Brownsville, TX 78520

J. Vargas-Camplis
INAFAP
P. O. Box 5109
Brownsville, TX 78523

Relative Sensitivity of Various Caribbean Strains of *Aedes aegypti* Larvae to Insecticides Based on a Simple Bioassay

INTRODUCTION The control of *Aedes aegypti* (Linnaeus), the only known vector of dengue in the Caribbean, is crucial to the prevention and control of dengue, dengue haemorrhagic fever, and dengue shock syndrome. Traditionally, one of the tools utilized in the control of *Ae. aegypti* has been the use of insecticides with varying degrees of success (1). The discovery of DDT in the 1940s led to its use as the insecticide of choice in eradication programmes. However, the appearance of resistance to DDT in the 1960s resulted in the development of, and subsequent switch to, organophosphate insecticides such as malathion and temephos (2,3).

In the Caribbean, organophosphate insecticides, specifically malathion and temephos, are used in the chemical control of *Ae. aegypti* during an outbreak of dengue or when the vector is detected during routine surveillance activities. Malathion is generally used as an adulticide whereas temephos (Abate®) is the larvicide of choice recommended for use in potable water by the World Health Organization (WHO).

The continued use of these insecticides has resulted in the development of resistance in some Caribbean strains of *Ae. aegypti* due to increased insecticidal selection pressure (4,5). Knowledge of the status of insecticide resistance in *Ae. aegypti* populations is important in the event of a dengue outbreak, so that alternative measures can be implemented in areas where these insecticides are no longer effective.

In this study, various Caribbean strains of *Ae. aegypti* larvae were subjected to specific concentrations of malathion and temephos to test their sensitivity in relation to the Caribbean Epidemiology Centre (CAREC) *Ae. aegypti* strain which has been maintained in the laboratory for the last 25 years, free of exposure. In addition to giving information on the sensitivity of these strains to these organophosphates, the method used is a rapid response tool that can be adopted in countries with minimal laboratory and technical resources, and tests can be done on site.

MATERIALS and METHODS Twenty-seven strains of *Ae. aegypti* were collected from 9 Caribbean countries by ovitraps (6) and tested for insecticide resistance at CAREC. Insecticide susceptibility tests to malathion and temephos were carried out on the CAREC strain in accordance with WHO recommended methods for determining the susceptibility of mosquito larvae to insecticides (7). Three replicates of 20 fourth instar *Aedes* larvae were exposed to different concentrations of malathion and temephos. Mortality counts were made after 24 hours and the results were probit analysed to determine the concentration required to kill 90% of the larvae, LC90. Tests were repeated three times with each insecticide.

Analysis of the CAREC strain gave an LC90 of 0.27mg/l for malathion and 0.02mg/l for temephos. Each of the 24 strains were then exposed to these insecticides at the respective dosages. Mortalities were determined after 24 hours and the relative sensitivity (RS) of each strain calculated as:

*RS = (% mortality of strain / mortality of reference strain) x 100

A relative sensitivity of 100% implies that the strain is as sensitive as the CAREC reference strain, <100% implies that the strain is less sensitive than the CAREC strain and therefore demonstrates some level of resistance. Relative sensitivity > 100% implies that the strain tested is more sensitive than the CAREC reference strain. Further subdivisions were made according to levels of relative sensitivity as follows:

0% no sensitivity
1- 30% very low sensitivity

31 - 60% low sensitivity
61 - 90% medium sensitivity
91 - 100+% sensitive

* Both strains exposed to dosage giving 90% mortality in CAREC reference strain

RESULTS Results showed that there were varying degrees of sensitivity to malathion and temephos among and within the countries from which the *Ae. aegypti* were taken (Tables 1 & 2). When subjected to malathion at 0.27mg/l, populations from 6 of the 9 countries showed very low to medium sensitivities with levels ranging from 2 - 85% (Table 1). Of the other 3 countries tested, the Bad Cox strain from Anguilla and the Gingerland and Charlestown strains from St. Kitts / Nevis were more sensitive than the CAREC reference strain (RS = 110%; 104% & 110% respectively). The Bridgetown strain, Barbados was as sensitive as the CAREC reference strain (RS = 100%).

Three strains out of the 27 tested (2 from the Dominican Republic and 1 from Barbados) registered medium sensitivity to malathion ranging from RS = 53 - 85% (Table 1). Low levels of relative sensitivity were observed in 5 populations. Over half of the tested strains (15 / 27) showed very low sensitivity to malathion with the least sensitive populations being from Antigua, the British Virgin Islands (BVI), Dominica, and Trinidad (Table 1).

When populations were exposed to temephos at a dosage of 0.02mg/l, none of the strains tested were as sensitive as the CAREC reference strain (Table 2). The most sensitive strains were the 3 from the Dominican Republic in which registered RS values of 89, 95, and 98%. Low levels of sensitivity were recorded in 2 of the 27 populations, whereas, in the majority of test populations (17 / 27), relative sensitivity was very low (Table 2). Interestingly, zero sensitivity to temephos was observed in the Beef Island population from BVI and all of the 4 Trinidadian populations tested (Table 2).

DISCUSSION The data indicate that there is some amount of reduced sensitivity to malathion and temephos in Caribbean populations of larval *Ae. aegypti* mosquitoes. This further confirms the results of previous studies by Georghiou et al (4) and Rawlins and Hing Wan (5). The variations in sensitivity among and within countries is important to note as this may mean that control strategies recommended may be different for each country.

Overall, populations are less sensitive to temephos than to malathion. This could be due to the treatment of habitats with temephos during routine inspections. Malathion on the other hand is used sporadically in emergency control situations. Malathion, which is usually used as an adulticide, was tested as a larvicide

Table 1: Relative Sensitivity (RS) of several Caribbean strains of Ae. aegypti larvae to malathion based on CAREC susceptible reference strain (LC90 - 0.27mg/l)

Country	Location	RS (%)	Level of Sensitivity
Anguilla	Bad Cox (Rock holes)	110	sensitive
Antigua	Barnes Hill	9	very low
	Gray's Farm	26	very low
	All Saints	47	very low
Aruba	Oranjestaat	14	very low
	Several combined	46	low
Barbados	Bridgetown	100	sensitive
	Queen Elizabeth Hospital, St. Michael	53	low
	Ministry of Health, St. Michael	88	medium
British Virgin Islands (BVI)	Beef Island	29	very low
	Durcell Estate	23	very low
	Carrot Bay	10	very low
Dominica	Canefield Airport	21	very low
	Ferry Terminal Building	11	very low
	Police Marine Base - Woodbridge	30	very low
	Woodbridge Bay Port	1	very low
	Roseau Fisheries	32	low
Dominican Republic	Barahona	53	low
	Puerta Plata	73	medium
	Dajabon	85	medium
St. Kitts / Nevis	Charlestown	29	sensitive
	Gingerland	36	sensitive
	Several combined	7	very low
Trinidad	Belmont	3	very low
	Coconte	2	very low
	Woodbrook	7	very low
	Federation Park	14	very low

Table 2: Relative Sensitivity (RS) of several Caribbean strains of *Ae. aegypti* larvae to temephos based on CAREC susceptible reference strain (LC90 - 0.02mg/l)

Country	Location	RS (%)	Level of Sensitivity
Anguilla	Bad Cox (Rock holes)	29	very low
Antigua	Barnes Hill	9	very low
	Gray's Farm	4	very low
	All Saints	3	very low
Aruba	Oranjestaat	11	very low
	Several combined	4	very low
Barbados	Bridgetown	9	very low
	Queen Elizabeth Hospital, St. Michael	21	very low
	Ministry of Health, St. Michael	46	low
British Virgin Islands (BVI)	Beef Island	0	none
	Durcell Estate	1	very low
	Carrot Bay	2	very low
Dominica	Canefield Airport	19	very low
	Ferry Terminal Building	6	very low
	Police Marine Base - Woodbridge	5	very low
	Woodbridge Bay Port	1	very low
	Roseau Fisheries	5	very low
Dominican Republic	Barahona	95	sensitive
	Puerta Plata	98	sensitive
	Dajabon	89	medium
St. Kitts / Nevis	Charlestown	29	very low
	Gingerland	36	low
	Several combined	7	very low
Trinidad	Belmont	0	none
	Coconte	0	none
	Woodbrook	0	none
	Federation Park	0	none

even though it is not recommended for use as such. Based on the results, it is possible that resistant genes in the adult populations may have been passed on to the larval populations, hence the indication of levels of insensitivity in some populations.

Although resistance to temephos has been documented before, its use as a larvicide continues to be prevalent in the region (8). This is because of its low mammalian toxicity (8600mg/ kg in male rats) and its recommendation for use in potable water by the WHO (3). Also, compared to some of the other insecticides such as carbamates and pyrethroids, temephos is relatively inexpensive hence the difficulty in switching to other insecticides. The use of insecticides for *Ae. aegypti* control is not recommended for routine operations. Source reduction through environmental sanitation is the method of choice by the Pan American Health Organization (PAHO).

However, there are situations such as emergencies and non-disposable water containers that warrant the use of these insecticides as has been done for over 25 years. The use of fish and copepods as a means of biological control of *Ae. aegypti* has been recommended in some situations (9,10).

At present, insecticide resistance tests for all CMCs are carried at CAREC. This involves persons collecting *Ae. aegypti* eggs in the country by ovitrapping (6) and shipping them to CAREC where they are reared through to F1 or F2 larval generations (depending on the amount of material received) before testing can commence. Normally, the actual larval bioassay entails the prior exposure of populations of hundreds of larvae to a range of insecticidal concentrations of a particular insecticide. This enables information on the percentage mortality to be obtained in order to ascertain the LC90 for each population. Such tests have to be done until repeatable results are obtained.

The current insecticide resistance testing process necessitates the availability of many resources that could be quite costly and may not be available in some countries. Firstly, the proper facilities such as a secure insectary for mass rearing of mosquitoes and a secured laboratory in which to conduct the bioassays are required. Secondly, in order to analyze the data from the tests, a special computer programme, Probit Analysis, or a complex statistical calculation is needed. This special software package is not accessible to Vector Control (VC) personnel in countries. Thirdly, a technologist requires intensive training in the rearing of large batches of mosquitoes to the right stage and in the actual conduct of bioassays and analysis of results. It takes approximately 1 hour for a technologist to do one run of a test, using at least 5 concentrations, which has to be repeated several times in order to obtain baseline data for each strain of mosquitoes. Each country needs to be able to quickly ascertain the insecticidal sensitivity of their target populations before carrying out any insecticidal control measures, especially in an epidemic. Time is therefore of the essence.

With this simple new method that we have demonstrated here, VC personnel would be able, without any elaborate training, to collect material directly from the field and test in country without the need for any special facilities or equipment. All that would be required is for them to expose a few mosquito larvae to a specific insecticide concentration already established by us at CAREC as being the Caribbean susceptible level for larval *Ae. aegypti*, and within 24 hours they would know whether or not they have a sensitive or resistant population to the particular insecticide. Later, if they wish, countries could also find their own susceptible strain to use as a reference. For example, with respect to temephos, the Bad Cox strain (a rock hole dwelling strain) in Anguilla (RS = 100%) could be the reference strain from that country.

As we have noticed, there are differences in the sensitivity levels of *Ae. aegypti* within each country and even within populations which share similar geographic locations. This is possibly due to *Ae. aegypti* living in discrete populations and thus not exhibiting a great deal of dispersal (11). Using our method, there is the possibility that a more comprehensive profile of the distribution of the insecticide sensitivity status of *Ae. aegypti* populations in each country can be obtained.

We emphasize that we do not advocate the usage of insecticides as the main offensive weapon in *Ae. aegypti* control programmes. Instead, source reduction strategies, especially in view of the observed levels of reduced sensitivity in the larval populations to the insecticides of choice, is recommended. However, we recognize that insecticides do have a place - in emergency outbreak situations. It is important for countries to be informed as to the current and changing status of insecticide sensitivity in *Ae. aegypti* as a matter of urgency. If countries can obtain such information by conducting rapid and simple sensitivity tests, then they would be able to implement alternate interventions in areas where the insecticides would be ineffective in the event of a dengue outbreak.

CAREC is at present obtaining base-line information on alternative insecticides such as pyrethroids. There is an urgent need for some CMCs to switch to alternative insecticides or biolarvicides since those currently have very little practical value. In any case evaluations of changing sensitivity can be done in country using this simplified method.

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K. Polson, S. Rawlins, and H. Hylton
Caribbean Epidemiology Centre
Port of Spain, Trinidad

Codling Moth: The Current Status of Insecticide Resistance in the Two Major Pip-fruit Growing Regions in Italy

INTRODUCTION During the last ten years the rising number of insecticide treatments in two major Italian pip-fruit growing regions did not prevent an increase in fruit damage due to codling moth (CM). Climate conditions (that are favourable to CM development) recorded in this period could not alone explain the increase in fruit damage. The reduction in the efficacy of chemical treatments was perceived to be a possible co-existing cause.

HISTORICAL SITUATION

Trentino: In the lower areas of the region, where codling moth was traditionally present, the number of insecticide treatments to control CM had recently passed from 23 to 4 - 5 per season. Moreover, the pest distribution has been changing and the area needing specific control has gone from 6,000 ha to 10,000 ha.

Emilia Romagna: Codling moth is present in the whole regional area. Whereas in the past, 34 treatments per season could control CM, in the last ten years insecticide application consistantly increased to approximately 67 treatments per season. In 1997, for the first time anomalous fruit damage caused by CM was reported in a small orchard. In the following year, the number of orchards with high levels of damage caused by CM (30%) grew and the situation became worrying as the phenomenon was spreading over other provinces. Damage was recorded both in IPM orchards (IGR+OP) and in traditionally managed orchards where more OPs were applied.

INSECTICIDE RESISTANCE MONITORING In order to verify the presumed presence of resistant populations of codling moth, a monitoring programme activity was started in 1998. Two investigation methods were applied to determine the susceptibility level of insecticides: the attracticide susceptibility test and the topical treatment of diapausing larvae. CM populations of 28 orchards situated in different fruit growing areas were studied for two years.

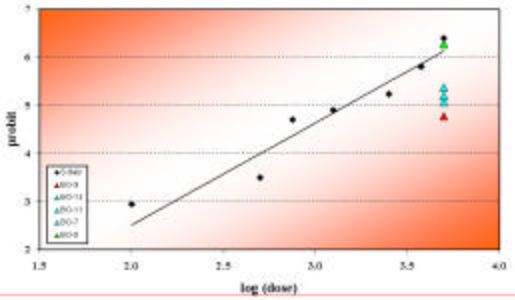
ATTRACTICIDE SUSCEPTIBILITY TEST (Azinphos methyl) Serial dilutions of technical insecticide (azinphos-methyl 92% purity) in acetone were prepared, mixed into warm entomological adhesive, and evenly spread on the surface of a trap bottom (Knight et al. 1990; Varela et al. 1993). Dose-mortality baseline for the susceptible strain was determined in the laboratory using newly emerged moths. For the field studies a discriminating concentration chosen could kill 92% of the sensible moths. Trap liners with the discriminating concentration, as well as the controls, were installed in 5 orchards during 1999 and in 6 orchards in 2000. The sticky bottoms were collected daily before 8 a.m. and then moved into the incubation room at 18°C. The mortality was assessed 40 hours after the sun set, i.e. the presumed contact time with the insecticide (Figure 1).

TOPICAL TREATMENT OF DIAPAUSING LARVAE (Diflubenzuron) Technical diflubenzuron in tetrahydrofuran solution was applied topically on the dorsum of fifth instar larvae with a microsyringe (Sauphanor et al., 1997). After determining the concentration-mortality baseline for the susceptible strain, the discriminating concentration chosen was the one that could kill 90% of the sensible larvae. Diapausing larvae from Trentino and Emilia-Romagna were collected in autumn 1998 and 1999 in 18 orchards

Results of the field survey carried out during 1999 and 2000 and significancy of the differences (▲ = N.S.; ▲ = P>0.01; ▲ = P>0.001) between the corrected mortality of the field populations and the laboratory one (92%) evaluated with $\chi 2$ test.

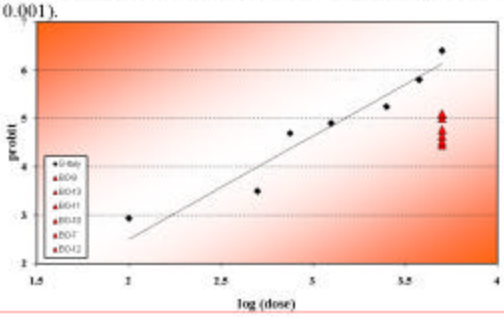
1999

During year 1999, codling moth populations of 5 orchards were tested using the attracticide susceptibility test. In 4 of the orchards the azinphos-m discriminating dose caused a significantly lower mortality rate (see blue and red triangle) than the one recorded in the laboratory for the susceptible population (3 at P>0.01 and 1 at P> 0.001).



2000

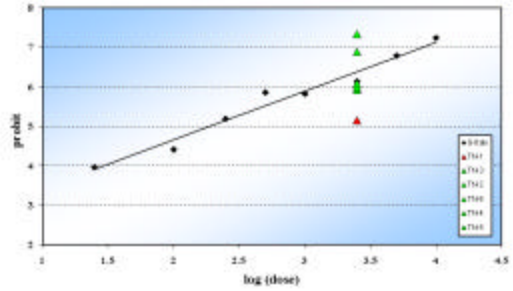
In the year 2000 the CM population of the orchard which had not resulted different from the susceptible strain the previous year was not tested whereas the investigation was extended on two further orchards. In each monitored orchard the azinphos-m discriminating dose caused significantly lower mortality rate (see red triangle) than the one recorded in the laboratory for the susceptible population (P> 0.001).



Results of the field survey carried out during 1998 and 1999 and significancy of the differences (▲ = N.S.; ▲ = P>0.05; ▲ = P>0.001) between the corrected mortality of the field populations and the laboratory one (88-90%) evaluated with $\chi 2$ test.

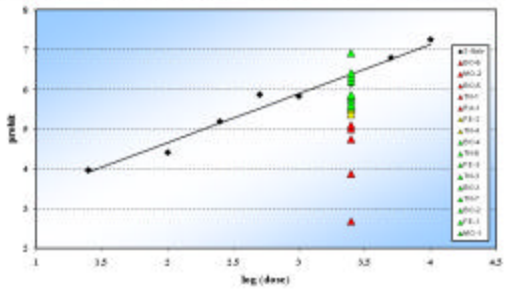
1998

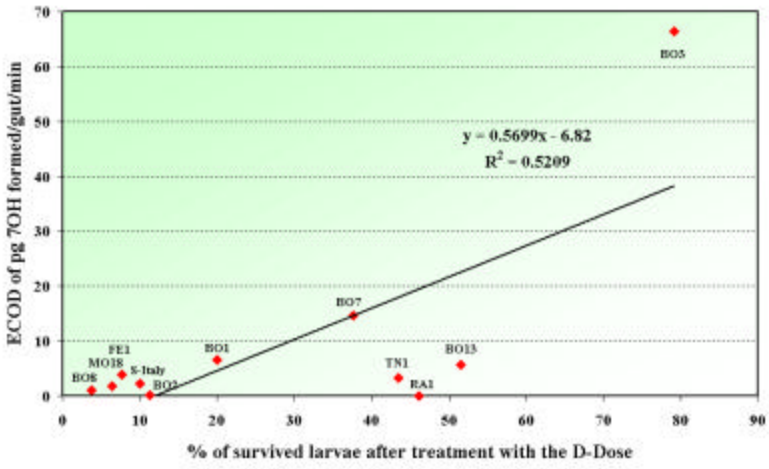
During the year 1998 we tested the codling moth populations of 6 orchards. The mortality rate of the overwintering larvae collected in five of them (see green triangle) was not different from that of the susceptible strain and only in one orchard (see red triangle) the codling moth population showed a statistically lower susceptible mortality rate (P>0.001).



1999

In 1999 we sampled 12 further orchards whose CM populations were tested in order to verify their resistance. On the whole, sixteen orchards were monitored; the mortality rate of the overwintering larvae collected in nine of them (see green triangle) did not differ from that of the susceptible strain. The CM populations sampled in the remaining seven orchards (see yellow and red triangle) showed a statistically different mortality rate (2 at P>0.05 and 5 at P> 0.001).





(apple, pear, and walnut) employing corrugated band traps. Larvae collected in the field were treated with the discriminating concentration and mortality was assessed 2 months later. When the suitable number of larvae collected in the cardboard traps was gathered, concentration-mortality regression lines were estimated by treating the larvae with at least 5 different concentrations (Figure 2).

ENZYME ASSAYS Mixed-function oxidase (MFO) and esterase activities were determined on fifth-instar diapausing larvae according to Bouvier et al. (1998). For each field population, 6 males and 6 females were used.

A few resistant populations were found to exhibit a significant increase in MFO (ECOD) activity, i.e. BO3 and BO7. Conversely the RA1 population expressed a 6.3-fold resistance ratio to diflubenzuron, compared to the susceptible strain, without enhanced MFO activity. This could be due to the heterogeneity of the population, or more likely to the involvement of the resistant mechanisms, such as Glutathion S-transferase which were not investigated. Esterase activities did not reveal any difference between the susceptible strain and the field populations (Figure 3).

CONCLUSIONS In both fruit growing regions, IRM strategies were applied shortly after the first detection of some resistance to insecticides.

In Trentino, a significant level of resistance to insecticide was found in only one of the monitored orchards. In 2000, a certain level of resistance was noticed in a second orchard where a resistant population of CM was released for an experimental purpose. As the level of CM population was generally low, MD is widely applied to control CM. In the last four years the surface treated with the MD increased from 310 ha to 1298 ha. In the non-MD orchards, at least one chlorpyrifos/ season is used.

In Emilia Romagna, the reduced efficacy of some of the insecticides used to control CM in the pip-fruit orchards, as well as the results of the resistance monitoring program for diflubenzuron and azinphos methyl (11 out of 18 monitored orchards showed significant level of resistance), caused some growers to change their control strategies. In order to evaluate the outcomes of IRM a sample of 31 orchards was kept under observation for three consecutive years. The average fruit harvest damage decreased from 30% (1998) to 5% (2000). The average number of IGR applications was reduced from 1.8 to 1.1. The same trend was recorded for azinphos-m, which passed from 1.7 to 1 treatment/year. On the contrary the average number of chlorpyrifos treatments increased from 0.7 to 2.3. In the year 2000, granulosis virus (GV) was registered in Italy for CM control. After the registration, CM-GV was largely applied in these orchards (2,7 treatments/orchard). Although any high level of fruit damage was recorded, IRM was applied also in the orchards close to the monitored ones where a high level of captures in the pheromone traps occurred. In these orchards, where the population level was still low, IRM mainly consisted in using CM-GV and mating disruption (1000 ha in the year 2000).

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C. Ioriatti
IASMA - S. Michele a/A
Trento, Italy
claudio.ioriatti@mail.ismaa.it

J. C. Bouvier
INRA
Avignon, France
jbouvier@avignon.inra.fr

A. Butturini
Servizio Fitosanitario Regionale
Bologna, Italy
abutturini@regione.emilia-romagna.it

R. Tiso
Servizio Fitosanitario Regionale
Bologna, Italy
rtiso@regione.emilia-romagna.it

L. Caroli
Centro Ricerche Produzioni Vegetali
Cesena, Italy
luigi.caroli@tin.it

R. Cornale
Centro Agricoltura Ambiente
Crevalcore-Bologna, Italy
rcornale@caa.it

Current Status of Resistance in Colorado Potato Beetle (CPB) Leptinotarsa Decemlineata (Say) in Poland

INTRODUCTION: Poland is a major producer of potatoes (1200 000 ha, Table 1) but the average potato crop in Poland is very low (1,7 t./ha. in comparison with the west European average of 35 t./ha) due to inadequate control of pests, diseases, weeds, and resistance. When the treatment is abandoned the damage may affect as much as 80-90% of the crop. The main pest of potato cultivation in Poland is Colorado potato beetle (*Leptinotarsa decemlineata* Say). The threshold of economic harmfulness of this pest is considerably exceeded every year Fig. 1 (50% of all insecticides used in Poland are devoted to this pest). The cost of chemical protection of potato fields in Poland amounts to an average of 20 US dollar/ha (30 000 000 US dollar in global scale and the yield losses resulted from the CPB feeding and insecticide resistance amount in average level 10-25% - 250 000 000 - 500 000 000 !!! US dollar). The constant selective pressure of insecticides on the population of polish CPB is a factor which accelerates the process of the growing resistance of that species (Fig.2 Table2).

CPB is still considered to be the Polish pest with the highest likelihood of developing insecticide resistance.

Tab 1. Croping area and insecticide pressure on the CPB in Poland and some others countries.

Country	Protection area in thousands ha	Number of treatment against CPB
Canada	130	0,9
France	34	0,2
Germany	124	0,4
Greece	48	1,2
Italy	72	0,8
Spain	121	0,7
Poland	1006	1,3
Portugal	177	2,1
Turkey	125	0,7
USA	525	0,9

Insecticide resistance in CPB has been a severe problem in Poland for a number of years. The dynamic and widespread nature of CPB resistance in Poland (organochlorines, organophosphates, carbamates) and the increase of tolerance to pyrethroids and nereistoxin analogues (bensultap) has created a need for resistance monitoring and to elaborate the strategies for the management of CPB resistance to syntetic insecticides. The studies on CPB susceptibility to main classes of insecticides have been performed in Poland for some time and are still continuing with the cooperation and support of Insecticide Resistance Action Committee (IRAC). At the present moment in Poland a bt of consideration is given to chloronicotinoids and phenylpyrazoles. Insecticides from the phenylpyrazoles and chloronicotinoids (neonicotinoids) classes are relatively new for the control of CPB in Poland (fipronil-1996, acetamiprid-1996, imidacloprid-1998, thiamethoxam-1999, thiacloprid-2001). The economic importance of both new classes of insecticides for CPB control in Poland have systematically increased.

Bioassays of this new classes insecticides at recommended field concentrations and laboratory susceptibility tests for resistance monitoring on CPB were performed in Institute of Plant Protection in Poznan.

METHOD: Commercially-available products were used for the field and laboratory inwestigations.

PHENYLPYRAZOLES: Regent 200 SC and Regent 800 WG-fipronil
CHLORONICOTINYLS: Confidor 200 SL-imidacloprid, Actara 25 WG-thiamethoxam, Stonkat 160 SL and Mospilan 20 SP- acetamiprid, Calypso 480 EC-thiacloprid
PYRETHROIDS: Decis 2,5 EC-deltamethrin, Cyperkil Super 25 EC- cypermethrin, Karate 025 EC-lambda-cyhalothrin, Fastac 100 EC-alpha-cypermethrin
NEREISTOXIN ANALOGUES: Bancol 50 WP-bensultap
CPB larvae and beetles were collected from the 7 geographically distant field populations:

1-Badrzychowice
2-Bolewice
3-Debki
4-Rarwino
5-Rogalinek
6-Skoki
7-Winna-Góra

Laboratory tests were conducted in 2001using IRAC method number. 7:

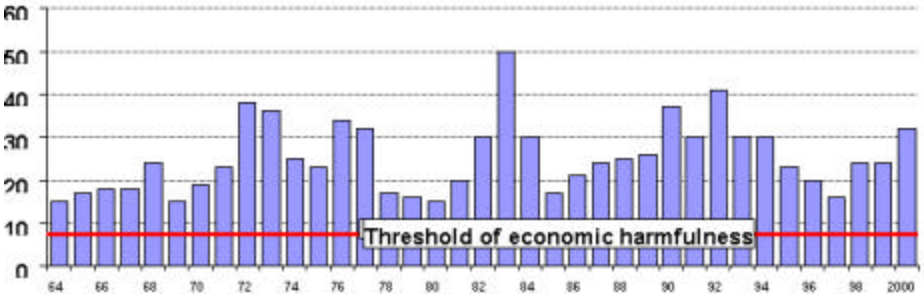
”-Collect a representative sample of insects in the field populations-CPB larvae or beetles suitable for immediate testing. The insects should not be subjected to temperature, humidity or starvation stress after collection. The species should be correctly identified.

”-Collect sufficient non-infested, untreated leaves to conduct the test. Whole leaves are preferred or, for some crops, the distal portions. Do not allow leaves to wilt.

”-Prepare accurate dilutions of the test compound from commercially-available products For initial studies 5-7 widely spaced rates are recommended. If a discriminating or single dose such as the LC95 of a

reference 'susceptible' strain is known, it is preferable to use this rather than 5-7 doses, and to determine 'percent srvivorship' at the single dose. The use of additional wetter is only recommended for highly waxed leaf materials, in which case this wetter solution is used for the "untreated" (control) solution in place of water alone.

Fig. 1 % of potato plants damaged by Colorado potato beetle in Poland



”-Dip leaves or leaf discs individually in the test liquid for 5 seconds with gentle agitation and place on paper towelling until the leaf surface is dry. Do not allow to wilt. Dip the same number of leaves per treatment and treat sufficient leaf material to avoid starvation stress in the "untreated" during the test. Start by dipping the "untreated" and work up through the test liquids from lowest to highest dose.

”-Place the treated surface-dry leaves in the labelled test containers, which must be suitable for holding enough leaf material in good condition for up to 3 days. The addition of a moistened filter paper or a thin layer of agar will help to maintain a humid micro-climate.

”-Add equal numbers of recently moulted L2 or L3 larvae or adult beetles to each container, ensuring that a total of 30-90 larvae or beetles are used per treatment, divided between five to eight replicate containers.

”-Store the containers in the dark or in an area where they are not exposed to direct sunlight or extremes of temperature. Record maximum and minimum temperatures, if possible. A mean temperature of 22-250 C is preferred.

”-In the case of rapidly acting compounds, a final assessment of larval mortalities is made after 48 h., beetles 72 h. For slowly acting compounds (eg. benzoylureas, bensultap, *Bacillus thuringiensis* etc.) a first assessment is made at 72 h, when the leaves are changed for fresh, untreated leaves. The containers are held for a further period before the final assessment, either for 48 h. or until larvae in the 'untreated' (control) have moulted again.

”-Express results as percent mortality at each dose, or survivorship at a single dose correcting for untreated (control) mortalities using Abbott's formula. Untreated mortality should be quoted.

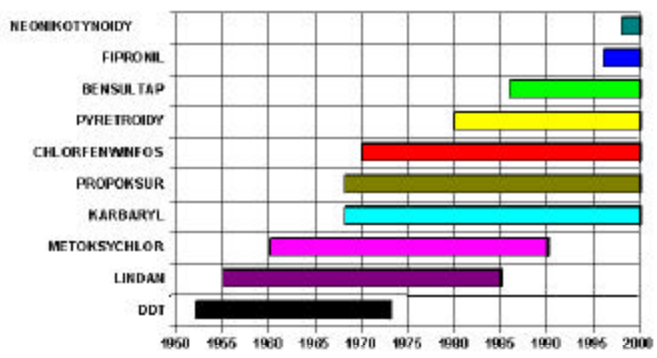
”-At each assessment, larvae and beetle are classed as either: (a) unaffected, giving a normal response (such as taking a co-ordinated step) when gently stimulated by touch or high temp. (30-350C during 10 min.), or (b) dead or affected, the latter giving an abnormal response to stimulation or showing abnormal growth which should be described. For some benzoylureas, colour changes are reliable indicators of effectiveness and should be recorded. Thus, % response ('mortality') will include both dead and affected.

”3. Corrected Mortality = 100 x (P-C/100-C) where P = % mortality in treatment, C = % mortality in controls.”

LC 50 and LC 95 were calculated using Finney probit analisys method and expressed in ppm. Field experiments on the commercial potato farms concerning fipronil, acetamiprid, thiamethoxam, alpha cypermethrin, cypermethrin, lambda cyhalothrin and deltamethrin activity against CPB larvae were conducted during 2001 on Badrzychowice population.

CPB larvae were counted before the treatment on the four potato plants rows (ten meters long) for any combination. The final assessment of larval mortalities were made on the succeding days after treatments. The efficacy of insecticides sprays were expressed in

Fig. 2 Periods of chemical protection of potato cultivation in Poland using particular insecticides



percent of larvae mortality.

Tab 2. CPB control scale in Poland within 1978-1999

YEARS	Croping area thousands ha	Protection area %	Number of treatment
1978	2360	76	1,2
1979	2441	78	1,2
1980	2344	50	1,1
1981	2257	72	1,2
1982	2178	78	1,2
1983	2220	96	1,6
1984	2147	78	1,1
1985	2095	37	1,1
1986	2009	70	1,1
1987	1934	75	1,1
1988	1886	82	1,2
1989	1858	89	1,3
1990	1835	87	1,4
1991	1732	84	1,4
1992	1757	89	1,7
1993	1761	89	1,4
1994	1697	83	1,4
1995	1522	97	1,6
1996	1342	98	1,6
1997	1303	68	1,3
1998	1250	42	1,3
1999	1255	83	1,4
22 years	average 1. 872. 000 ha	average 77,3%	average 1,3

RESULTS:

Tab. 3. Field efficacy of some active ingredients on CPB larvae L_2

Compound /dose-concentration (ppm)		% mortality after		
		5 days	10 days	14 days
acetamiprid	50 ppm	90,24	83,39	69,96
acetamiprid	67 ppm	90,94	86,52	76,11
thiamethoxam	67 ppm	99,38	99,86	100
fipronil	67 ppm	100	100	100
cypermethrin	80 ppm	95,11	91,58	85,84
alpha-cypermethrin	34 ppm	96,25	91,79	85,46
lambda-cyhalothrin	17 ppm	97,61	95,72	92,02
deltamethrin	17 ppm	95,21	92,66	87,38
deltamethrin	25 ppm	90,15	95,81	92,11
bensultap	500 ppm	97,90	100	100

Tab. 4 Maximal and minimal susceptibility level of CPB larvae (L_2) and beetels to some active substances - comparison with recommended concentrations (laboratory data from 8 populations - 1998 - 2001 year, IRAC method no. 7)

active substances	recommended field concentration (in ppm)	LC_{50} larvae (ppm)	LC_{95} larvae (ppm)	LC_{50} beetles (ppm)	LC_{95} beetles (ppm)
fipronil	50	0,06-0,17	0,28-0,70	0,25-0,75	1,20-2,20
thiamethoxam	50	0,20-0,35	0,801,55	1,20-2,50	1,90-5,10
acetamiprid	40	0,06-0,20	0,95-2,40	0,90-1,85	4,45-16,80
imidacloprid	50	0,30-0,80	1,0-2,8	1,50-3,70	7,80-18,40
bensultap	375	9,20-21,3	19,84-65,80	6,6-148,9	306-38707
chlorfenvinphos	625	55,57-121,7	427,5-898,2	38-187	1100-6100
deltamethrin	12,5	2,50-5,3	8,79-17,65	7,15-28,8	76,2-311,9
cypermethrin	62,5	6,50-22,34	18,46-67,32	18,9-128,9	139,6-380,0
lambda-cyhalothrin	25	No data	No data	17,4-73,7	31,3-165,9
alpha-cypermethrin	25	No data	No data	17,5-43,9	53,5-250,9

CONCLUSIONS:

1. CPB strains tolerant to nereistoxin analogue (bensultap), pyrethroids (deltamethrine, cypermethrine, alpha-cypermethrin, lambda-cyhalothrin) and organophosphorous (chlorfenvinphos) insecticides are still excellently controlled with imidacloprid, thiamethoxam, acetamiprid, thiacloprid and fipronil so no cases of resistance has been reported so far.
2. Field and laboratory investigations give no indication on rasistance or cross resistance elicited by tested populations of CPB to this insecticides.
3. To maintain as long as possible the high insecticidal potency of new and older chemical classes of insecticides there is a necessity to follow the general resistance management guidelines, which were elaborated in Institute of Plant Protection in Poznan-Poland with cooperation of IRAC, and could be adopted in all areas of potato insecticide protection in Poland.

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Pawel Wegorek
60-318 Poznan Institute of Plant Protection
Poland
p.wegorek@ior.poznan.pl

Insecticide Resistance Management Strategy for Colorado Potato Beetle (*Leptinotarsa Decemlineata* Say) in Poland

Poznan 2002 Publication sponsored by the Insecticide Resistance Action Committee

INTRODUCTION: "Resistance is the naturally occurring, inheritable adjustment in ability of individuals in a population to survive a plant protection product treatment that would normally give effective control."

"Practical resistance is the term used for loss of field control due to a shift in sensitivity."

Management of Colorado Potato Beetle (CPB) in Poland is mainly based on insecticide use. The very extensive acreage of potato cultivation (1,19 mln ha in 2001) and the constant need for intensive chemical protection is due to the concentration and size of the CPB populations that considerably exceed the economic damage threshold in occurrence area. Nearly 50 years of constant selective pressure on the CPB has brought about the development of local populations with lower susceptibility to some insecticides. This phenomenon, known as resistance, or tolerance, leads to big losses in potato crop and restriction in use and sales of less effective insecticides. In Poland, resistance proved highest in with the organophosphate, trade name-Enolofos 44 EC (the present trade name - Enolofos 500 EC), containing chlorfenvinphos as the biologically active ingredient. Resistance also appeared earlier, though on a lesser scale, in the case of chlorinated hydrocarbons insecticides - DDT, lindane, methoxychlor and the carbamates - carbaryl and propoxur. At present populations with increased tolerance to insecticides from the pyrethroids group and nereistoxin analogue can be observed. Problems caused by the pests growing resistance usually result from an inappropriate or simplified way of applying the chemicals, with successive applications being carried out in a given area using one or more products, of the same class. Study of resistance mechanisms show that selection based on one mode of action, after a period of time leads to development of genetically inherited features in the target population, which allows individuals carrying those genes to survive the action in ever bigger numbers. CPB is a species with a very high natural resistance to all kinds of poisons and tolerates well both plant toxins (xenobiotics) and synthetic insecticides, and for this reason only a limited number of chemical compounds can be effectively used to fight it. In the years 1994 - 1997, following a commission by IRAC, monitoring of the susceptibility level of a large number of populations of CPB was carried out in Poland in different regions of the country evaluate the effectiveness of -bensultap (trade name-Bancol 50 WP), cypermethrin (trade name-Sherpa 10 EC) and chlorfenvinphos (trade name-Enolofos 500 EC). The study revealed a high degree of variation in susceptibility to each of the insecticides both in the same areas, and in geographically distant regions. This suggests an advanced selection, which in near future may lead to development in the CPB of high resistance to each of the biologically active ingredient under investigation. To combat this situation, a controlled and rational use of the management insecticides is necessary in Poland, following the strategy worked out by Polish research institutes in cooperation with IRAC.

FACTORS INFLUENCING RESISTANCE DEVELOPMENT IN COLORADO POTATO BEETLE: There are a number

factors that condition insects resistance. Genetic, biological, ecological and agronomic factors play a major role.

In the case of genetic factors, the genetic potential of the species is of considerable importance. Estimates of frequencies of alleles responsible for resistance to insecticides cover a large range (10 -3 10 -13). In European CPB populations chemical selection factors decrease susceptibility after several generations (15 - 20), with development of high resistance in a short period of time. Classical and molecular analysis of CPB genome can be used to identify resistance genes and their interaction in resistance phenomenon.

In Polish conditions, bio - ecological factors that influence resistance development also seem to favor CPB. This is determined by population concentration, fertility, length of larval growth, diapause occurrence, migration capability and variation in ecological conditions as well as too low natural selection.

Factors affecting the level of CPB resistance risk include:

1. insecticide factors

-type of compound (if the compound is new molecule of established chemistry, the risk can be assumed to correspond to other compounds in the same chemical class, unless demonstrated to be different. In the case of a completely new type of compound (fipronil, imidacloprid, thiamethoxam, acetamiprid, thiacloprid) of the possibility with of resistance development with extensive use could be assumed. If the active ingredient is persistent the level of risk should be assumed to be higher (imidacloprid for systemic applications).

-mode of action (a single site mode of action is considered to be at a higher risk of resistance development than a multi-site mode of action.

-cross-resistance (-the existence of this phenomenon between a new compound and other of the same or other chemical class means that resistance occurs already in the CPB populations (chlorinated hydrocarbons and pyrethroids) if the compound is known to have a structure that can be easily degraded (such as by esterification or hydrolysis) the risk of CPB resistance development should be considered to be high.

2. agronomic factors

-the intensity of insecticide selective pressure (in Poland, there are on average 1.2-1.6 applications per year over almost the total potato acreage, as every year the economic damage threshold is exceeded, the life stage of the target pest, the level of mortality achieved, the way of application and the type of the used product.

-lack of diversity of available alternative CPB control measures in Poland.

PHYSIOLOGICAL MECHANISMS OF POTATO BEETLE RESISTANCE: CPB has demonstrated all resistance mechanisms identified to cause insecticide resistance.The main mechanisms that cause resistance of the CPB to insecticides are the following:

-increased metabolism of biologically active ingredient by specific detoxicating enzymes (oxidative metabolism, altered acetylcholinesterase, increased carboxyloesterase activity, knock - down resistance)

-limitation of biologically active ingredient penetration through body cuticle. (the reduced penetration mechanism will potentiate the oxidative metabolic factor)

-increase in excretion (rapid excretion coupled with reduced penetration could prevent internal levels of insecticide from reaching toxic level).

-reduction of the nervous system susceptibility at the site of insecticide action,

-avoidance of areas treated with a chemical product (behavioral resistance) (CPB populations have one or more combinations of five resistance mechanisms).

ACTUAL LIST OF INSECTICIDES FOR POTATO BEETLE MANAGEMENT RECOMMENDED IN POLAND (2002)

Chemical class	Trade name - active substance - toxicity	Dose in g, ml kg /1ha	Optimal action temp ^{0}C	PHI in days
1. Bacillus thuringiensis	*Novodor 02 SC - Bacillus thuringiensis endotoxins (CryIIIA protein) - IV*	3000-5000	over 18	0
2. Chloronicotinyls (neonicotinoids)	Actara 25 WG - *thiamethoxam - IV*	80	no influence	14
	Calypso 480 SC - thiacloprid - III	50-100	over 15	3
	Gaucho 350 FS - *imidacloprid - III*	36 ml/100 kg	no influence	-
	Mospilan 20 SP- *acetamiprid - III*	60 - 100	no influence	3
	Stonkat 160 SL - *acetamiprid - III*	100	no influence	3
3. Phenylopyrazoles	Regent 200 SC - *fipronil - III*	100	no influence	14
4. Organophosphorous	Cotnion-Metyl 200 EC - *azinophos-methyl - II*	1500 - 2000	over 15	7
	Danacap 450 CS - *parathion-methyl - III*	1000 - 1500	over 15	48
	Danadim 400 EC - *dimethoate - III*	1000	over 15	21
	Enolofos 500 EC - *chlorfenvinphos - I*	500 - 750	over 15	14
	Hostation 40 EC - *triazophos - II*	900	over 15	14
	Ofunack 400 EC - *pyridaphenthion - IV*	1000	over 15	28
	Zolone 350 EC - *phosalon - IV*	1500 - 2000	over 15	15
5. Chitin synthesis inhibitors	Andalin 250 DC - *flucycloxuron - III*	250 - 300	over 18	7
	Ekos 100 EC - *hexaflumuron - IV*	250	over 18	7
	Mat 050 EC - *lufenuron - IV*	300	over 18	1
	Nomolot 150 S.C. *teflubenzuron - IV*	200 - 250	over 18	14
6. Carbamates	Diafuran 5 GR - *carbofuran - II*	20000	-	
	Furadan 5 GR - *carbofuran - II*	20000	-	-
	Marshal 250 CS - *carbosulfan - II*	1000	over 15	7
	Marshal 250 EC - carbosulfan - II	1000-1500	over 15	7
	Oncol 120 EC - *benfuracarb - III*	1000 - 1700	over 18	35
	Oncol 200 EC - *benfuracarb - II*	600 - 1000	over 18	35
	Propxan 500 WP - *propoxur - II*	600 - 1000	over 15	7
7. Nereistoxin analogues	Bancol 50 WP - *bensultap - III*	300 - 400	From 10 to 35	3
	Bancol Super 500 SC - *bensultap - III*	400	from 10 to 35	3
8. Pyrethroids	Alfamor 050 S.C. - *alpha-cypermethrin - IV*	200	Below 20	7
	Alfazot 050 EC- *alpha-cypermethrin - III*	200	Below 20	7
	Alphaguard 100 EC- alpha-cypermethrin - III	100	Below 20	
	Alphatop 100 EC -alpha-cypermethrin	100	Below 20	7
	Ammo 250 EC - *cypermethrin - III*	100 - 120	Below 20	7
	Bulldock 025 EC - *beta-cyfluthrin - III*	200 - 300	Below 20	14
	Cyperkil 25 EC- *cypermethrin - III*	100 - 120	Below 20	7
	Cyperkil Super 25 EC- *cypermethrin - III*	100 - 120	Below 20	14
	Decis 2,5 EC - *deltamethrin - III*	200 - 300	Below 20	14
	Decis 005 UL - *deltamethrin - IV*	1000 - 1500	Below 20	7
	Decis koncentrat 015 UL - deltamethrin - IV	300 - 500	Below 20	
	Decistab TB - *deltamethrin - III*	8-12 tab	Below 20	7
	Fastac 100 EC- *alpha-cypermethrin - III*	80 -100	Below 20	7
	Fury 100 EC -*zeta-cypermethrin - III*	100	Below 20	7
	Fury 100 EW - *zeta-cypermethrin - III*	150 - 200	Below 20	7
	Karate 0,25 EC - *lambda- cyhalothrin - III*	200 - 300	Below 20	14
	Karate 25 WG - *lambda- cyhalothrin - III*	200 - 300	Below 20	14
	Karate Zeon 050 CS- *lambda-cyhalothrin III*	120-150	Below 20	7
	Karate Zeon 100 CS- *lambda-cyhalothrin - III*	60 - 80	Below 20	7
	Patriot 2,5 EC - *deltamethrin - III*	200 - 300	Below 20	7
	Patriot 100 EC - *deltamethrin - III*	50 - 75	Below 20	7
	Ripcord 10 EC- *cypermethrin - III*	250 - 300	Below 20	7
	Ripcord Nowy 0,50 EC - *alpha-cypermethrin - III*	200	Below 20	7
	Ripcord Super 050 EC - *alpha-cypermethrin - III*	200	Below 20	14
	Sherpa 10 EC - *cypermethrin - VI*	250 - 300	Below 20	7
	Sumi -Alpha 050 EC - *esfenvalerate - III*	200 - 250	Below 20	7
	Sumicidin 20 EC - *fenvalerate - III*	300 - 400	Below 20	14
	Trebon 10 SC - *ethofenprox - IV*	450 - 600	Below 20	14
	Trebon 30 EC - *ethofenprox - IV*	300	Below 20	7
Mixtures	Chlormezyl 500 EC - *chlorpirifos+ dimethoate - III*	1000 - 1500	From 15 to 30	30
	Decisquick 425 EC - deltamethrin+heptenophos - II	300	from 15 to 20	
	Enduro 258 EC *betacyfluthrin+oxydemethon-methyl - II*	400 - 600	from 10 to 25	7
	Nurelle D 550 EC - *chlorpirysphos+cypermethrine - III*	400 - 600	from 15 to 25	28
	Prestige 290 FS - *imidachloprid+penoxuron - III*	75 ml/100 kg	-	30

MECHANISMS OF INSECTICIDE ACTION ON POTATO BEETLE: A majority of CPB insecticides registered in Poland affects the pests nervous system. Carbamates and organophosphate compounds block the action of acetylcholinesterase, an enzyme responsible for the decomposition of the neurotransmitter-acetylcholine. Pyrethroids block the sodium channels in the insects neurons, causing first overexcitation of the nervous system and then paralysis of the insect. The nereistoxine analogues (bensultap) act as antagonist and neonicotinoids acts as agonist (or partial agonist) of the nicotinic acetylcholine receptor-obstruct the activity of acetylcholine by interaction with the proteins receptors of the transmitter. Phenylpyrazoles affect the receptor of another neurotransmitter-gamma-aminobutyl acid (GABA). Insecticides of the chitin biosynthesis inhibitors group obstruct chitin biosynthesis in CPB larvae. They also affect females laying eggs, causing anatomical damage to the hatching larvae. Their biologically active ingredients interact with metabolites and block the enzymes responsible for chitin formation in the epithelium cells. The biologically active ingredients of the bacteria group products is in the form of crystals of active protein of the bacterium *Bacillus thuringiensis* subsp. *tenebrionsis*, which damage the beetles digestive system, mainly in its middle section, by disintegrating the cells.

Thus, 57 insecticides (34 active ingredients) for CPB control in Poland are classified into 1 biological class and 7 chemical classes, two of which clearly predominate in this country: These are pyrethroids class (9 active ingredients) and the nereistoxine analogue (1 active ingredient). As much as 80-90% of pest management action in Poland involves Bancol 50 WP (bensultap) and one of pyrethroids insecticide. If this tendency to apply only two of the above chemical classes continues, it may in effect increase the risk of resistance development in local CPB populations to the products belonging to these classes. The studies in resistance management carried out in research institutes (Institute of Plant Protection in Poznan, Plant Breeding and Acclimatization Institute Department in Bonin and Institute of Organic Industry in Warsaw have shown a highly diverse susceptibility of the monitored beetle populations to biologically active ingredients of three insecticides. Since in one growing season CPB usually produces only one complete generation, and on average 1.5 applications are used on it, there is small risk of a fast development of high resistance to a given biologically active ingredient or a chemical group over a few seasons. However, a continual selection pressure with similar product must be avoided, and insecticides from different chemical classes and with different mechanisms of action should be used.

NEW CHEMICAL CLASSES FOR COLORADO POTATO BEETLE CONTROL IN POLAND: Insecticides from the phenylpyrazoles and neonicotinoids classes are relatively new for the control of CPB in Poland (fipronil - 1996, acetamiprid - 1996, imidacloprid - 1998, thiamethoxam - 1999, thiacloprid 2001). The importance of both new classes of insecticides for CPB control in Poland systematically increase. These new insecticides are neurotoxins and act on the neurotransmitter receptors ion channel complex. The main target of neonicotinoids are acetylcholine receptors in the insect central nervous system. They interact with the receptors as acetylcholine agonists, and are not degraded by acetylcholinesterase that results in a long period of excitation followed by paralysis and death of insect. Neonicotinoids are very active, effective and show very rapid action in knock-down and killing CPB. Their chemical structures are practically not vulnerable to hydrolytic attack by metabolising and sequestering esterases of resistant pests.

Phenylpyrazole insecticide (fipronil) interact with receptors of gamma -aminobutanoic acid (GABA) - inhibitory neurotransmitter in insect central nervous system and nerve - muscle synapses, cause the strong block of GABA - regulated chloride channel. Fipronil is a GABA - antagonistic. CPB poisoned with fipronil fall in convulsions, followed by death. This insecticide has practically no effect on acetylcholine esterase.

Bioassays of this new classes insecticides at recommended field concentrations for resistance monitoring on CPB were performed in Institute of Plant Protection in Poznan and Plant Breeding and Acclimatization Institute in Bonin, where field and laboratory investigations gave no indications on resistance elicited by tested populations of CPB to those insecticides today. CPB strains more tolerant to nereistoxin analogue (bensultap), pyrethroids and organophosphorous (chlorfenvinphos) insecticides are still excellently controlled with imidacloprid, thiamethoxam, acetamiprid and fipronil. No cases of resistance has been reported in Poland so far.

Using new classes of insecticides against CPB in Poland we must remember that this pest insect is multiply and cross - resistant to five major groups of insecticides in other countries. Resistant to *Bacillus thuringiensis* Berliner (CryIIIA) abamectine, imidacloprid and tolerant to fipronil too. CPB is still considered to be the Polish pest with the highest likelihood of developing insecticide resistance. To maintain as long as possible the high insecticidal potency of new and older chemical classes of insecticides there is a necessity to follow the general resistance management guideline, which were elaborated in Institute of Plant Protection in Poznan with cooperation of IRAC and should be adopted in all area of potato insecticide protection in Poland.

POTATO CROPS PROTECTION STRATEGY FOR PREVENTING CPB RESISTANCE DEVELOPMENT TO RECOMMENDED PRODUCTS: Having decided that the resistance risk in the CPB in Poland is too high the integrated use of combinations of different strategies is recommended.

1. One of the main tools against resistance should to be constant monitoring of the pests susceptibility level using standard methods recommended by IRAC (Method number 7). Monitoring has allowed re-introduction of Enolofos 500 EC, withdrawn earlier from the western regions of Poland, because of CPB resistance. Resistant populations often show less adaptation and vitality (lower fertility, shorter life span, etc). Following the withdrawal of a selection factor, after a period of time the interbreeding of individuals from different populations can dilute the resistance gene(s) and bring back high susceptibility to the given factor. Monitoring before the commercial introduction of an active substance establishes the baseline sensitivity of CPB and should bee a continuous process after registration. The results of the monitoring indicate whether resistance is developing and management strategies may need to be introduced or modified.

2. It is recommended that the products are applied in the full effective dose rates recommended by the producer. Reduced doses (sublethal) quickly select populations with average level of tolerance, while too large ones lead to resistance development at a very high level. Thus, the choice of appropriate spray equipment and the technique of application (correct amount of water, suitable liquid pressure for uniform spray coverage optimal temperature, etc) is of vital importance.

3. Timing of the application must coincide with moment of the greatest susceptibility in the life stage of the pest to the particular product. Application to young larvae can be more effective then on later stages or adults.

4. In case of using insecticide mixture, it should be ensured that the compounds belong to different classes and are applied in effective equivalent control rates. The CPB can develop tolerance to components of the insecticide mixture if it has been used long enough, and the resulting resistance may be more permanent and more difficult to manage than one developed separately to each of the biologically active ingredient present in the mixture.

5. If the activity of the product proves ineffective and must be repeated, the reasons of ineffectiveness must be defined and, if necessary, a product of different class should be used.

6. If the local CPB population has been found to be resistant to compounds of some specific class, products with similar action mechanism should not be used in the rotation strategy.

7. Withholding the use of the product that the CPB has developed resistance to must be continued until the pest shows high susceptibility.

8. Attention must be paid to protection of beneficial organisms natural enemies of CPB, since these play a major role in the management of resistance. They limit the CPB population, irrespective of the pest resistance and act against the selection of resistant populations.

Aware of the fact that in Poland the choice of an insecticide for farm protection is mainly based on price, speedy effectiveness, ease of application, the post harvest interval and toxicity, we encourage farmers to follow the strategy outlined in this paper. Also, we recommend the use of unconventional products with new modes of action (Novodor 02 SC, Andalin 250 DC, Ekos 100 EC, Mat 050 EC, Nomolt 150 SC) which, even if less popular because of their price, are highly effective when appropriately used, while one of their greatest advantages is environment protection and the safety of the user (IPM and IRM compatibility is an important characteristic of these insecticides).

THE INSECTICIDE SUSCEPTIBILITY TEST METHOD FOR CPB RESISTANCE DETECTION: Plant protection advisors and farmers should consider using the simple IRAC method nr. 7 before "high-risk" CPB population treatment to detect the field efficacy of insecticides for CPB control.

1. Collect a representative sample (300 - 400) of CPB larvae L2 (or L3) stage in the different places of the field.

2. Collect sufficient non infested, untreated leaves to perform the test.

3. Wear solvent-proof gloves, syringe or pipette and prepare accurate recommended (field concentration) water dilutions from commercially-available product.

4. Dip leaves in water for untreated control and other leaves in the tested liquid for 5 seconds and place on paper towel to let it dry up.

5. Place the untreated and treated dry leaves in the containers, which must be suitable for keeping enough leaf material in good condition for up 2 -3 days.

6. Add equal numbers of L2 (or L3) CPB larvae to each container (one container should be use for untreated control) but no more than 20 larvae/container and store the containers in the area where they are not exposed to direct sunlight or extremes temperature (mean temperature of 22-240 C is preferred).

7. For rapidly acting insecticides (pyrethroids, neonicotinoids, phenylopyrazoles, organophosphores and carbamates) a final assessment of larval mortalities is made after 48 h. For slowly acting insecticides (bensultap, Bacillus thuringiensis, etc.) after 120 h.

Larval mortality in control container should be less than 10%.

Larval mortality in treatment should to be 100%.

If 1 or more larvaes survive the treatment test the product should not be recommended to control tested population. The resistance management strategy presented aim is extending the effective performance of all products recommended for control the CPB in Poland.

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Pawel Wegorek
Institute of Plant Protection
Poznan
Poland

Maria Pawinska
Plant Breeding and Acclimatization Institute
Department in Bonin
Poland

Elzbieta Przybysz
Institute of Organic Industry
Warsaw
Poland

Robert Dutton
Dow Agro Sciences
United Kingdom

Insecticide Resistance Action Committee

Field evaluation of transgenic peas for the design of strategies to minimise the evolution of resistance in pea weevil (*Bruchus pisorum*)

The pea weevil (Bruchidae: *Bruchus pisorum*) is the most damaging insect pest of field peas (Pisum sativum) in Australia, causing economic loss by consuming seed contents, (Smith, 1990). Pea weevils appear to be capable of increasing population sizes more than 200-fold per generation.

To control the pea weevil, the gene for a-amylase inhibitor (*a*-AI-1) has been transferred from the common bean (*Phaseolus vulgaris*) to field peas. In seeds that express the *a-ai*1 gene, larval development is blocked at an early stage, i.e. first or second instar, (Schroeder et al. 1995).

Our preliminary attempts to select for resistance to these peas in pea weevil have been unsuccessful, but the sample size tested (2,400 eggs) implies that any dominant genes would have to be rarer than 10-3 (Roush and Miller 1986). Still, resistance must be considered a possibility. As shown in Figure 1, resistance is a serious threat, especially if resistance allele frequencies are high (toward 10-3), unless the survival of heterozygous larvae on the peas is low (say, 10%).

The strategy investigated to reduce the onset of resistance was the provision of a refuge; the larger the refuge the better (Figure 1). To evaluate this, a field

trial of 100%, 90%, 80% and 0% transgenic peas was run in both 1999 and 2000.

The results indicated that, relative to the non-transgenic plots, population growth rates of weevils were reduced by a factor of 0.11 - 0.13 in the 80% plots, from 0.12 - 0.04 in the 90% plots and from 0.03 - 0.02 in the 100% transgenic plots. However, there was considerable variation between years, such that the average population growth rate across all of the 80-90% refuge plots was 19.4 fold per year, compared to 171 fold per year in the non-transgenic plots (Table 1).

These results suggest that seed mixtures including 10 - 20% non-transgenic seed can significantly dampen population growth, while preserving susceptible genotypes to dilute and slow the evolution of resistance. However, such seed mixtures don't dampen population growth sufficiently to stop pest population growth. Better yet will be to combine the transgenic peas with early harvest of the peas, which prevents many of the larvae from completing their growth. Early harvest alone is not sufficient or practical on farms to control the pea weevils, but the combination of transgenic pea plants and modestly early harvest is (Figure 2).

In summary, modelling studies suggest that resistance can be delayed by the provision of refuges, the larger the better. For these refuges to be acceptable, additional control tactics will still need to be applied. Such tactics may not be sufficiently effective to their own (such as earlier harvest), but could contribute to preventing crop damage in transgenic seed mixtures.

Figure.1. Simulations of the effect of initial resistance allele frequency and proportion of the population in refuge for the evolution of resistance to pea weevil on transgenic peas. Except as noted, the survival of heterozygous (RS) pea weevil larvae on transgenic peas is 50%. All RR homozygous pea weevils are assumed to survive without fitness costs; all Ss homozygous feeding on peas are assumed to die.

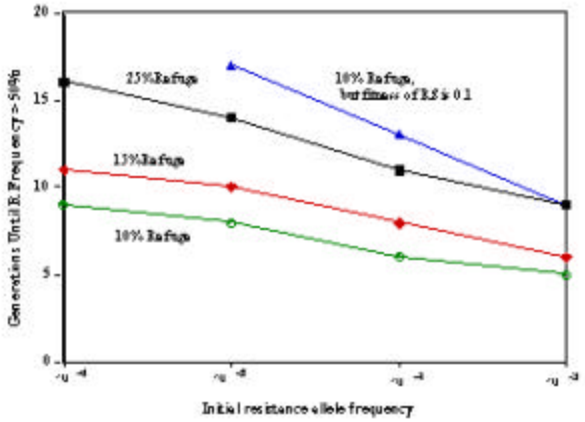
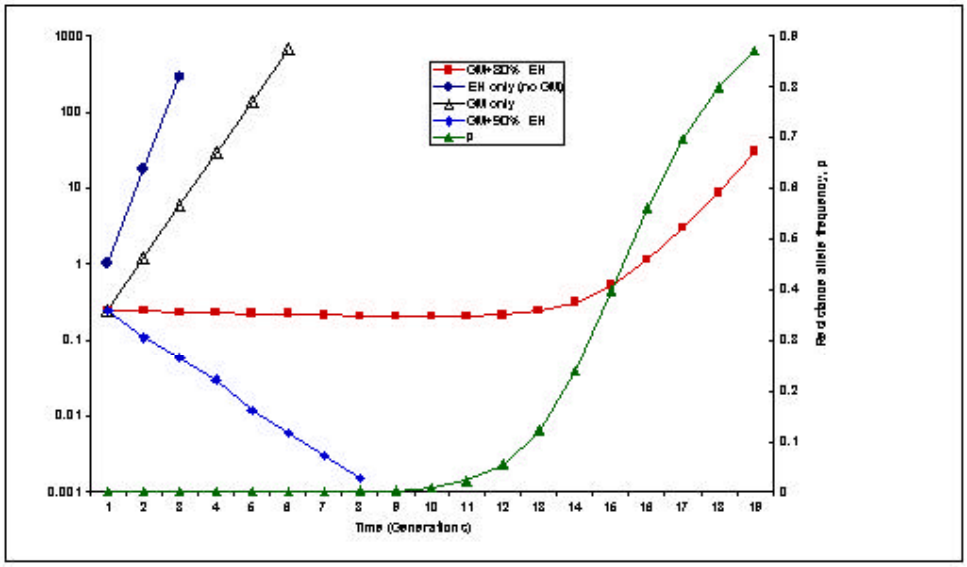


Table.1. Summary of the estimated initial and final pea weevil populations in the different treatment during the two field trials. The ratio of population increase (Ro) and the reduction of Ro in the different treatments is also shown.

Years	Actual % T	Estimated initial number PW /hectare	Estimated adult PW produced /hectare	Ro	Ratio of actual to potential Ro
1999	0%T	2.05×10^4	6.15×10^6	333	1
2000	0%T	1.38×10^5	1.12×10^6	9	1
1999	74%T	1.45×10^4	6.40×10^5	38	0.11
2000	76%T	1.38×10^5	1.68×10^5	1	0.13
1999	85%T	2.45×10^4	6.08×10^5	38	0.12
2000	90%T	2.31×10^5	1.02×10^5	0.4	0.04
1999	100%T	2.49×10^4	1.47×10^5	11	0.03
2000	100%T	1.85×10^5	2.55×10^4	0.2	0.02

Figure.2. Simulated effect of refuge on resistance evolution and pea weevil populations in the presence or absence of additional control method, early harvest, using estimates from Table 1 and assuming an initial resistance allele frequency of 10-6. In the absence of transgenic peas, populations grow quickly (an average of 171 fold per generation averaged across both years), even with early harvest (EH) killing 90% of the larvae ("EH only"). The transgenics alone, with a 10-25% refuge to delay resistance, allowed an average of a 19.4 fold per year increase, but the combination of trangenics ("GM") with 80% or even 90% early harvest mortality kept weevils to a low density or even a declining population.



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ACKNOWLEDGEMENTS: This research was supported by GRC (Grain Research Committee).

M. J de Sousa Majer
Department of Environmental Biology
Curtin University of Technology
Perth, WA, 6845
Australia
and
Agriculture Western Australia
Baron-Hay Court, South Perth, 6151
Western Australia

R. T. Roush
Department of Applied and Molecular Ecology
Waite Campus
The University of Adelaide
Glen Osmond, SA, 5064
Australia

D. C. Hardie
Agriculture Western Australia
Baron-Hay Court, South Perth, 6151
Western Australia

R. Morton and T. J. V. Higgins
CSIRO Plant Industry
Canberra, ACT, 2601
Australia

Fungicide Resistance in Cucurbit Powdery Mildew: Experiences and Challenges

Application of fungicides is presently the principal practice in most cucurbit crops for managing powdery mildew, which is a major production problem in many areas of the world (McGrath et al. 1996). Fungicides that are systemic or have translaminar activity are needed to obtain adequate protection of abaxial leaf surfaces, where conditions are more favorable for development of the pathogen than on adaxial surfaces. Unfortunately, these fungicides generally have a high risk of developing resistance because they have specific modes of action, and powdery mildew fungi have a high potential for resistance development. This has been especially true for the cucurbit powdery mildew fungus, Podosphaera (sect. Sphaerotheca) xanthii (Castagne) U. Braun & N. Shishkoff (also known as Sphaerotheca fusca (Fr.) S. Blumer and S. fuliginea (Schlechtend.:Fr.) Pollacci). It has developed resistance to several fungicide classes, including benzimidazoles, demethylation inhibitors (DMIs), organophosphates, hydroxypyrimidines, Qo inhibitors (QoIs; aka strobilurins), and quinoxalines. Resistance has developed quickly in some situations. Strains resistant to benzimidazoles, DMIs, hydroxypyrimidines or QoIs were detected after only 1 to 2 years of intensive use (Dekker 1977; Ohtsuka et al. 1988). The high frequency of resistant strains detected in Australia in the early 1990s prompted the conclusion that there was a need for control programs less reliant on single site mode of action fungicides (O'Brien 1994).

Currently, alternating between QoIs and DMIs is recommended within the management program for cucurbit powdery mildew where efficacy of the particular fungicides used has not been compromised by resistance. Resistance to DMIs is quantitative. The level of DMI resistance in the United States is such that triadimefon is no longer effective while myclobutanil is effective, but sometimes only when used at the highest labeled rate. Using full rate and maintaining manufacturer's labeled application interval is recommended when resistance is quantitative in order to minimize selection of phenotypes with intermediate fungicide sensitivity. In addition to alternating between two at-risk fungicides with different modes of action (different cross-resistance groups), the fungicide program should also include multi-site fungicides that have a low resistance risk. The current recommendation for cucurbit powdery mildew is to apply a QoI in alternation with a tank-mix of a DMI with a multi-site fungicide. At-risk fungicides should be used only when needed most. The most critical time to use them for resistance management is early in an epidemic when the pathogen population is small. Multi-site contact fungicides should be used alone late in the growing season where they have been shown to provide sufficient disease control to protect yield. A disease threshold approach has been recommended for initiating fungicide applications for powdery mildew (McGrath 1996; McGrath et al. 1996). An alternative approach is to use contact fungicides during early crop growth, when better spray coverage is possible beginning before powdery mildew has started to develop, then include at-risk fungicides after fruit set when powdery mildew begins to develop. This tactic is recommended by the Australian Fungicide Resistance Action Committee. When one crop could serve as a source of inoculum for a subsequent crop, the alternation scheme among at-risk fungicides should be continued between successive crops such that the first at-risk fungicide applied to a crop belongs to a different cross-resistance group than the last at-risk fungicide applied to the previous crop. The management program should also include other practices that minimize the need for fungicides, such as resistant cultivars, when possible.

Considerable effort is being made to obtain information needed to respond proactively rather than reactively to resistance. This includes determining baseline sensitivity. These data are being generated by both private- and public-sector scientists. Pathogen sensitivity also is being monitored after registration. Chemical companies consider these activities to be essential components of product stewardship.

Effectively managing resistance has been and likely will continue to be challenged by many factors. Resistance risk of a new fungicide is difficult to predict. Risk cannot always be predicted solely from the mode of action. Additionally, resistance development in model systems with yeasts or non-obligate pathogens is not always similar to that in obligate pathogens. For example, the QoI fungicides were initially thought to have a low to medium resistance risk and resistance was predicted to be quantitative based on their mode of action (inhibition of respiration) and on results of research with yeast. However, resistance developed quickly and in a disruptive manner.

Identifying the most appropriate resistance management strategy for a fungicide is often challenged by lack of understanding of the mechanism of resistance (e.g., quantitative or qualitative) and of the mode of action (e.g., active pre- or post-symptom). Fungicide rate is important with quantitative resistance.

While a resistance management program for a new fungicide is needed at the time of registration, it cannot be evaluated before pathogen strains with reduced sensitivity have been found. Releasing laboratory-generated resistant strains is very risky because they cannot be contained. Furthermore, evaluation may need to be conducted over several successive crops.

At any given time, effective companion fungicides often have not been available. Registering new fungicides is a lengthy process. At any given time, only one effective at-risk fungicide typically has been available for growers to use. For example, in the United States, the first at-risk fungicide registered for cucurbit powdery mildew was the benzimidazole fungicide benomyl in 1972. When the next at-risk fungicide with a different mode of action, the DMI triadimefon, was registered in 1984, resistance to benomyl was already widespread based on the reduced efficacy and control failures with benomyl that had occurred in several fungicide efficacy experiments. Resistance to triadimefon was widespread when the first QoI fungicide, azoxystrobin, was registered in 1999. For azoxystrobin, an effective at-risk companion fungicide with different mode of action, myclobutanil, was not widely available until 2000. In addition, registrations of some multi-site companion fungicides have been cancelled or are under review in the United States through implementation of the Food Quality Protection Act (FQPA). Others have been lost because the market was too small and thus uneconomical for the manufacturer. This is the reason the main multi-site companion fungicide used previously in Australia, quinomethionate, is no longer registered.

Product withdrawal after resistance development, followed by later re-introduction, is not a viable option for some fungicides. For this management tactic to be effective, resistant strains must be less fit than sensitive strains. However, some resistant strains have been able to persist in the absence of selection pressure from fungicide use. Benzimidazole-resistant strains have persisted for many years after use was discontinued (O'Brien 1994; Bardin et al. 1995). In addition, this tactic is unlikely to be implemented if the target fungicide is highly effective for controlling other diseases on the same crop, as is the case with azoxystrobin which, in addition to powdery mildew, also controls anthracnose, belly rot, downy mildew, gummy stem blight and leaf spots caused by Alternaria and Cercospora.

The labeled rate for a fungicide might not be the best rate to use for delaying resistance development with quantitative resistance. Efficacy experiments to identify rates are usually conducted before fungicides are registered for commercial use. At this time, resistant strains would likely be at too low a frequency to impact performance. The use rate selected for registration of a new fungicide typically is the lowest rate providing consistent control in the efficacy experiments. The lowest effective rate might not be the best rate to use for delaying resistance development as it may permit strains with intermediate resistance to survive.

Cross-resistance is common among fungicides within a chemical group (Schepers 1985; Kendall 1986). Consequently, using one fungicide (e.g., triadimefon) might select for strains less sensitive to another fungicide in the same group (e.g., myclobutanil), thereby affecting its efficacy. Use rates are not based on cross-resistance data for new fungicides belonging to a fungicide group (e.g., the DMIs) to which the pathogen has already developed quantitative resistance.

Because strains have been detected with resistance to as many as four classes of fungicides, it is clear that the cucurbit powdery mildew fungus is capable of thwarting a complex fungicide program (Tsay et al. 1992; O'Brien 1994; del Pino et al. 1999).

The life cycle of the cucurbit powdery mildew fungus needs to be better understood in order to manage resistance and assess resistance risk more effectively. For example, if the fungus survives between crops as cleistothecia or on a weedy reservoir host, then growers need to consider fungicides applied to the previous crop when selecting the fungicide program for the current crop. If verbena or another ornamental plant is an important source of inoculum for powdery mildew epidemics in cucurbits, then fungicides used on this other host, which could include products not yet registered on cucurbits, need to be considered when developing fungicide programs for cucurbits. A product newly registered for cucurbits could be compromised by previous selection of resistant strains on the ornamental host.

With highly mobile pathogens such as cucurbit powdery mildew, successful management may require regional implementation. Otherwise, growers using an at-risk fungicide exclusively may select resistant strains and thereby thwart efforts of growers who are using a resistance management program.

Monitoring pathogen sensitivity to fungicides is useful for documenting shifts. However, current techniques, which entail expensive sampling and laboratory screening, are limited by their inability to detect rare resistant strains.

Fungicide cost and efficacy are greater concerns for growers than resistance. Implementation may be difficult when the resistance management program is more expensive or less effective than using the at-risk fungicide alone full-season. Inexpensive fungicides are likely to be used intensively, whereas expensive fungicides are likely to be used at reduced rates or extended spray intervals. Growers may not be easily convinced to use a multi-site contact companion fungicide with an at-risk fungicide when current-season disease control is not improved. Although addition of a contact fungicide may manage resistance to a highly effective fungicide, it is not expected to provide a detectable increase in disease control in early stages of resistance development, because strains with reduced sensitivity to the at-risk fungicide would be at

too low a frequency in the pathogen population to affect disease development.

Another risk is that development of resistance may be overlooked when a multi-site contact companion fungicide is used. This type of companion fungicide will effectively control any resistant strains on adaxial leaf surfaces, but selection of resistant strains may still progress on abaxial surfaces where spray deposit is poor. This can easily be missed because abaxial surfaces are not readily visible unless leaves are turned over.

Just as many diseases and insect pests are managed after they appear, some growers become concerned about managing resistance only after it has developed. They do not recognize that the primary goal of resistance management is to delay its development rather than to manage resistant strains. Consequently, resistance management programs are not always implemented when at-risk fungicides become available for commercial agricultural use.

Resistance management programs are not enforceable. Therefore, prepacked mixtures are considered the only practical strategy for delaying development of resistance generally (Chin 1987).

Managing resistance with full-rate mixtures is at odds with the public desire to reduce pesticide use. Full-rate mixtures comprise a greater quantity of fungicide than using one at-risk fungicide exclusively at a rate near the MIC for sensitive strains.

IPM tactics that delay applications until after disease detection and extend spray intervals until disease-favorable conditions have occurred may be in conflict with the accepted resistance management tactics of avoiding curative (eradicant) treatments and maintaining recommended intervals. Resistance can develop quickly when fungicides are used curatively. Each IPM tactic needs to be considered in terms of the size of the pathogen population at application and the potential impact on resistance management. Fortunately, the action threshold for cucurbit powdery mildew of one leaf with symptoms per 50 old (most susceptible) leaves is considered to be below the disease level that would correspond to a curative treatment. However, the potential problem of delayed applications needs to be kept in mind if other IPM tactics are considered in the future. For example, the threshold for initiating fungicide applications for carrot leaf blights in Canada is considerably higher, being a disease incidence of 100% for early carrots and 50% for late carrots (Kushalappa et al. 1989). A forecasting system that times applications by predicting future occurrence of conditions favorable for infection would be compatible with resistance management whereas a disease-warning system that alerts users to when conditions were favorable, such as TOM-CAST (Gleason et al. 1995), could result in curative treatment. However, resistance is currently not a concern with either carrot leaf blights or Tom-Cast because chlorothalonil is the primary fungicide being used in these situations. Fungicide mode of action should also be considered. Applications of fungicides that inhibit spore germination, such as the QoIs, should be started earlier in disease development than fungicides with post-infection activity, such as the DMIs.

Although effectively managing resistance will continue to be challenged by biological, economic, and political factors, an understanding of these challenges and the current proactive approach to their resolution being taken ensures that we are now in a good position to address fungicide resistance in cucurbit powdery mildew.

A longer article on this topic was published in March 2001 in Plant Disease 85(3):236-245.

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Margaret Tuttle McGrath
Department of Plant Pathology
Long Island Horticultural Research and Extension Center
Cornell University
3059 Sound Avenue
Riverhead, New York 11901-1098
E-mail: mtm3@cornell.edu
Phone: 631-727-3595

Sensitivity Profiles of *Cercospora beticola* Populations to Several Fungicide Groups in Greece

ABSTRACT In Greece control of sugar beet leaf-spot disease caused by *Cercospora beticola* is mainly achieved by fungicide spray applications. The fungicides used are the fentin-derivatives and the sterol demethylation inhibitors (DMIs) mixed with maneb or chlorothalonil. Additionally benzimidazole fungicides have been used in some areas during the last few years, after an interval of about 15 years, because of the resistance development. Resistance or decreased sensitivity of *C. beticola* populations to some of these fungicide classes has been reported in the past. To prevent disease control failures caused by resistance development, a monitoring programme for sensitivity determination has been carried out yearly. During the autumn of 2000 pathogen isolates were obtained from sugar beet fields of 4 areas of Greece, Imathia, Larissa, Serres, and Orestiada, with different fungicide use history. The sensitivity of these isolates to fentin-acetate, flutriafol, and benomyl was determined by using an in vitro assay at a single discriminatory dose of each fungicide. Sensitivity determination to fentin-acetate, for first time after the detection of resistance in 1978, showed that through the years the resistance frequency was significantly decreased, ranging from 1.9 % in the area of Serres to 8.6 % in the area of Orestiada. This decrease of resistance frequency is related to the decreased competitive ability of fentin-resistant isolates in the absence of selection pressure since fentin applications are carried out only in the beginning of the season, followed by applications of DMIs. In the areas of Larissa and Orestiada no shift toward decreased sensitivity to flutriafol was detected, while in the remaining two, which were more heavily treated with DMIs, the resistance frequency was 10 and 20%, respectively. Significant differences were detected among the four sampled areas regarding the sensitivity to benomyl. These differences reflect to the different benomyl use history among these areas. In the areas of Orestiada and Larissa where benomyl has been used in one spray application per year, after the reintroduction of the fungicide into the spray programm, the resistance frequency had values of 80 and 65%, respectively. In the remaining two areas, Imathia and Serres where benomyl has not been used since the detection of resistance early in 70`s the resistance frequency was significantly lower with values of 23 and 14% respectively.

INTRODUCTION Sugar beet leaf-spot, caused by *Cercospora beticola*, is the most important foliar disease of sugar beet in warm and humid areas of Southern Europe. It can cause serious yield losses in the absence of treatments, ranging from 25 to 50% (Byford, 1996). Control of sugar beet leaf-spot, in Greece is mainly obtained by fungicide treatments, while alternatives, like use of resistant varieties and culture rotation, can merely contribute to successful disease control.

The benzimidazole derivatives were the first systemic fungicides that became available for *C. beticola* control. In Greece commercial applications of benomyl began in 1971 with excellent results but within two years the fungal populations developed resistance and as a consequence fungicide efficacy was completely lost (Georgopoulos and Dovas, 1973). After the emergence of benzimidazole resistance the use of benomyl was discontinued. However, in an extensive monitoring program carried out in 1995 was found that resistance frequency to benomyl had been significantly decreased in comparison to that detected in 1972 and since then, benomyl is used in one or two applications early in the season in some areas of Greece (Karadimos et al., 2000).

Fentin derivatives were the only available fungicides providing satisfactory control of *C. beticola*, after the emergence of benomyl resistance. However, during the 1976 and 1977 growing seasons the control of Cercospora leaf-spot, achieved by fentin fungicides was unsatisfactory and laboratory tests showed that resistance had been developed (Giannopolitis, 1978). Since then the use of fentin derivatives was restricted

to 2 or 3 applications in mixture with maneb, early in the season.

Demethylation inhibitors (DMIs) have been used in Greece, for the control of the disease, since 1979. These fungicides are used always in mixture with a protectant fungicide, either maneb or chlorothalonil. However, after 1990 a reduction in the performance of DMI fungicides was observed in field experiments for fungicide evaluation, on sugar beet, in Northern Greece and an extensive monitoring program showed that fungal populations had shifted towards decreased sensitivity (Karaoglanidis et al., 2000). The current study was conducted in order to determine the sensitivity of *C. beticola* populations to benomyl, fentin-acetate, and flutriafol after the end of the 2000 spraying period.

MATERIALS and METHODS

Sampling Sites:

Four different areas in Northern and Central Greece, Larissa, Imathia Serres, and Orestiada, with different histories of fungicide use, were sampled. During 2000 in all the four areas fentin-acetate mixed with maneb was used in the first 2-3 sprays of the season. DMIs in mixture with maneb or chlorothalonil were applied later in the season in 3-4 spray applications. In the past, Imathia and Serres were the areas received the higher DMIs selection pressure. Additionally in the areas of Larissa and Orestiada one or two benomyl applications, in tank-mixture with maneb, were carried out. The use of benomyl had been discontinued after the emergence of resistance strains in 1972 and reintroduced into the spray programs in 1996 in the area of Larissa and in 2000 in the area of Orestiada.

Pathogen Isolation:

Sugar beet leaves with distinct sporulating lesions were collected, from about ten fields in each sampling area, after the end of the spraying period during October 2000. Single-lesion isolates of *C. beticola* were obtained by transferring conidia, with the aid of a fine glass needle, to Aspergillus Complete Medium (ACM), composed of 20gr agar (Oxoid, Unipath Ltd, Basingstoke, England), 10gr dextrose (Merck, Darmstadt, Germany) and 1gr yeast extract (Oxoid) per litre and acidified with lactic acid in order to suppress bacterial growth. Conidia from only one lesion per leaf were transferred to the culture medium. From each of the four sampling sites, 150 isolates were obtained. After two days of incubation at 25o C, in the dark, the fungal colonies were transferred to fresh ACM. In each petri dish 6 colonies were started and incubated for 10 days at 25^{o} C.

Sensitivity Tests:

Fungicides used in this study were the commercial formulations of benomyl (Benlate 50WP, DuPont Agro Hellas), fentin-acetate (Brestan 60WP, Agrevo Hellas S.A.) and flutriafol (Impact, 12.5 EC, Zeneca Hellas, Greece). Autoclaved ACM was cooled to 50o C and amended with aqueous fungicide solutions at discriminatory concentrations. The discriminatory concentration for benomyl was 1 µg ml-1, a concentration completely inhibitory for the sensitive isolates but not at all for the resistant isolates. For fentin-acetate the isolates were classified in three sensitivity groups, resistant, moderately resistant and sensitive, according to their vegetative growth at 2 µg ml-1. For the measurement of sensitivity to flutriafol isolates 1 µg ml-1 was used as discriminatory concentration. Control petri dishes were not amended with fungicide. Tests for each isolate were replicated twice per concentration of each fungicide.

Mycelial plugs of 5mm diameter were removed from the colony margins and placed upside down on the fungicide-amended and fungicide-free petri dishes and incubated at 25o C, in the dark. Radial growth of each isolate was measured (minus the diameter of inoculation plug) after 7 days by calculating the mean of two perpendicular colony diameters. The mean diameters of colonies were expressed as percentages of the colony diameters in control treatments and the relative growth (RG) was estimated. Chi-square analysis was used to analyze the sensitivity distribution of the populations based on the relative growth of the isolates from the four areas tested.

RESULTS and DISCUSSION

Sensitivity to Benomyl:

Since resistance to benomyl has a qualitative character (Georgopoulos and Skylakakis, 1986), the sensitivity of *C. beticola* was studied with a discriminating concentration. Measurement of sensitivity to benomyl showed that in the two benlate - treated areas, Orestiada and Larissa, resistance frequencies had values of 80 and 65%, respectively (Fig.1). Conversely, the resistance frequency in the remaining two areas, Imathia and Serres was significantly lower with values of 22 and 15%, respectively (Fig. 1). In a previous monitoring program, carried out during 1995-1996, had been found that resistance frequency to benomyl had been decreased to values of 20-25% (Karadimos et al., 2000). According to the findings of the current study in

the two areas in which benomyl have not been reintroduced into the spray program resistance frequency continues dropping slowly, despite the predictions for a stable resistance frequency due to the equal competitive ability of resistant and sensitive strains (Dovas et al., 1976, Ruppel, 1975). A similar decrease of benomyl resistance frequency has also been reported for *Mycosphaerella fijiensis* in banana (Smith, 1988). Converserly the values of resistance frequency in the areas of Larissa and Orestiada were extremely high and reached the levels of resistance frequency in the early 70`s when for first time resistant to benomyl had been detected (Georgopoulos and Dovas, 1973), suggesting that even one spray application of benomyl can cause a significant increase of the resistant subpopulation. Such results suggest that the reintroduction of benomyl into the spray programs can hardly be suggested for the control of sugar beet leaf-spot.

Table.1. Summary of the estimated initial and final pea weevil populations in the different treatment during the two field trials. The ratio of population increase (Ro) and the reduction of Ro in the different treatments is also shown.

Years	Actual % T	Estimated initial number PW /hectare	Estimated adult PW produced /hectare	Ro	Ratio of actual to potential Ro
1999	0%T	2.05×10^4	6.15×10^6	333	1
2000	0%T	1.38×10^5	1.12×10^6	9	1
1999	74%T	1.45×10^4	6.40×10^5	38	0.11
2000	76%T	1.38×10^5	1.68×10^5	1	0.13
1999	85%T	2.45×10^4	6.08×10^5	38	0.12
2000	90%T	2.31×10^5	1.02×10^5	0.4	0.04
1999	100%T	2.49×10^4	1.47×10^5	11	0.03
2000	100%T	1.85×10^5	2.55×10^4	0.2	0.02

Sensitivity to Fentin-acetate:

In the current study was measured for first time after the emergence of resistance the sensitivity of *C. beticola* populations to fentin-acetate, in Greece. For the measurement of sensitivity was used the discriminatory concentration suggested by Giannopolitis (1978). Results showed that in all the three areas sampled the resistance frequency has been significantly decreased in comparison to the levels of resistance detected by Giannopolitis (1978). In all the three areas the percentage of resistant strains was lower than 10% while had a value of 29% when for first time resistant had been detected (Table 1). The lower resistance frequency is related to the fact that after the emergence of resistance, fentin fungicides are used only in two or three spray applications always early in the season (Ioannidis, 1994; Ioannidis and Karaoglanidis, 2000). Such a tactic allows the elimination of the resistant strains selected under the selection force of the fentin fungicides, since in the absence of fentin fungicides the resistance strains do not compete well with the sensitive strains (Giannopolitis and Chrysayi-Tokousbalides, 1980).

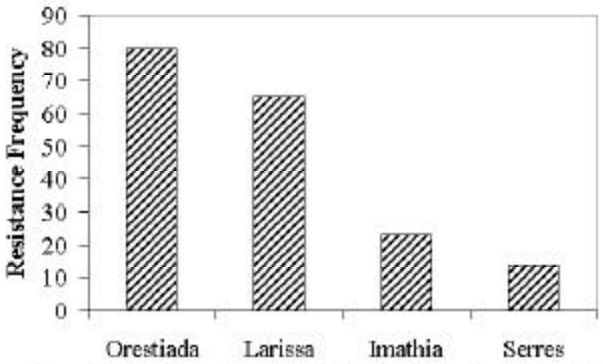

Figure 1. Resistance frequency (%) to the benzimidazole fungicide benomyl of *Cercospora beticola* populations in 4 areas of Greece

Sensitivity to Flutriafol:

Resistance to flutriafol was measured by using a discriminatory concentration despite the fact that resistance to DMIs has a quantitative character (Georgopoulos and Skylakakis, 1986). Such a procedure has also been accomplished for the measurement of sensitivity to DMIs in several other pathogens (Smith et al., 1991; Peever and Milgroom, 1992, Romero and Sutton, 1997) but also for the measurement of the sensitivity of *C. beticola* to bitertanol (Georgopoulos, 1987). The concentration of 1 μg ml-1 flutriafol was selected since has been found that RG values at this concentration have the higher correlation factor with the EC50 value of each individual isolate (Karaoglanidis, 2000).

Results showed that sensitivity distributions of the *C. beticola* populations in the areas of Imathia and Serres have been clearly shifted towards decreased sensitivity while in the remaining two areas, Larissa and Orestiada, the populations are more sensitive (Fig 2.). Differences among the sampled areas are correlated to the DMIs application history of each area. Imathia and particularly Serres areas were heavily treated with DMIs for about 20 years while in the other two areas DMIs were used less often. However, resistance frequencies detected in the current study in the areas of Imathia and Serres were lower compared to those found in a previous monitoring program carried out for a 4-year period (1996-1999) in the same areas (Karaoglanidis et al., 2001). This lower resistance frequency could be correlated to the elimination of the number of DMI spray applications from 5-6 to 3 spray applications per season.

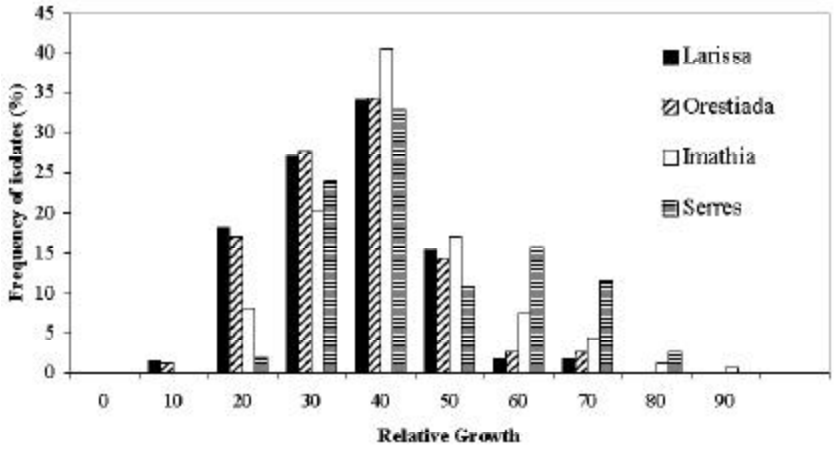

Figure 2. Sensitivity distribution of *Cercospora beticola* isolates according to their relative growth at 1 μg ml^{-1} flutriafol.

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Ioannidis, P. M. and Karaoglanidis, G. S.
Hellenic Sugar Industry S.A.
Plant Protection Department
59032, Platy Imathias
Greece

Ioannidis, P. I. and Karadimos, D. A.
Hellenic Sugar Industry S.A.
Plant Protection Department
41110, Larisa,
Greece

*Author for correspondence:
D. A. Karadimos
Hellenic Sugar Industry S.A., Plant Protection Department
41110, Larisa, Greece
e-mail address: karajim@otenet.gr

Sethoxydim Resistance: A Point Mutation in the *Alopecurus myosuroides* ACCase Gene

INTRODUCTION: Two of the most important classes of herbicides used in the control of *Alopecurus myosuroides* (black-grass) are the aryloxyphenoxypropionates (AOPP/ 'fops') and the cyclohexadiones (CHD/ 'dims'), which act specifically on grass-weeds by inhibiting the plastid-localised, multi-domain acetyl-coenzyme A carboxylase (ACCase: E.C. 6.4.1.2), a key enzyme in the regulation of fatty acid biosynthesis (1). Resistance to the CHD herbicide sethoxydim has been demonstrated to result from an alteration in the target ACCase (2), and populations of *A. myosuroides* from the UK have been observed to have a proportion of individuals possessing insensitive ACCase. A preliminary report by Zhang & Devine (3) identified a point mutation, in the region of the plastidic ACCase gene encoding the carboxyl transferase (CT) domain of a sethoxydim resistant biotype of *Setaria viridis*, which conferred an isoleucine to leucine substitution. This has also been confirmed by Délye et al. (4), and a similar change has been reported in the ACCase genes of sethoxydim resistant *Avena fatua* (5) and *Lolium rigidum* (6). The purpose of the present study was to examine the corresponding regions of *A. myosuroides* plastidic ACCase genes from herbicide sensitive and resistant plants for the presence of resistance specific mutations.

MATERIALS and METHODS: Three populations of *A. myosuroides* were used: a standard sensitive population, Rothamsted (Roth.), and the sethoxydim resistant populations: Notts. A1 and Lincs. E1, which have been previously identified by enzyme assays to contain individuals with sethoxydim-insensitive ACCase (7). Three hundred plants from each population were grown in single pots in a heated glasshouse (17°C for 10 h light, and 14 h dark at 11°C). At the two-to-three leaf stage, plants were cut at soil level, the tissue was snap frozen in liquid nitrogen and the plants were allowed to re-grow for three weeks before being sprayed with sethoxydim, at a rate of 290g a. i. ha-1, using a laboratory sprayer. Twenty-eight days after spraying the plants were assessed visually as either: sensitive, intermediate, or resistant, and DNA and RNA extracted from five single plants for each population and resistance group category. Degenerate primers were designed from maize and wheat ACCase gene sequences (from the Genbank Entrez database, accession numbers: U58598, Z24449, and AF029895, U39321, respectively) corresponding to the region of the *S. viridis* ACCase gene in which Zhang & Devine (3) had identified a point mutation associated with herbicide resistance. Forward primer sequences were: Primer A 5'GAW ARY GGW GAA AYY AGR T3', and Primer B 5'TGG RTT RTT GAY WCY RT3'; reverse primer sequences were: Primer C 5'AAT GMA GHG TAA ATG TCT C3', and Primer D 5'GAR TAR GCA CAC TGG CAA T3'. PCR and RT-PCR methods were used to amplify the corresponding region of the A. myosuroides ACCase gene (7).

RESULTS and DISCUSSION: After spraying, all the plants from the Roth. population were killed and therefore categoris ed as sensitive. The Notts. A1 population was found to contain a mixture of sensitive and highly resistant plants, with 86% of the population resistant. The Lincs. E1 biotype comprised of three categories of individuals: sensitive (85%), resistant (4%) and intermediate (11%). The gDNA and cDNA sequences from the Roth. and Notts. A1-sensitive plants had an A, conferring an isoleucine, in the CT domain of the ACCase gene, which had previously been associated with herbicide sensitivity (3). For the five Notts. A1-resistant individuals each sequence had a T substitution, conferring a leucine. There was no evidence for the presence of heterozygotes in either the Roth. and Notts. A1 populations. All of the individuals from the Lincs. E1 population carried the leucine substitution, consistent with the fact that they are not killed by sethoxydim. The sequences from the sensitive and intermediate plants, however, showed that most are heterozygous for the mutation, possessing both the leucine and isoleucine encoding sequences present in either the gDNA or the cDNA. Since plants from both the intermediate and sensitive groups were found to be heterozygous it is possible that variation in expression of the two alleles could explain why some individuals are completely sensitive and killed by the treatment, whilst others are only partially affected.

CONCLUSIONS: These results confirm that sethoxydim-resistance in *A. myosuroides* is associated with the CT domain leucine substitution previously found in resistant S. viridis (3, 4), *A. fatua* (5) and *L. rigidum* (6) (Fig. 1), and adds to the evidence for a functional role for this mutation. There were no other differences found within this region of the ACCase gene, however given the varying cross resistance patterns seen from different populations it is likely that other mutations may exist in other regions of the ACCase gene. This is the first time that this mutation has been reported in populations of *A. myosuroides*.

In the present study only a small region of the *A. myosuroides* ACCase gene has been examined and one cannot rule out the possibility of other mutations being

Figure 1: Comparison of findings from *A. myosuroides* with other sethoxydim-resistant grass weeds

Key: S= sensitive, R= resistant.

involved in the production of insensitive enzyme. However, in *S. viridis* it has been shown that the corresponding mutation is not accompanied by other changes and it is speculated that the single isoleucine to leucine substitution can either reduced herbicide binding or affect access to the target site (3). It is also clear from the mutagenesis work of Zagnitko et al. (6) that changing the leucine back to isoleucine returns the sensitivity of the wheat ACCase. Christoffers et al. (8) found other mutations present in the ACCase genes from fenoxaprop-resistant *A. fatua*, and the presence of additional mutations could explain the complex resistance profile observed to AOPP and CHD herbicides in other populations of *A. myosuroides* and *A. fatua*. This will be the subject of future studies.

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Figure 1: Comparison of findings from A. myosuroides with other sethoxydim-resistant grass weeds Key: S= sensitive, R= resistant.

Amanda Brown
IACR-Rothamsted
Harpenden, Herts. AL5 2JQ
United Kingdom

Correspondence:
The Wolfson Institute for Biomedical Research
University College London
Gower Street London WC1E 6BT
United Kingdom
Amanda.Brown@ucl.ac.uk

Stephen Moss
IACR-Rothamsted
Harpenden, Herts. AL5 2JQ
United Kingdom

Zoe Wilson
University of Nottingham
Sutton Bonington Campus
Loughborough, Leics. LE12 5RD
United Kingdom

Lin Field
IACR-Rothamsted
Harpenden, Herts. AL5 2JQ
United Kingdom

Fenoxaprop-Resistant Wild Oat (*Avena strerilis*) Biotypes in Turkey

INTRODUCTION Wheat, which covers 9.5 million ha, has been traditionally an important crop in Turkey where agriculture maintains its importance economically and socially. The Çukurova Region

(Eastern Mediterranean area), is one of the foremost agricultural areas of Turkey. Adana province is the major part of Çukurova Region that produces 6-8% of the wheat grown in Turkey.

Wild oat (*Avena spp*.) infests almost all regions of Turkey. Kadioglu (1989) identified three wild oat species in the Çukurova Region, with *A. sterilis* the most common species. He counted up to 315 wild oat shoots/m2 in some fields. Uygur et al. (1993) reported that wild oat had been found in 86% of surveyed fields in the mid 80's; it was seen in almost all fields in the early 90's. On the other hand, the overall average density of wild oat decreased over the same period from 32 to 21 shoots/m2. Chemical control of wild oat have been used since the 1970's (KKGM, 1987), with graminicides use on less than 5000 ha in wheat and some barley fields. At present, graminicide are applied on more 200,000 ha. In earlier years, flamoprop and difenzequat were in use but were replaced with diclofop in the 1980's. Fenoxaprop has been one of the most common herbicides for the last decade. Clodinafop use has been increasing due to increased infestation with *Phalaris spp*. In addition to these herbicides, imazamethabenz and tralkoxydim are recommended in cereals in Turkey (KKGM, 1999) but not widely used in the Çukurova Region.

In spite of worldwide distribution of herbicide-resistant weeds (Heap, 2000), there was no study on weed resistance to herbicides in Turkey, except for a few attempts to confirm trifluralin resistance in *Setaria verticilliata* (Demirci and Nemli, 1997; Uzun and Topuz, 1997). The first claim of resistance came from a farmer who had grown wheat for over 30 years in the same field and had used graminicides since they were introduced to Turkey. A preliminary experiment conducted in 1999 showed that wild oat collected in 1998 was resistant even at 4X the recommended rate of fenoxaprop. In this paper we verify fenoxaprop resistance in wild oat.

MATERIALS AND METHODS Before seed shedding in May 2000, wild oat seeds were collected from 20 fields from plants that survived fenoxaprop or clodinafop application during 1999-2000 cropping season. Wild oat seeds were also collected from two untreated areas to serve as control populations. Eight seeds from each population were dehulled, and soaked in 0.1% KNO3 solution for twodays, before planted in a pot containing potting medium. The pots were placed in a tarp covered greenhouse, which had natural light and temperatures of the local winter.

At 2 to 3 leaf stage, oat seedlings were thinned to 5 plants/pot and fenoxaprop was applied POSTat 0.25X, 0.5X, 1X, 2X, 4X, and 8X the recommended field rate (45 g a.i./ha). Four weeks after application, plant shoots were harvested, dried at 60ºC and weighed. Experimental design was RCBD with 4 replications. The experiment was repeated twice and the data from two experiments were combined. Dose response curves were obtained using log logistic model (Seefeldt et al., 1995) on dry weight data, and the ED50 value (herbicide concentration causing 50% growth inhibition) was calculated for each population. Comparisons were based on differences between possible resistant and susceptible (wild type) populations.

RESULTS AND DISCUSSION ED50 values and field histories of the last 10 years for some populations are presented in Table 1.

In order to identify the resistant populations, we arbitrarily decided that all populations conferring R ratio values below 2, will be considered susceptible. Seven populations were identified as resistant to fenoxaprop (Table 1). It is evident that these populations were collected from fields which have had wheat as a monoculture and continuous FOP herbicide applications. Populations KRL1 and CYL were also collected from a wheat monoculture without being resistant to fenoxaprop. The possible reason for this

Table 1. ED50 values, resistance ratios and crop and herbicide use histories of various wild oat populations

Populations	ED50 (g/ha)	Ratio*	Field History
AKR1	201	5.0	Continuous wheat and FOP
AKR2	134	3.4	Continuous wheat and FOP
BLG	55	1.4	Cotton in every 3-4 years
BTP1	62	1.6	Cotton or watermelon in every 3 years
BTP2	46	1.2	Cotton or watermelon in every 3 years
CBY	51	1.3	Wheat-lentil rotation, continuous FOP/DIM application
CYL	52	1.3	Continuous wheat and FOP
DZC	2605.77	6.5	
GKY1	>360	>8.0	Continuous wheat and FOP
GKY2	62	1.6	
GKY3	>360	>8.0	
GRD	50	1.2	
HZL	44	1.1	Wheat-cotton rotation
KMP	155	3.9	Continuous wheat and FOP
KMT	95	2.4	Continuous wheat and FOP
KRL1	48	1.2	Continuous wheat and FOP
KRL2	41	1.0	Cotton in every 3 years
KRL3	52	1.3	Barley or oat in some years
MKU	50	1.2	Rotation with different crops
SAL	56	1.4	
Susceptible	40	1.00	No herbicide treatment

* Ratio between the population's ED50 divided by the ED50 of the susceptible population.

discrepancies might be wrong information supplied by the farmer.

These results demonstrate for the first time that fenoxaprop-resistant wild oat has evolved in Turkey. Our observations show that the problem has been increasing and is closely associated with monoculture and lack of awareness among the farmers and extension personnel. Further studies are in progress to test for possible evolution of resistance to other herbicides in wheat.

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Ahmet Uludag
Plant Protection Research Institute
01321 Adana
Turkey

Yildiz Nemli
Ege University
Izmir
Turkey

Baruch Rubin
Hebrew University
Rehovot
Israel

Research in Resistance Management

Evidence of the shift in susceptibility to *Bacillus thuringiensis* delta-endotoxin CryIAc in Australian *Helicoverpa armigera* (Lepidoptera: Noctuidae)

INTRODUCTION *H. armigera* and *H. punctigera* are important pests of cotton in Australia. Transgenic cotton (producing CryIAc protein) has been commercially grown in Australia for six years. The susceptibility of both species to *Bacillus thuringiensis* (Bt) toxins in Australian field populations has been monitored since 1993.

Three Bt commercial products containing different proteins have been used for testing the survivorship of *Helicoverpa spps* larvae, namely Dipel(R), Xentari(R), and MVP(R) (Table 1). The results of monitoring against Dipel(R) and Xentari(R) have not been changed to date. However, the survivorship of a *Helicoverpa spps* larval tested against MVP (contains CryIAc, Table 1) significantly increased during the early part of the 2000/01 cotton season, especially *H. armigera*.

This paper reports the results of Bt resistance monitoring to date and evidence of the shift in the susceptibility with possible esterase mediated mechanism of resistance in *H. armigera*.

Table 1: Products tested against *H.armigera* and *H.punctigera* in the Bt Resistance Monitoring Survey(DD=Discriminating Dose)

Dipel® (DD=2mg/ml)
Cry 1Aa, Cry 1Ab, Cry 1Ac, Cry2A, Cry2B, spore

Xentari® (DD=2mg/ml)
Cry 1Aa, Cry 1Ab, Cry1C, Cry1D, Cry 2B, spore

MVP/MVP2® (DD=3ul/ml)
Cry 1Ac encapsulated in dead *seudomonas flourescens* cells

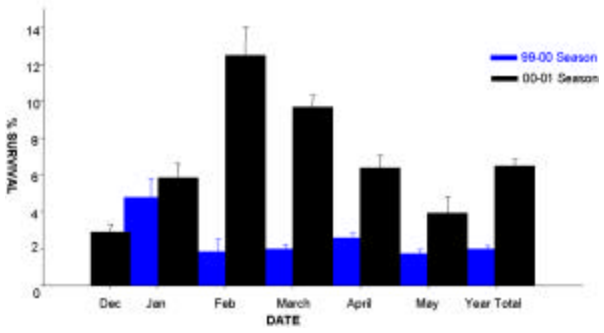
MATERIALS AND METHODS

1. Bioassays in resistance monitoring program

Helicoverpa eggs were collected throughout the season in cotton and other summer crops in cotton growing regions in Australia. Eggs were then transferred onto an artificial diet, hatched, and maintained to early third instar larvae. Larvae were then, transferred onto a "testing diet" into which a discriminating dose of the Bt product had been incorporated (Fig 1). Dipel(R), Xentari(R), and MVP(R) were used for screening (Table 1). Larval mortality was assessed after seven and ten days of exposure to the testing diet, respectively. The discriminating dose for each product was the LC99 for tested species. Survivorship of larvae on diet incorporated with the discriminating dose of Bt products was monitored.

In collaboration with Monsanto Australia, mortality was assessed using larvae hatched from the over wintering pupal populations collected in August, 2001.

Figure 1: Percent survival of *H. armigera* (MVP – 3ul/ml)



2. Preliminary study on genetics of resistance

Since February, 2001, the survival of field populations *H. armigera* was found to be significantly increased. Experiments were carried out to investigate the possible mode of inheritance of the resistant trait.

H. armigera larvae surviving the discriminating dose of MVP (3ul/ml diet) were removed from the "testing diet" and maintained on the feeding diet (without MVP) until pupation. Surviving moths were bred to produce selected resistant strains (called Silver). Probit analysis was run on bioassay results of various generations of the Silver strain in comparison with that of the susceptible laboratory strain and field non-selected strain.

Silver F4 female moths were used for crossing with male moths of the susceptible strain (KO) to produce the hybrid strain (Silko F1). Silko F1 was bioassayed in comparison with other strains.

3. Assessment of resistance on whole plant and in field trial

3.1 Whole plant bioassays

Larvae from resistant, Silver (F4), and KO strains were placed on caged plants under greenhouse conditions. Temperature was maintained at 20/30C for night and day. Relative humidity was

maintained at 50-80%. Two neonates were introduced into each caged plant at 50 days after seeding (squaring stage). Larval and pupal survivorship monitored and pupal weight recorded.

3.2 Field performance of transgenic cotton

During the commercial cotton season 2000/01, in collaboration with Delta Pine International and Cotton Grower Services (CGS), a trial was conducted to monitor the efficacy of commercial transgenic cotton varieties throughout the crop season.

One-gene transgenic varieties were planted together with the conventional (non-Bt) and two gene varieties in the field trials. The varieties were planted in randomised blocks and replicated four times. Each variety was planted in four 20-metre rows for each replicate. Ten leaves were collected randomly from each replicate for each variety for bioassay. They were newly opened leaves at the top node of the plant. Bioassay was carried out weekly starting from five weeks after seeding through to maturity.

The insects used in this experiment were field collected from the southern districts of Queensland, Australia in October/November, 2000.

One day fed neonate was placed with one leaf into the air-tight Falcon(R) petridish and mortality was assessed at five days after.

Table 2: Summary of resistance monitoring survey 1996 - 2001

Bt Product	Year/1	*H. armigera*		*H. punctigera*	
		% Survival	Number Tested	% Survival	Number Tested
Dipel®	1996/97	0.3	6149	0.5	1788
	1997/98	0.7	7580	1.3	1699
	1998/99	0.6	9974	1.4	974
	1999/00	0.7	14295	0.2	1496
	2000/01	1.0	5143	0.5	1393
Xentari®	1996/97	0.4	4980	0.5	1155
	1997/98	0.2	3130	0.4	974
	2000/01	0.8	3698	0.6	1059
MVP®	1997/98*	0	2575	0	1217
	1999/00**	2.6	11275	2.3	1884
	2000/01**	7.1	11572	3.7	4385

* Data based on screening with neonates
** Data based on screening with 3rd instar larvae
1/Results of 96-99 survey from Dr N W Forrester.

Table 3: Percent survival of *H. armigera* field and laboratory strains tested in september 2001[1]

Insect Colony[2]	No. Tested	% Survival
Thebo	308	9.09
Kununurra	162	8.64
Agriland	256	16.8
Warren	330	4.55
Boggabri	42	4.76
Dalby	481	11.23
SF4–Lab strain	96	43.7
KO–Lab strain	72	0

1. Third instar larvae tested with diet containing MVP (3 ul/ml diet), mortality assessed at 10 days after introduction.
2. Field strains are offspring of pupae collected from cotton fields in august, 2001. Laboratory strain: SF4 (Silver F4, resistant strain) and KO (susceptible strain).

4 Resistance mechanism studies

The Envirologix test kit was used to conduct a preliminary analysis for the presence of toxin CryIAc in surviving and dead larval from the MVP screen. The result revealed that dead larvae contained higher levels of CryIAc than surviving larvae indicating that there was difference in the rate of detoxification in the individuals Subsequently, we investigated the difference in esterase activity in susceptible and Silver F2 strain.

Homogenates from mid-gut of susceptible (KO) and Silver F2 *H. armigera* larvae were incubated with concentrations of purified CryIAc. Total esterase activity was detected using Ł-naphthyl acetate as a substrate. Incubates was then run on polyacrylamide gels and stained for esterase activity.

RESULTS AND DISCUSSIONS

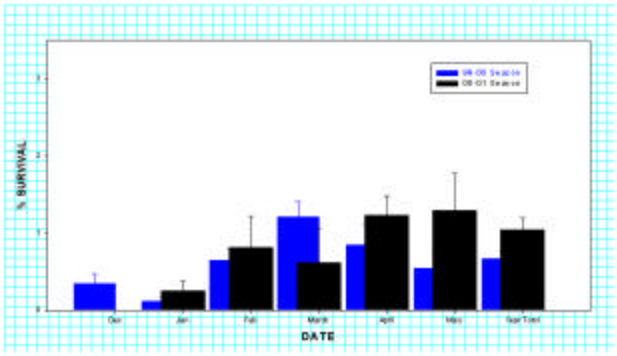
1. Result of Bt resistance monitoring program

The percentage survival of *Helicoverpa armigera* and *H. punctigera* against the discriminating dose for each product form 1996/97 to 2000/2001 seasons is presented in Table 2. Monitoring for Dipel(R) resistance has been conducted since 1996 and there has been no change in susceptibility of field populations to this product. A similar result was obtained for Xentari(R). On the other hand, survival of both species to MVP(R), which contains the single Bt toxin (CryIAc), increased significantly. Average survival of *H. armigera* against

MVP(R) for the crop season 1999/2000 and 2000/01 was 2.6% and 7.1% respectively. Bioassays of *H.armigera* field collections conducted in August, 2001 by Monsanto across six cotton growing regions indicated that the survival had increased to 9.2% (average of six regions - Table 3)

The differences in survival for *H. armigera* against MVP(R) and Dipel(R) in the two years 1999/2000 and 2000/01 are shown in Fig 1 and 2.

Figure 2:Percent survival of *H.armigera* (Dipel – 2mg/ml)



A closer examination of the increase in survival of *H. armigera* during 2000/01 (Fig. 1), indicates a significant increase in *H. armigera* survival in February and March. In later months of the crop season, the survivorship declined. The extent of *H. armigera*'s survival appears to be related to the to the level of Bt expression in transgenic cotton throughout the season.

Table 4: LC99, LC50 and Resistance Factor (RF) of selected ,cross and field strains of *H. armigera* tested against MVP

Strain	Selection	LC99	LC50	RF*
Silver F1	+	201	3.099	118 fold
Silver F2	+	321	4.033	187
Silver F4	-	860	1.674	503
SILKO(SF4 X KO) F1	-	73	.431	43
KO(Susceptible strain)	-	1.71	0.084	1
Emerald (Field strain)	-	132	1.303	77

2. Inheritance of resistance in selected strain Table 4 shows the LC50, LC99 and resistance factor of different Silver generations (selected strains), field, susceptible strains, and the cross between susceptible and selected strain (SilkoF1).

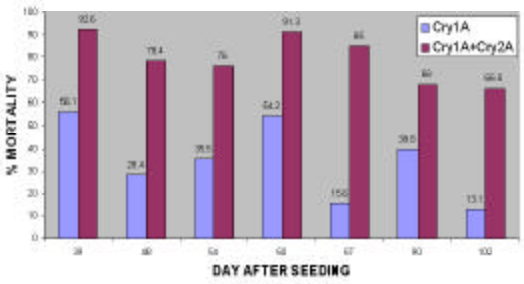
The resistance factor increased as the strains being selected and reduced when crossed with susceptible strain. The resistant trait proved to be heritable characteristic.

3. Larval survival on whole plant and in field trial Table 5 shows the survivorship from neonates to pupae of Silver F4 (selected strain) and KO (susceptible strain) on caged plants under greenhouse conditions. Larvae of KO strain died after the first week of exposure, as compared to 10.75% of larvae of Silver F4 strain which survived through to pupation.

In the field trial, the larval mortality of field collected strain on single gene Bt transgenic varieties (average of four varieties) was lower than that on two-gene variety (Fig. 3). The mortality was corrected using mortality on non-Bt conventional variety. It is apparent that the single gene transgenic varieties had reduced efficacy against the *H. armigera* field collected insects.

Leaf samples assessed for CryIAc content

Fig.3 : Mortality of *H. armigera* larvae tested on transgenic cotton leaves .



(Envirologix Test Kit) indicated that CryIAc was present at high concentration until the plants were about 100 days old. However, mortality of insects was very low even at the start of the season. At the peak of Bt expression in single gene transgenic varieties (at 48 and 54 days after seeding -DAS), there was 64% and 53% reduction in efficacy as compared to two-gene variety. If the field collected strain was susceptible to CryIAc, such reduction in efficacy would not be expected. Thus, the field strain used in this experiment which was collected in October/November, 2000 might have been less susceptible to CryIAc.

4. Resistance mechanism - Esterase sequestration in resistant strain

Polyacrylamide gels developed from this study showed that the selected resistant strain had greatly increased esterase activity (Fig 4) and there were considerable differences in esterase banding patterns between these strains. Data in Fig 5 show that CryIAc inhibited esterase activity in the resistant strain, where as there was no detectable binding of CryIAc to esterase in the susceptible strain.

Esterases are enzymes in *H. armigera* and other insect pests, which detoxify many insecticides by hydrolysis and sequestration. Sequestration by esterases has been characterised as the primary cause of pyrethrod resistance in Australian *H. armigera*.

Table 5: Percentage survivorship of *H. armigera* on greenhouse plants[1]

Insect	Plant Type[2]	No. of Plants	Larval Survival		Pupal Survival	Pupal Weight (mg)
			7 DAI[3]	14 DAI		
Silver F4	Ingard	200	25.50	13.25	10.75	325.1
Silver F4	Convention	40	92.0	80.0	55.0	327.1
KO	Ingard	40	1.25	0	0	-
KO	Convention	40	82.0	78.0	57.0	304.5

1. Two first instar larvae were introduced to each caged plant at 50 days old.
2. Ingard: Sioct 289i, Convention: Sicot 189

3.DAI=Day after introduction .

Figure 4: Polyacrylamide gels showing effects of Cry1Ac on esterase activity in Cry1Ac -susceptible and Cry1Ac-resistant Australian *H. armigera*

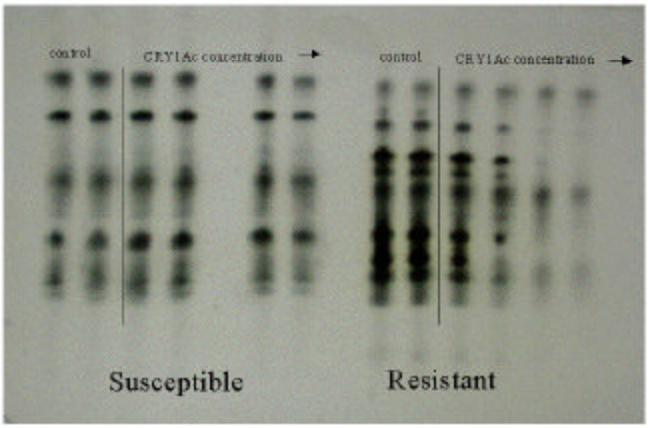
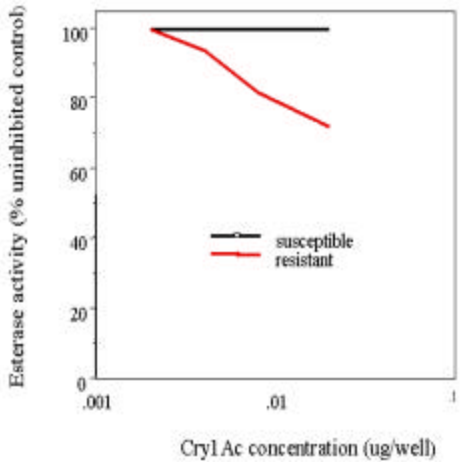


Figure 5: Inhibitory effects of Cry1Ac on total esterase activity in Cry1Ac -susceptible and Cry1Ac-resistant Australian *H. armigera*



Moores (IACR, Rithamsted, UK), has shown that activity of esterase in the gut of the Bt resistant *H. armigera* strain binds readily for the CryIAc pro-toxin. Given the greatly increased esterase activity in the resistant strain, it is likely that considerable amount of CryIAc pro-toxin could be sequestered before reaching the target site. Our preliminary data in comparing the extent of CryIAc sequestration in various *H. armigera* strains showed that levels of sequestration are decreasing in the following order (1) Silver F2 (resistant strain), (2) Emerald (field strain), (3) SF4 x KO (cross between resistant and susceptible strain) and KO(susceptible strain). Similar order is also observed for the resistance factor of these strains (Table 4) , Thus, the level of esterase sequestration is closely related to the resistance factor found in probit analysis.

SUMMARY Ingard(R) cotton provides a valuable tool for the management of *Helicoverpa spps* in the Australian cotton industry. Maintaining susceptibility of insect populations to Bt proteins as well as to new chemistries is essential for the industries sustainability. The actual impact of efficacy of transgenic crops due to development of resistance is difficult to measure. The current change in *H. armigera* susceptibility would not be detected by commercial field checks for *Helicoverpa* infestations. Also, any loss of efficacy in transgenic crops would be difficult to establish as the majority of transgenic cotton crops in Australia are regularly sprayed with larvicides after flowering. The use of non-Bt conventional insecticides in transgenic crops would mask the reduced efficacy.

The evidence of the shift in susceptibility demonstrated in our studies might have impact on the field performance of one gene transgenic varieties. Further research focussing on the assessment of field performance of Ingard cotton would be required to ascertain this possibility. Such study will determine not only the impact of the shift in susceptibility on the field efficacy of the Bt toxin in one gene transgenic crop but also effect on the efficacy of the future two-gene transgenic cotton. While the mechanisms of Bt resistance in *Helicoverpa* field populations are not well understood, enzymatic

sequestration of toxin recognised by Bt research workers as a potential resistance mechanism. In the case of *H. armigera*, it is likely that considerable amount of CryAc pro-toxin is being sequestered. Esterase sequestration however might be only one of a number of mechanisms involved in Bt resistance.

A number of the assumptions that was made in the development of the current resistance management strategy for Bt cotton have proved to be unsubstantiated (Daly and Olsen, 2000; Tabashnik et al, 2000). Improving our understanding of the mechanisms and genetics of resistance to CryIAc and new Bt toxins is seen as a priority in the development of future resistance management strategy.

ACKNOWLEDGMENTS: We are thankful to the Cotton Research and Development Corporation (CRDC-Australia) for funding this research project. Thanks to Dr Neil Forrester (Deltapine International) and Mr Philip Armitage (Cotton Grower Service) and Mr Stewart Addison (Monsanto Australia) for their assistance in various studies. Many thanks to all members of the team including Mrs J.Chapman, Mrs C. Dang, Mrs D. Richardson and Mrs K. Stanford for the bioassays.

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Ho.T Dang
Australian Cotton Research Institute
NSW Agriculture
Wee Waa Road
Narrabri
Australia
hod@mv.pi.csiro.au

Robin Gunning
NSW Agriculture
Calala Lane
Tamworth
Australia
robin.gunning@agric.nsw.gov.au

Abstracts

Sensitivity of *Phytophthora infestans* Isolates from Northern Ireland to Phenylamides and Fluazinam

In Northern Ireland, potato late blight is controlled using a wide range of fungicides. One-three applications of formulations containing phenylamides are applied to up to 50% of the crop area. The non-systemic fluazinam, introduced as a single active ingredient product in 1994, is currently applied to *c.* 20% of the crop area.

The Northern Ireland *Phytophthora infestans* population was characterised for phenylamide resistance and fluazinam sensitivity and for markers including mating type, allozyme genotype and mitochondrial DNA (mtDNA) haplotype. Phenylamide resistance was tested using the floating leaf disc method and fluazinam sensitivity by a zoospore motility assay.

In the period 1995-2000, all isolates save one were of the A1 mating type. Phenylamide-resistant strains were present in c. 30-60% of isolates. There was a marked association between mtDNA haplotype and phenylamide resistance. Of the two common haplotypes (Ia and IIa), haplotype IIa predominated (in contrast to other parts of Europe) and was associated with sensitivity to metalaxyl, whilst haplotype Ia was associated with metalaxyl resistance. Haplotype IIa was less frequent in the year 2000 and this was accompanied by increased occurrence of isolates containing phenylamide-resistant strains.

Northern Ireland isolates proved very sensitive to fluazinam with most inhibited by <50 mg fluazinam/litre. For comparison, isolates were obtained from selected Dutch and Swedish potato crops in 1999. Fluazinam has been very intensively used in the Netherlands since the mid 1990s. Of these isolates, 15 (of 39) Dutch and 9 (of 9) Swedish isolates were derived from fluazinam-treated crops. Most isolates (39) were of the A1 mating type, but 9 were A2. These isolates proved as sensitive to fluazinam as those from Northern Ireland with zoospore motility generally completely inhibited by 10 - 60 mg fluazinam/litre.

L. R. Cooke,
Applied Plant Science Division
Department of Agriculture & Rural Development
Newforge Lane
Belfast, Northern Ireland, BT9 5PX

and
Department of Applied Plant Science
Queen's University
Newforge Lane
Belfast, Northern Ireland, BT9 5PX,
United Kingdom

D J Carlisle
Department of Applied Plant Science
Queen's University
Newforge Lane
Belfast, Northern Ireland, BT9 5PX,
United Kingdom

M Quinn and D G Wilson
Applied Plant Science Division
Department of Agriculture & Rural Development
Newforge Lane
Belfast, Northern Ireland, BT9 5PX
United Kingdom

DMI Sensitivity in *Rhynchosporium secalis* as Influenced by Fungicide Programmes

Trials over three years in Northern Ireland and southwest England on winter barley evaluated two-spray programmes based on the DMI epoxiconazole alone and in combination or alternation with fenpropimorph (a morpholine), cyprodinil (an anilinopyrimidine) and azoxystrobin (strobilurin) for their effect on *Rhynchosporium secalis*. Epoxiconazole, used alone, gave the lowest disease control. Addition of another fungicide consistently improved control, cyprodinil being marginally the best partner. Despite its relatively poorer disease control, epoxiconazole gave a good yield response compared with untreated plots. However, all three partner fungicides increased yield further, azoxystrobin and cyprodinil being better than fenpropimorph. Samples of leaf blotch were collected from field trials before and after fungicide application and where possible, *R. secalis* was isolated and tested for sensitivity to epoxiconazole. Sufficient isolates to permit comparisons before and after treatment were only obtained in 1998 and 2000. The proportion of isolates with lower MIC (minimum inhibitory concentration) values (1 mg epoxiconazole/litre or less) decreased after treatment and the least sensitive were obtained from epoxiconazole-treated plots.

L. R. Cooke, R. Coll, L. Black, P. J. Taggart, and P. C. Mercer
Department of Applied Plant Science
Queen's University
Newforge Lane
Belfast, Northern Ireland, BT9 5PX
United Kingdom

T. Locke and K. D. Lockley
ADAS Rosemaund
Preston Wynne, Hereford, HR1 3PG
United Kingdom

A. N. Phillips and M. D. S. Sadiq
ADAS Wolverhampton, 'Woodthorne'
Wergs Road, Wolverhampton, WV6 8TQ
United Kingdom

Syngenta Quick Test: A Rapid Diagnostic Test for Detecting Herbicide Resistant Weeds

A diagnostic test (Syngenta Quick Test, QT) has been designed for testing grass weed survivors to herbicides from the field. The test is robust, simple, quick, and reliable to perform with no complicated equipment required. Glasshouse studies revealed that the test can be used for grass weed species tested including blackgrass (*Alopecurus myosuroides* Huds.), foxtails (*Setaria* spp.), ryegrass (*Lolium* spp.), wild oats (*Avena* spp.), dicots eg. *Chenopodium* spp., and wild radish (*Raphanus raphanistrum*). Resistance of known herbicide resistant blackgrass biotypes to aryloxyphenoxy-propionate herbicides and to phenylurea herbicides was verified by the QT. The findings were similar to herbicide treatment of seedlings. For field evaluation of the QT, ryegrass from suspect resistant fields in South Australia were sampled and sent by post to Switzerland. Resistance was confirmed in less than 4 weeks and verified that reported field failures and identified herbicides that were still effective. Recent field trials in Australia and in several European countries evaluating the QT have shown that herbicide resistance in ryegrass, blackgrass, wild oats, and wild radish was confirmed in 3-4 weeks. The QT has clear advantages over current resistance tests including a likely fit for in-season testing of weed survivors with possible follow-up action in certain cases.

To view the published paper, see:

Weed Technology: A Journal of the Weed Science Society of America. Vol. 15, No. 2 (Apr/June 2001): p. 257-263.

Dr. Peter Boutsalis
Syngenta
Herbicide Resistance Support, Jeallot's Hill
Bracknell, Berkshire RG42 6EY
United Kingdom
E-mail: peter.boutsallis@syngenta.com

Managing Insecticide Resistance in Australian *Helicoverpa armigera*

Insecticide Resistance Management (IRM) strategies for Australian cotton were first developed in 1983/84 and have been evolving ever since. This paper aims to discuss some of the main components of the proposed 2000/2001 strategy, including chemical and non-chemical components. Australian field populations of *H. armigera* have developed high frequencies of resistance to pyrethroids carbamates, endosulfan, and organophosphates. Historically, new groups of insecticides or new technologies have become available one at a time. This has resulted in overuse of each sequential technology as it was released as well as in selection for resistance. Once resistance is detected, we have attempted to manage it re-actively through the IRM strategies. The Australian IRM strategies are second to none and have successfully preserved older groups of insecticides and bought time for the development of new technologies and new approaches. However, once resistance problems to any insecticide group or technology are detected, they are established in the field and very difficult to slow down or reverse.

The 'history' of resistance and cross-resistance to older insecticide groups places increased selection pressure on new technologies (such as Ingardâ) and new conventional chemistry and emphasizes the need for a pro-active approach to resistance management. For the first time, we have a suite of concurrent new insecticides and technologies that are available for use in Australian cotton. None of these new technologies have an established resistance problem (yet), but none of them are resistance proof. The way that we implement these products now will have a tremendous bearing on how quickly our major cotton pests develop resistance to them. We have a tremendous opportunity to develop pro-active resistance management strategies to preserve these new tools for the longer term. Successful implementation of pro-active IRM will balance the risk of selection for resistance between different insecticide groups and prevent selection from being channelled towards any single group. This should result not only in the preservation of the new technologies, but also a lifting in selection pressure and a benefit to the older groups with established resistance problems. Both chemical and non-chemical approaches need to be incorporated into integrated pest management guidelines, which will complement and support IRM.

Chemical approaches include the separation of the target pest species and insecticide selection pressure in time (alternations, rotations and window strategies) and separation of the target pest species and insecticide selection pressure in space (mosaic and refuge strategies). They include the use of synergists or mixtures where appropriate to overcome metabolic resistance and restrictions in the total number of applications of a particular insecticide group used. The success of these approaches will depend on the range (and cost) of chemical groups available, their impact on the major beneficial insects and their resistance status which needs to be thoroughly monitored. Once resistance is detected in the field, or preferably artificially selected for in the laboratory, then an understanding of the major resistance mechanisms and a thorough understanding of the ecology of the pest are vital for determining appropriate IRM tactics.

Complementary non-chemical approaches will have to emphasise a systems approach to help to reduce pest population pressure and reduce insecticide use. This will include:

- matching the variety and its agronomic management to the region,
- optimising planting windows,
- realizing early season thresholds,
- understanding the crops' compensatory capacity for damage,
- noting classical or genetically modified host plant resistance,
- using trap crops to concentrate pests for management,
- using refuges for the preservation of susceptible genes,
- stimulating the physical destruction of over-wintering pupae, and
- utilizing area-wide management.

These components have been incorporated into Integrated Pest Management (IPM) guidelines designed to complement and support IRM.

Our understanding of many of these components is growing but far from complete. The challenges encountered in integrating these approaches into a coordinated strategy and regularly updating this strategy to cope with the dynamic nature of the resistance problem will be discussed using the Australian Cotton IRM Strategy as an example.

J. W. Holloway
formerly NSW Agriculture
Australian Cotton Research Institute
Aventis Crop Science
Narrabri, NSW 2390
Australia
E-mail: jonathan.holloway@ aventis.com

N. W. Forrester
DeltaPine International
60 Maitland St.
Narrabri, NSW 2390
Australia
E-mail: neilandk@turboweb.net.au

Current Status of Resistance in Colorado Potato Beetle (CPB) *Leptinotarsa Decemlineata* (Say) in Poland

ABSTRACT: The studies on CPB susceptibility to main classes of insecticides have been performed in Poland for a very long time and are still continuing with the cooperation and support of Insecticide Resistance Action Committee (IRAC). At present, a lot of consideration is being given to neonicotinoids and phenylpyrazoles. Insecticides from the phenylpyrazoles and neonicotinoids classes are relatively new for the control of CPB in Poland (fipronil - 1996, acetamiprid - 1996, imidacolprid - 1998, thiamethoxam 1999, thiacloprid - 2001). The importance of both new classes of insecticides for CPB control in Poland has systematically increased. Bioassays of this insecticides with recommended field concentrations and the laboratory tests concerning susceptibility level are realized in the Institute of Plant Protection in Poznan for resistance monitoring in CPB.

CONCLUSIONS:

1. CPB strains are more tolerant to nereistoxin analogue (bensultap) pyrethroids, and organophosphorous (chlorfenvinphos) insecticides are still good controlled with imidacloprid, thiamethoxan, acetamiprid, thiacloprid, and fipronil so no cases of resistance has been reported in Poland so far.

2. Field and laboratory investigations give no indication on resistance or cross resistance elicited by tested populations of CPB to these insecticides today.

3. Using new classes of insecticides in Poland we must remember that we have CPB strains more tolerant to nereistoxin analogue (bensultap) pyrethroids and organophosphorous (chlorofenvinphos) insecticides and that this insect pest is multiply cross-resistant to five major groups of insecticides in United States. CPB has developed resistance to *Bacillus thuringiensis* and abamectine imidacloprid, and has shown tolerance to fipronil too. To maintain as long as possible the high insecticidal potency of new and older chemical classes of insecticides there is a necessity to follow the general resistance management guidelines, which were elaborated in Institute of Plant Protection in Poznan with cooperation of IRAC and could be adopted in all area of potato insecticide protection in Poland.

Tab. 2. Field efficacy of some active ingredients on CPB larvae L_2 (data from 1998-2000)

Compound /dose-concentration (ppm)		% mortality after		
		5 days	10 days	14 days
acetamiprid	50 ppm	90,24	83,39	69,96
acetamiprid	67 ppm	90,94	86,52	76,11
thiamethoxam	67 ppm	99,38	99,86	100
fipronil	67 ppm	100	100	100
cypermethrin	80 ppm	95,11	91,58	85,84
alpha-cypermethrin	34 ppm	96,25	91,79	85,46
lambda-cyhalothrin	17 ppm	97,61	95,72	92,02
deltamethrin	17 ppm	95,21	92,66	87,38
deltamethrin	25 ppm	90,15	95,81	92,11
bensultap	500 ppm	97,90	100	100

Tab. 1 Maximal and minimal susceptibility level of CPB larvae (L2) and beetels to some active substances - comparison with recommended concentrations (data from 8 populations in1998 - 2001 year, IRAC method no. 7).

active substances	recommended field concentration (in ppm)	LC_{50} larvae (ppm)	LC_{90} larvae (ppm)	LC_{50} beetles (ppm)	LC_{90} beetles (ppm)
fipronil	50	0,06-0,17	0,28-0,70	0,25-0,75	1,20-2,20
thiamethoxam	50	0,20-0,35	0,80-1,55	1,20-2,50	1,90-5,10
acetamiprid	40	0,06-0,20	0,95-2,40	0,90-1,85	4,45-16,80
imidacloprid	50	0,30-0,80	1,0-2,8	1,50-3,70	7,80-18,40
bensultap	375	9,20-21,3	19,84-65,80	6,6-148,9	306-38307
chlorfenvinphos	625	55,57-121,7	427,5-898,2	38-187	1100-6100
deltamethrin	12,5	2,50-5,3	8,79-17,65	7,15-28,8	76,2-311,9
cypermethrin	62,5	6,50-22,34	18,46-67,32	18,9-128,9	139,6-380,0
lamb-da-cyhalothrin	25	No data	No data	17,4-73,7	31,3-165,9
alpha-cypermethrin	25	No data	No data	17,5-43,9	53,5-250,9

Pawel Wegorek
60-318 Poznan Institute of Plant Protection
Poland
p.wegorek@ior.poznan.pl

Strategy for Managing Colorado Potato Beetle *Leptinotarsa Decemlineata* (Say) Resistance in Poland

ABSTRACT: Colorado Potato Beetle (CPB) is still considered to be the Polish pest with the highest likelihood of developing insecticide resistance. Management of Colorado Potato Beetle (CPB) in Poland is mainly based on insecticide use. The very extensive acreage of potato cultivation (1, 19 mln ha in 2001) and the constant need for intensive chemical protection is due to the concentration and size of the CPB populations that considerably exceed the economic damage threshold in occurrence area. Nearly 50 years of constant selective pressure on the CPB has brought about the development of local populations with lower susceptibility to some insecticides. This phenomenon, known as resistance, resistivity or tolerance, leads to big losses in potato crop and restriction in use and sales of less effective insecticides. In Poland, resistance proved highest in the organophosphate, especially those with the trade name - Enolofos 44 EC (the present trade name - Enolofos 500 EC), containing chlorfenvinphos as the biologically active ingredient. Resistance also appeared earlier, though on a lesser scale, in the case of chlorinated hydrocarbons insecticides - DDT, lindane, methoxychlor and the carbamates - carbaryl and propoxur. At present populations with increased tolerance to insecticides from the pyrethroids group and nereistoxin analogue can be observed. Problems caused by the pest's growing resistance usually result from an inappropriate or simplified way of applying the chemicals, with successive applications being carried out in a given area using one or more products, of the same class. Study of resistance mechanisms show that selection based on one mode of action, after a period of time leads to development of genetically inherited features in target populations, which allow individuals carrying those genes to survive the action in ever bigger numbers. CPB is a species with a very high natural resistance to all kinds of poisons and tolerates well both plants toxins (xenobiotics) and synthetic insecticides, and for this reason only a limited number of chemical compounds can be effectively used to fight it. Limited numbers of chemical compounds can be effectively used to fight it. In the years 1994 - 1997, following a commission by IRAC, monitoring of the susceptibility level of a large number of populations of CPB was carried out in Poland in different regions of the country, to evaluate the effectiveness of - bensultap (trade name - Bancol 50 WP), cypermethrin (trade name - Sherpa 10 EC) and chlorfenvinphos (trade name - Enolofos 500 EC). The study revealed a high degree of variation in susceptibility to each of the insecticides both in the same areas, and in geographically distant regions. This suggests an advanced selection, which in the near future, may lead to CPB development of high resistance to each of the biologically active ingredient under investigation. To combat this situation, a controlled and rational use of management insecticides is necessary in Poland, following the strategy worked out by Polish research institutes in cooperation with IRAC.

Pawel Wegorek
60-318 Poznan Institute of Plant Protection
Poland
p.wegorek@ior.poznan.pl

Negative Cross-Resistance between Indoxacarb and Pyrethroids in Australian *Helicoverpa armigera*: A Tool for Resistance Management

Helicoverpa armigera is Australia's most serious insect pest on crops and chemical control of this insect is considered essential, especially on cotton. Esterase-mediated pyrethroid resistance in *H. armigera*, is an enduring threat to the economic production of cotton in Australia. Esterase isoenzymes sequester and metabolise pyrethroids and resistance factor increases with esterase titre. Insecticide use against *Helicoverpa spp*. on cotton is subject to an insecticide resistance management strategy and we constantly seek the registration of new insecticides with novel modes of action.

Indoxacarb (Dupont's Steward(R)), has been recently registered for the control of *Helicoverpa spp*. on cotton in Australia. Indoxacarb is novel insecticide requiring bio-activation to a toxic metabolite. Our studies have shown that *H. armigera* activates indoxacarb using the same esterase enzymes that are involved in pyrethroid resistance. Increased esterase activity leads to increased activation and therefore increased susceptibility to indoxacarb. Bioassay data confirm negative cross-resistance between pyrethroids and indoxacarb in *H. armigera*. The importance of indoxacarb, as a tool for resistance management cannot be underestimated.

Robin V. Gunning
NSW Agriculture
Tamworth Centre for Crop Improvement
RMB 944 Calala Lane
Tamworth, NSW 2340
Australia

Alan L. Devonshire
IACR-Rothhamsted
Harpenden, Herts, AL5 2JQ
United Kingdom

Symposia

HRAC-NA MINUTES
February 11, 2002
Reno, NV

Sharlene Matten, Ph..D. Office of Pesticide Programs, Biopesticides & Pollution Prevention Division , US EPA presented information on the USEPA guidelines: PR Notice 2001-5, June 2001 [http://www.epa.gov/opppmsd1/PR_Notices/] which outlines the voluntary labeling of pesticides by site of action and USDA/APHIS and EPA - Stewardship plans for herbicides used on herbicide-tolerant crops (HTCs).

Dr. Matten stressed the harmonization between the US and Canadian effort to label pesticides by site of action.

Gregory Bridger, Ph.D, Evaluation Officer, Herbicide and Plant Growth Regulators Section Efficacy & Sustainability Assessment Division, PMRA reported on the status of labeling of herbicides by site of action in Canada Directive 99-06 [http:/www.hc.sc.gc.ca/pmra-arla].

Dr. Bridger indicated that 24 of the 1800 pesticides have listed the site of action on the label, 20 of which are herbicides.

Steve Moss, IACR-Rothamsted updated the group on the Baseline sensitivity to herbicides a guide to methodologies. In Europe there has been an effort to establish a baseline of tolerance of plants to herbicides. This information would be required for the registration of new pesticides. This would allow the detection of small shifts in sensitivity to pesticides and warn of possible resistance problems. Maintenance of seed supplies, size and the populations used in the baseline testing were discussed.

Martin Parnham, from Herbiseed inquired about the possible demand for standardized populations of resistant weed seed. There did not seem to be a demand for such seed by either industry or university researchers.

Ian Heap, WeedSmart, gave an update on the current listing of herbicide resistant biotypes list on his website [www.weedscience.com]. There are currently 257 biotypes that exhibit resistance to herbicides. These biotypes are in 156 different species. There are 94 monocot species and 62 dicot species. There were nine new biotypes added in 2001. There were 1.2 million page views to the website in 2001.

Ian has been able to identify 3000+ links to the weedscience.com site. In the round table discussion the following new resistant biotypes are being investigated: glyphosate resistant horseweed (*Conyza canadensis*) in TN, *Phalaris minor* resistance to sethoxydim, field pennycress (*Thlaspi arvense*) resistance to ALS herbicides in Canada, populations of waterhemp (*Amaranthus rudis*) resistance to PPO and glyphosate herbicides in KS, and Eastern black nightshade (*Solanum ptycanthum*) and common ragweed (*Ambrosia artemisiifolia*) populations resistant to ALS herbicides in ND.

Steve Moss indicated that ALS resistant poppies (*Papaver sp*.) and chickweed (*Stellaria uredia*) in the UK.

ACTION ITEMS

1. Dr. Terry Wright - Dow AgroSciences, is requesting, before the next HRAC-NA meeting, University Extension and Corporate publications that would be used to educate growers, dealers and crop advisiors on resistance management labeling.

   PLEASE SEND ONE COPY of Resistance Management materials to:

   Dr. Terry Wright
   Dow AgroSciences
   753 Hwy 438
   Greenville, MS 38701
   (662) 379-8990

2. HRAC-NA will try to search to secure some funding sources to assist in the publication of herbicide resistance labeling education guides.
3. The next HRAC-NA meeting will be at the 2003 WSSA Meeting in Jacksonville FL in a joint session with the Herbicide Tolerant Plants and Extension committees.

Submission Deadlines and Announcements

Welcome to the new electronic version of the Resistant Pest Management Newsletter! The site is located at www.cips.msu.edu/whalonlab/rpmnews/. We hope that this format proves to be both informative and useful and allows enhanced communication of resistance issues around the globe.

We ask that you try to be patient if you encounter any problems while navigating the site - we'll do our best to fix any glitches and to increase the ease of your searching. If you have any problems, please let us know about them so that they may be remedied and others won't suffer the same difficulties.

Since the Newsletter is now electronic, it is likely that we will cease to issue printed copies except to libraries that request a printed version.

Also, we would like to have new issues for you to read more than two times yearly. For this to be a possibility, we need your assistance. 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.

The next two **submission deadlines** are:

Monday, June 17th
Monday, September 16th

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

In the future, this page will notify you of any changes to the Newsletter, corrections to articles, and announcements. We appreciate your patronage and hope to hear from you. Please let us know what you think - and again, welcome!

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