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. 14, No. 1 (Fall 2004)

Table of Contents

Letter from the Editor

IMPORTANT NOTE

When we first began publication of this Newsletter over 10 years ago, it was intended primarily to be an informal communication device for people interested in and working on pesticide resistance. With the aim of fostering communication, we solicited and published brief reports on research in progress and abstracts of presentations at meetings. From time to time, we have stressed that the Resistant Pest Management Newsletter is not a referred publication and that, as such, it should not be used as a source for publication of full-length papers or as a primary literature citation source.

Nevertheless, over time, some of the research reports have expanded to become full-length referred journal-like papers. We believe publishing complete papers is not an appropriate use of the Newsletter. We did give some transient thought to making this an e-journal with one section devoted to refereed papers on

resistance. However, this is not a workload or responsibility any of the editorial staff are prepared for, and there is already a variety of well-respected refereed journals that publish original research on resistance. Therefore, starting with the next issue, Vol. 14, No. 1 - Fall 2004, of the Newsletter, we will not publish full-length articles that we judge would be more appropriate for mainstream scientific refereed publications.

We very much hope that you will continue to provide brief reports and abstracts of resistance research. Except in unusual circumstances or survey reports, the size limitations of Newsletter articles should be limited to 2 1/2 pages plus one figure or table. We will include longer articles that constitute regional or countrywide survey information from time to time.

Web-Based Resistance Data Entry Coming. We are getting close to the completion of another project that has occupied us for the last year or so. This is the development of a web-based entry system for the arthropod resistance database. The database already exists and can be found at http://www.pesticideresistance.org/DB/. It contains about 3000 records of the development of resistance in arthropods based on the species, selecting compound and location. We owe a great debt to the late George Georghiou who initiated this cataloging of resistance in the 1970s and whose database published in 1991 forms the historical backbone. Keeping this database up to date has not been an easy task since finances to run it have been hard to come by and there have been stretches when nothing was being done to add new records. Still, incomplete as it is, we believe it is the only one of its kind and has utility for those who work in the resistance field.

Recently we obtained financial assistance from IRAC, Michigan State University and the US Department of Agriculture to develop a user-based interface for entry of new records. This will be ready for use in 2004. Anyone who wishes to enter a resistance episode may do so and it will be incorporated into the database after editorial review (assuming it meets basic editorial criteria).

This web-based survey system is designed to record resistance where field failures have been investigated and demonstrated to be attributable to a genetic change in the target population. In other words, resistance instances where other possible explanations such as weather-related attenuation, misapplication, etc. have been eliminated with scientifically-based bioassays or where verified field discriminating dosage studies have been carried out.

We anticipate that the data resulting from this web-based survey mechanism will be timelier and potentially more spatially comprehensive than the fragmented and limited records we have to date. The survey tool features a series of drop-down menus and cloning tools to ease respondent burden. In preliminary testing, the survey tool has demonstrated a reasonably transparent data entry process for uninitiated first-time users. Each resistance case submission will be given an accession number and, after review and acceptance, should be citable as a contribution to the database.

We hope that you will evaluate and use this web-based resistance survey system. Your comments would be most useful! With regular input from resistance scientists around the globe, our database should be increasingly comprehensive and more current. Thus we will collectively produce a historical record of the occurrence and development of resistance, but also provide global, regional and, in time, a local "snapshot" of the resistance status of important pests, individual compounds and groups of compounds (modes of action).

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

Resistance Management Reviews

Mating Interactions of *Bemisia tabaci* Biotypes in Cyprus
By Dr Terry Mabbett

Most farmers and growers in the tropics and sub tropics are confronted with *Bemisia tabaci*. A sap sucking bug (Homoptera: Aleyrodidae), and variously called tobacco whitefly, cotton whitefly and sweet potato whitefly, *B. tabaci* feeds and breeds on a massive range of agricultural and horticultural crops and associated weeds. Key crops include tomato, sweet pepper, *Phaseolus* beans, cotton, tobacco, sweet potato and cassava.

This sucking insect pest with a winged adult stage and a sessile and scale-like larva has increasingly spread within high value horticultural crops around the world. Typical of those affected are tomato, sweet pepper and other crops in warm temperate areas of southern Europe and the Mediterranean, where year round cropping using greenhouses and polytunnels during winter is standard.

In addition to the debilitating effect on crop plants, mostly through larval feeding, adult whiteflies are vectors of many of the most virulent and damaging plant virus diseases. One prime example is the Tomato Yellow Leaf Curl Virus (TYLCV). TYLCV is widespread throughout the Mediterranean area and capable of inflicting total crop loss.

Repeated attempts to establish sustainable chemical control of *B. tabaci* have been thwarted by the development of insecticide insensitivity (resistance). This occurred initially with use of carbamate and organophosphorus insecticides and more recently with introduction of the synthetic pyrethroid and neonioctinoid insecticide chemistries.

Compounding the *B. tabaci* pest problem and its management on a huge range of high value crops is the existence of distinct biotypes, of which the 'B' and 'Q' biotypes are of key interest in southern Europe.

The island of Cyprus in the Mediterranean with its crucially important agricultural and horticultural systems and high value export crops is one of the countries in this region having to cope with the worsening whitefly problem. The suspected arrival of 'Q' biotypes to join well-established and insecticide resistant populations of 'B' biotypes on Cyprus is giving cause for concern.

Margarita C. Hadjistylli from Cyprus and postgraduate student on the MSc Applied Entomology Course at Imperial College (London) studied the mating interactions between the 'B' and 'Q' biotypes, to highlight and understand the factors that influence their dynamic distribution, with implications for both pest management and insecticide resistance management.

Separate studies were set up to investigate:

1. The potential of the two biotypes to interbreed via crossing experiments.
2. Whether behavioural aspects of mating and courtship act as barriers to successful copulation and therefore interbreeding.

The main findings of the experiments were:

- Successful mating is possible through reciprocal, inter-biotype crosses, even though the rate of hybridisation was significantly lower ($P<0.001$) than in intra-biotype crosses. Progeny from these crosses were both viable and fertile.
- Inter-biotype pairs spent more time courting than did intra-biotype pairs, with copulation in inter-biotype pairs occuring in only rare instances. In addition, results of observed interactions suggest that these behavioural patterns could account for unsuccessful copulation in the inter-biotype pairs, leading to assortative mating.
- Males of the 'Q' biotype were more active and courted females of either biotype more readily than did 'B' biotype males. This could be indicative, said Margarita, of a competitive advantage of 'Q' males over 'B' males when both biotypes exist in the same area or location.

Bemisia tabaci populations collected in Cyprus were then tested for biotype status, to establish information on the occurrence and resistance status of 'B' and 'Q' biotypes. Insects were bioassayed with three insecticides - cypermethrin (synthetic pyrethroid), methamidophos (organophosphate) and imidacloprid (neonicotinoid) - all currently used on commercial crops in Cyprus.

Results from these investigations showed that:

- Both 'B' and 'Q' biotypes are present in Cyprus although they appear to occur within distinct geographical areas of the island. The 'B' biotype had been reported before but this is the first time that the 'Q' biotype has been identified from the island, said Margarita.
- The 'Q' biotype population exhibited very high levels of resistance to all three insecticides (>100 fold) compared to asusceptible, laboratory-based strain.

The results of this work strongly suggest that existing chemical control options using currently available products and established insecticide chemistry will be increasingly inadequate to control resistant populations like the one tested here. In view of this, says Margarita, pest management strategies for *Bemisia tabaci* need to be re-assessed and re-evaluated as a matter of some urgency.

Further information from:

Dr Simon Leather
The Course Director

Department of Biological Sciences
Imperial College London, Silwood Park Campus
Ascot SL5 7PY
United Kingdom
Tel: +44 (0) 207 594 2316
Fax: +44 (0) 207 594 2339
http://www.imperial.ac.uk/biologicalsciences

or

Dr Terry Mabbett
2 Albemarle Avenue
Potters Bar
Hertfordshire EN6 1TD
United Kingdom
Tel & Fax: +44 (0) 1707 644953

Insecticide Resistance Disadvantage for *Myzus persicae*

By Dr Terry Mabbett

Some insect pest species can adapt to most climatic conditions and are able to feed on a huge range of different crops. As such they are truly cosmopolitan and are almost global in distribution. *Myzus persicae*, variously called the green peach aphid or peach-potato aphid, is one such example. This polyphagous sucking pest feeds and breeds on a massive range of crops and wild plants including beans, potato, sugar beet, sugar cane, brassicas, citrus and tobacco.

In addition to extensive direct damage caused by the combined feeding activity of large colonies of wingless forms, the winged form has the capacity to carry and transmit more than 100 different plant virus diseases.

Populations of this aphid are associated with a large web of natural enemies - predators, parasitoids and parasites - which help to manage populations by biological control. But as such a well-established, widespread and damaging insect pest, *M. persicae* has clearly been at the receiving end of a succession of insecticide chemistry.

Initially it was the carbamates and organophosphates and more recently synthetic pyrethroid and neonicotinoid insecticides. Response has been development of insecticide resistant populations selected out by the routine use and abuse of chemical control. But there is increasing evidence that insecticide resistance may impair the ability of aphids including *M. persicae* to respond to attack by parasitoids.

In a project placement, Monique Tomiczek, postgraduate student on the MSc Applied Entomology course at Imperial College London (Silwood Park Campus), looked at comparative vulnerabilities of insecticide-resistant and insecticide susceptible *M. persicae* to attack by the parasitoid wasp, *Diaeretiella rapae*. This work was supervised by Dr Steve Forster, who is a senior member of the Insecticide Resistance Group within the Plant and Invertebrate Ecology Division, Rothamsted Research, Hertfordshire in the United Kingdom.

More specifically, Monique tested whether the pleiotropic effects on the responsiveness to aphid alarm pheromone, of genes conferring *kdr* and esterase-based insecticide resistance, result in greater vulnerability of *M. persicae* to parasitoid attack on in the absence of insecticides.

Various types of defensive and avoidance behaviours exhibited by *M. persicae*, in response to attack by the parasitoid, were measured. This was carried out in both the presence and absence of the alarm pheromone ((E)-*B*-farnesene) for a range of insecticide resistant and insecticide susceptible genotypes of *M. persicae*.

Results showed evidence that extreme esterase-based (R3) resistance and combined *kdr* and esterase-based resistance (RR/R3) mechanisms are associated with increased vulnerability to parasitoid attack (manifested in a number of behaviours).

Monique concluded that enhancement or preservation of parasitoids in sustainable agricultural and horticultural systems would have dual benefits in the fight against insecticide resistance. Firstly, it could confer the direct benefit of reducing the relative fitness of insect pests carrying resistance genes. Secondly, it offers the indirect benefit of reducing reliance on insecticides and therefore lessens the intensity of selection in favour of insecticide resistant genes.

Insecticide resistance was traditionally considered to be pure benefit for the development and success of insect pest populations, but it may turn out to be a 'double edged sword' in relation to the progress of some insect pest species, particularly during times of stress.

Further information from:

Dr Simon Leather
The Course Director
Department of Biological Sciences
Imperial College London, Silwood Park Campus
Ascot SL5 7PY
United Kingdom
Tel: +44 (0) 207 594 2316
Fax: +44 (0) 207 594 2339
http://www.imperial.ac.uk/biologicalsciences

or

Dr Terry Mabbett

2 Albemarle Avenue
Potters Bar
Hertfordshire EN6 1TD

United Kingdom
Tel & Fax: +44 (0) 1707 644953

Resistance Management from Around the Globe

Baseline Resistance Information

Generating Base Line Data for Insecticide Resistance Monitoring in Coffee Green Scale, *Coccus viridis* (Green)

ABSTRACT Investigations were carried out to generate data on base line toxicity of thiamethoxam, imidacloprid and dimethoate to *Coccus viridis* (Green) by conducting acute toxicity studies. The LC_{50} of thiamethoxam, imidacloprid and dimethoate to first generation of *C. viridis* was 1.4287, 3.2278 and 11.4155 ppm respectively. The LC_{50} obtained for subsequent three generations without selection pressure to these insecticides did not vary much, indicating no development of resistance. Based on the study, discriminating doses of 35, 66 and 500 ppm were fixed for thiamethoxam, imidacloprid and dimethoate respectively

INTRODUCTION Coffee occupies a place of pride among plantation crops grown in India. Cultivation of this crop is mainly confined to the southern states of Karnataka, Kerala, Tamil Nadu, and Andhra Pradesh. Arabica and Robusta are the two types of coffee cultivated on a commercial scale. The area under coffee in India is around 3, 40,306 ha with an average annual production of 2, 50,000 metric tonnes, of which 75 per cent accounts for foreign exchange as an export commodity (CCRI, 2000).

An array of insects including borers, leaf feeders, sap feeders and root feeders were found to infest coffee (Regupathy et al., 2003). The super family, Coccoidea, comprising scale insects and mealy bugs, is important in causing severe damage to coffee plantations. These pests suck the plant sap and devitalize them. Although large numbers of Coccoids infesting coffee have been recorded, only a few are of economic importance (Uma Narasimham, 1987). Earlier an array of insecticidal compounds belonging to organophosphates (OP's) and organochlorine (OC) groups was recommended to combat these pests. Indiscriminate use of pesticides in coffee resulted in development of resistance by *Coccus viridis* (Green) to the commonly used pesticides (Venkataramiah and D' Souza, 1974). However, no concrete and systematic work was carried out for resistance monitoring of this pest as carried out for other pests in Tamil Nadu. Hence, keeping this in view a study was undertaken to generate baseline toxicity data for future monitoring studies.

MATERIALS AND METHODS Acute toxicity studies were carried out for two neonicotinoid compounds, thiamethoxam and imidacloprid, and for dimethoate against the major sucking pest of coffee, *C. viridis*, for four successive generations. The scale insect, *C. viridis*, mass cultured in the Toxicology Laboratory Glass house, Department of Agricultural Entomology, Tamil Nadu Agricultural University, was used in the study.

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 logconcentration-probit-mortality (l cpm) lines (Regupathy and Dhamu, 2001).

Infested coffee leaves were dipped in appropriate dilutions of the test insecticide solution and shade dried. Leaves dipped in water alone served as control. The petiole of the treated leaves was wrapped with moist cotton swabs placed in vials containing water to maintain the turgidity. Each treatment was replicated three times. Observations on the mortality of green bug were recorded after 48 h and the experiment was terminated after 96 h.

Mortality was corrected using Abbot's correction (Abbott, 1925). Median lethal concentration was estimated by probit analyses as prescribed by Finney (1971). Differences in mortality were considered significant when fiducial limits did not overlap. Susceptibility indices were calculated based on LC_{50} and LC_{95} obtained for the final generation of *C. viridis*, maintained without insecticide exposure in the glass house (Regupathy and Dhamu, 2001).

$$\text{Susceptibility index} = \frac{\text{LC50 of F1}}{\text{LC50 of Fn}} \quad \text{or} \quad \frac{\text{LC95 of F1}}{\text{LC95 of Fn}}$$

The rate resistance decline (R) used to quantify the rate of changing LC_{50} when the selection pressure is stopped was estimated by the formula;

$$R = \frac{\text{Log (final LC50) - log (initial LC50)}}{n}$$

Where,

- n is the number of generations not exposed to insecticide
- Final LC_{50} is the LC_{50} after n generations without selection, and
- Initial LC_{50} is the LC_{50} of the parental generation before n generations of selection.

The number of generations (G) required for ten-fold decrease in the LC_{50} value was calculated using the formula.

$$G = R \wedge -1$$

RESULTS AND DISCUSSION Among the different insecticides tested, thiamethoxam was found to be highly toxic to *C. viridis*, followed by imidacloprid and dimethoate. The median lethal concentration (LC_{50}) of these insecticides to the first generation of *C. viridis* was 1.4287, 3.2278 and 11.4155 ppm for thiamethoxam, imidacloprid and dimethoate, respectively (Table 1). The LC_{95} values being 45.3135, 75.2177 and 656.7360 ppm, respectively for the three insecticides tested.

Table 1. Susceptibility of C. viridis to various insecticides

Generation	Regression equation	χ^2	LC_{50} (ppm)	Fiducial limits LL		UL	LC_{95}	Fiducial limits LL		UL
Thiamethoxam										
1	Y =0.4476 + 1.0957 x	1.3914	1.4287	1.1722	-	1.7413	45.3135	37.1777	-	55.2296
2	Y =0.3597 + 1.1267 x	0.9295	1.3142	1.0868	-	1.5892	37.9009	31.3417	-	45.8328
3	Y =0.3852 + 1.1221 x	0.9694	1.2957	1.0691	-	1.5702	37.8753	31.2529	-	45.9011
4	Y =0.3437 + 1.1351 x	1.0544	1.2646	1.0461	-	1.5287	35.5649	29.4201	-	42.993
Imidacloprid										
1	Y =-0.4239 + 1.2029 x	1.7144	3.2278	2.6613	-	3.915	75.2177	62.0156	-	91.2304
2	Y =-0.4836 + 1.2194 x	1.9546	3.1382	2.5931	-	3.7978	70.0694	57.8985	-	84.7988
3	Y =-0.3579 + 1.2004 x	1.9086	2.9059	2.4025	-	3.5148	68.161	56.3532	-	82.4429
4	Y =-0.3475 + 1.2019 x	2.487	2.8127	2.3298	-	3.3956	65.7178	54.4355	-	79.3385
Dimethoate										
1	Y =1.2076 + 0.9347 x	0.3754	11.4155	9.1688	-	14.2127	656.736	527.482	-	817.663
2	Y =1.2052 + 0.9388 x	0.3186	11.0257	8.8661	-	13.7113	623.19	501.127	-	774.984
3	Y =1.2395 + 0.9352 x	0.2899	10.4943	8.4163	-	13.0855	602.264	483.005	-	750.97
4	Y =1.2615 + 0.9348 x	0.3666	9.986	7.9987	-	12.4672	574.243	459.959	-	716.922

No marked increase in susceptibility of the insects to the chemicals was noticed with the advancement through four generations. This was evident by the overlapping fiducial limits of both LC_{50} and LC_{95} values for all the insecticides tested. The susceptibility index varied between 1.13 and 1.15 based on LC_{50} and it was in the range of 1.14 to 1.27 based on LC_{95} values for all the chemistries tested. The rate of resistance decline was negative for all the chemicals tested, indicating less or no development of resistance by the test insect. In terms of the number of generations required for 10-fold decrease in LC_{50}, a numerically high value was obtained for thiamethoxam (75.6) followed by dimethoate (68.8) and imidacloprid (66.9) (Table 2). The susceptibility baseline data are not generated so far for these insecticides taken up for the study. Hence, the LC_{95}s of the insecticides were considered as discriminating doses for monitoring the field populations for their resistance to these insecticides. From the acute toxicity studies conducted in our laboratory, the discriminating doses 35, 65 and 575 ppm for thiamethoxam, imidacloprid and dimethoate, respectively were fixed for resistance monitoring in future.

Table 2. Susceptibility index and rate of resistance decline in C. viridis

Insecticides	Susceptibility Index LC_{50} (ppm)	Susceptibility Index LC_{95} (ppm)	Rate of Resistance Decline R	Rate of Resistance Decline G
Thiamethoxam	1.13	1.27	-0.0132	75.6
Imidacloprid	1.15	1.14	-0.015	66.9
Dimethoate	1.14	1.14	-0.0145	68.8

There was no development of resistance to all the insecticides tested as revealed by the subtle changes in LC_{50} values with the advancement of generations. Furthermore, the development of resistance in *C. viridis* to insecticides will be less, as it is a seasonal pest and its infestation appears in summer months only. However, the toxicity data obtained from the present studies could be used for fixing discriminating doses and can be used in future insecticide resistance management programmes.

REFERENCES

Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol., 18: 265- 267

CCRI, 2000. Coffee guide, Central coffee research institute. 210p.

Finney, D. J. 1971. Probit Analysis, 3rd ed. Cambridge University Press. Cambridge.

Regupathy, A. and K.P. Dhamu. 2001. Statistics Work Book for Insecticide Toxicology-Second Edition. Softech Publishers, Coimbatore. 206p.

Regupathy, A., N. Chandramohan, S. Palanisamy and K. Gunathilagaraj. 2003. A. Guide on Crop Pests. 3rd Edition. Sheeba Printers, Coimbatore. 276p.

Uma Narashimham, A. 1987. Scale insects and mealybugs on coffee, tea and cardamom and their natural enemies. J. Coffee Res., 17(1): 7-13.

Venkataramaiah, G. H. and G. I. D' Souza. 1974. Resistance to parathion by green bug, Coccus viridis. J. Coffee Res., 4: 7-12. C.

C. M. Senthil Kumar and A. Regupathy
Department of Agricultural Entomology
Agricultural College and Research Institute
Tamil Nadu Agricultural University

Coimbatore 641 003 India

Arthropod Resistance

Stability of Insecticide Resistance in Diamondback Moth, *Plutella xylostella* (L.)

Diamondback moth has a long history of eventually becoming resistant to every insecticide used extensively against it and in many countries it has become resistant to every synthetic insecticide used in the field. Quick induction of resistance and reversion of susceptibility upon relaxation of selection pressure has been the unique feature of resistance in DBM.

MATERIAL AND METHODS A composite of diamondback moth larvae collected from 5 fields within 2-3 kms around Belgaum was continuously reared in laboratory under pesticide free conditions on cabbage, cauliflower, mustard and knol khol following the method described by Liu and Sun, (1984). At every fifth generation third instar (0.5±0.15 cm; 1.65±0.20 mg) F1 larvae were subjected to discriminating dose screen (Sannaveerappanavar, 1995). For discriminating dose tests with endosulfan, monocrotophos, methomyl and fenvalerate, required doses were prepared using commercial grade insecticide with analytical grade acetone (99.5 percent purity). Topical application method (FAO method no. 21) was adopted. Minimums of a hundred third instar larvae were treated in batches of ten each. For each chemical, 0.25 µl of insecticide solution was applied manually on the dorsal region with the help of micro-applicator (Burkard). Controls were treated with acetone alone. The treated larvae were transferred to plastic petri-plates (10x2.5 cm) containing cabbage leaf disc (6 cm dia). Mortality counts were taken up to 48 hr at an interval of 24 hr. In the case of cartap hydrochloride (Padan 50 WP) and *Bacillus thuringiensis* var. *kurstaki* (Biobit 50 HPWP), leaf dip method was used (Tabashnik and Cushing, 1987). Whenever the control mortality exceeded 20 per cent the data was rejected and fresh batch of larvae were used for the treatment. Survival percent was calculated using the formula outlined in NRI Manual (Anon., 1993-95).

RESULTS AND DISCUSSION Varying levels of resistance were obtained with F1 larvae of field population reared on different hosts. Variation in survival percent of the larvae reared on different host plants was evident between as well as within the test insecticides. Decline in survival percent of field-collected larvae upon removal of selection pressure is given in Table 1.

Table 1: Stability of insecticide resistance in DBM

Sl. No.	Insecticides	Host Plants	Per cent survival					
			F1	F5	F10	F15	F20	F25
1	Endosulfan	Cauliflower	70.0 ± 8.2	70.0 ± 7.1	68.9 ± 7.8	65.0 ± 7.6	56.0 ± 8.0	53.3 ± 3.3
		Cabbage	70.0 ± 8.2	-*	68.0 ± 3.0	68.0 ± 2.5	62.0 ± 7.5	57.6 ± 4.2
		Knol khol	78.0 ± 6.7	72.8 ± 2.8	66.0 ± 1.2	56.0 ± 8.0	54.0 ± 5.5	51.0 ± 3.4
		Mustard	76.0 ± 4.5	-	70.0 ± 8.2	68.0 ± 1.3	44.0 ± 1.2	40.4 ± 3.0
		Mean	**73.5 ± 3.6**	**71.4 ± 1.4**	**68.2 ±1.5**	**64.3 ± 4.9**	**54.0 ± 6.5**	**50.6 ±6.3**
2	Monocrotophos	Cauliflower	88.0 ± 4.0	76.7 ± 4.7	75.0 ± 5.5	74.0 ± 5.5	46.7 ± 1.7	42.3 ± 2.3
		Cabbage	88.0 ± 4.0	-	74.7 ± 4.7	72.0 ± 8.4	56.7 ± 4.7	50.8 ± 2.7
		Knol khol	86.0 ± 8.0	70.2 ± 2.1	68.0 ± 2.4	68.0 ± 2.0	66.0 ± 5.5	55.5 ± 3.5
		Mustard	88.0 ± 4.5	-	74.7 ± 4.7	72.0 ± 8.4	56.7 ± 7.4	54.6 ± 5.6
		Mean	**87.5 ± 0.9**	**73.6 ± 3.3**	**73.1 ± 2.9**	**71.5 ± 2.2**	**56.5 ± 6.8**	**50.8 ± 5.2**
3	Methomyl	Cauliflower	85.0 ± 6.7	76.7 ± 4.7	74.0 ± 8.7	74.0 ± 1.4	60.0 ± 8.0	52.3 ± 4.3
		Cabbage	90.0 ± 8.0	-	76.7 ± 4.7	72.0 ± 8.4	56.7 ± 4.7	49.1 ± 2.1
		Knol khol	76.7 ± 8.5	76.0 ± 4.7	76.0 ± 3.5	61.0 ± 1.2	56.0 ± 8.0	50.2 ± 2.2
		Mustard	77.0 ± 8.0	-	76.7 ± 4.7	76.0 ± 5.5	50.0 ± 1.4	48.2 ± 1.2
		Mean	**82.2 ± 5.6**	**76.4 ± 0.4**	**75.9 ± 1.1**	**70.8 ± 5.8**	**55.7 ± 3.6**	**50.0 ± 1.5**
4	Fenvalerate	Cauliflower	85.0 ± 7.6	76.7 ± 4.7	74.0 ± 8.7	74.0 ± 1.4	66.0 ± 8.7	58.7 ± 3.7
		Cabbage	83.3 ± 8.4	-	76.7 ± 7.4	76.0 ± 4.0	68.0 ± 3.0	57.3 ± 3.5
		Knol khol	92.0 ± 8.0	87.0 ± 8.0	76.7 ± 5.4	76.0 ± 6.5	74.0 ± 8.0	63.4 ± 2.8
		Mustard	92.0 ± 6.0	-	68.0 ± 8.7	76.7 ± 7.4	66.0 ± 5.5	59.8 ± 3.2
		Mean	**88.1 ± 4.0**	**81.9 ± 5.2**	**78.9 ± 5.4**	**75.7 ± 1.0**	**68.5 ± 3.3**	**59.8 ± 2.3**
5	Cartap hydrochloride	Cauliflower	20.0 ±0.0	10.0 ± 0.0	8.2 ± 2.5	4.0 ± 0.5	0.0± 0.0	0.0 ± 0.0
		Cabbage	20.0 ± 0.0	-	4.0 ± 1.0	3.0 ± 1.0	0.0 ± 0.0	0.0 ± 0.0
		Knol khol	20.0 ± 0.0	10.0 ± 1.0	4.0 ± 1.2	3.0 ± 1.0	2.0 ± 0.0	0.0 ± 0.0
		Mustard	20.0 ± 0.0	-	7.0 ± 1.7	5.0 ± 0.5	4.0 ± 1.5	0.0 ± 0.0
		Mean	**20.0 ± 0.0**	**10.0 ± 0.0**	**5.8 ± 1.8**	**3.8 ± 0.8**	**2.1 ± 1.9**	**0.0 ± 0.0**
6	**B.thuringiensis**	Cauliflower	3.3 ± 0.7	2.0 ± 0.5	2.0 ± 0.0	1.0 ± 0.2	1.0 ± 0.2	0.0 ± 0.0
		Cabbage	3.3 ± 0.7	-	1.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
		Knol khol	3.3 ± 0.7	2.0 ± 0.5	1.0 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
		Mustard	4.0 ± 1.5	-	1.0 ± 0.0	1.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
		Mean	**3.5 ± 0.3**	**2.0 ± 0.0**	**1.3 ± 0.4**	**0.5 ± 0.5**	**0.3 ± 0.0**	**0.0 ±0.0**

*not tested due to insufficient larvae

Survival percent for the four conventional insecticides declined slowly between F5 to F15 and more rapidly between F15 to F25. Since the initial survival percent was low, susceptibility was completely restored by the end of F20 and F25 for *B. thuringiensis* and cartap hydrochloride respectively. At the end of 25th generation, survival per cent on different hosts ranged from 40.4-57.6, 42.3-55.5, 48.2-52.3 and 57.3-59.8 in endosulfan, monocrotophos, methomyl and fenvalerate, respectively.

Resistance to endosulfan, monocrotophos, methomyl and fenvalerate was very persistent. While quickest decline was noticed in the case of monocrotophos resistance, it was slowest for fenvalerate. Resistance to cartap hydrochloride and *B. thuringiensis*, however, declined quickly and dissipated completely by the end of F15. Decline in persistence of

insecticide resistance, in the absence of selection pressure, varies with the resistant genotype, nature of selecting agent and intensity of resistance. Thus resistance declined slowly among the chemicals with high intensity of resistance (endosulfan, monocrotophos, methomyl and fenvalerate) compared to cartap hydrochloride and *B. thuringiensis*, in which the level of resistance was low to moderate.

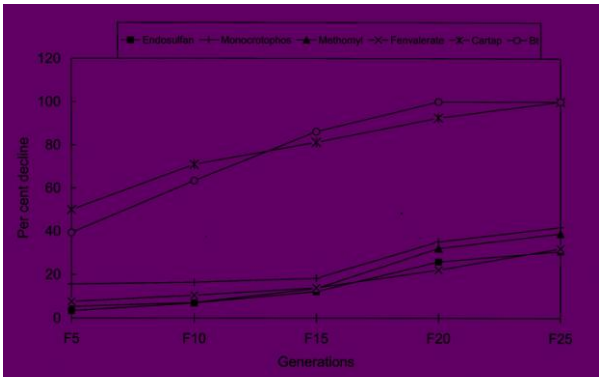


Fig. 1: Decline of insecticide resistance in DBM

These findings are in close agreement with those of Chen and Sun (1986), who opined that in moderately resistant populations, resistance to some organophosphate insecticides declined drastically after only a few generations; while in highly resistant populations, significant decline was observed only after 10-20 generations.

Instability of *B. thuringiensis* resistance in the absence of selection pressure is well documented, but at least in one case, removal of selection pressure did not influence resistance (Tabashnik *et al*., 1995). Further, coupled with the fact that revertant colonies subjected to reselection rapidly regained resistance has lead some workers (Tang and Shelton, 1995) to speculate that it could be problematic for future resistance management of DBM.

Larvae reared on cauliflower showed largest decline in monocrotophos resistance followed by methomyl and fenvalerate, while it was least in endosulfan. On cabbage, resistance to endosulfan also declined slowly compared to other conventional insecticides. Larvae reared on knol khol did not show any appreciable variation in the decline to any of the conventional insecticides. However, larvae reared on mustard recorded greatest decline in endosulfan resistance than other conventional test insecticides. Except *B. thuringiensis* and cartap hydrochloride, all test insecticides showed stable resistance, where the decline in resistance was < 50% at the end of F25. However, continued rearing up to F25, the decline in resistance seems to have attained a plateau.

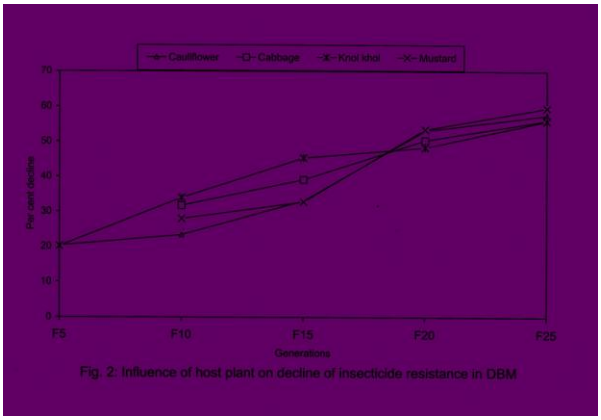


Fig. 2: Influence of host plant on decline of insecticide resistance in DBM

Thus, in the absence of selection pressure, host plants had no significant influence on the decline of resistance although resistance declined slowly over generations.

The concept of rotation assumes that individuals that may be resistant to one chemical have substantially lower biotic fitness than the susceptible individuals, so that their frequency declines during the interval between applications of the same compound. Stable resistance in the context of IRM could entail total replacement of chemicals, at least temporarily, with those unaffected by resistance problems. Though reduced biotic fitness associated with resistance is the most likely cause of instability it has not been associated with resistance in DBM.

REFERENCES

Anonymous, 1993-95, Monitoring and management of insecticide resistance in Helicoverpa armigera. NRI Manual, 33 p.

Liu, M. Y. And Sun, C. N., 1984, Rearing diamondback moth, (Lepidoptera: Plutellidae) on rape seedlings by a modification of the Koshihara and Yamada mehtod. Journal of Economic Entomology, 77: 1608-1609.

Sannaveerappanavar, V. T., 1995, Studies on insecticide resistance in the diamondback moth Plutella xylostella (L.) (Lepidoptera: Yponomeutidae) and strategies for its management. Ph.D. thesis, University of Agricultural Sciences, Bangalore.

Tabashnik, B. E. and Cushing, N. L., 1987, Leaf residue vs topical bioassays for assessing insecticide resistance in the diamondback moth, Plutella xylostella L. FAO Plant Protection Bulletin, 35: 11-14.

Tabashnik, B.E., Finson, N., Johnson, M.W. and Heckel, D.G., 1995, Prolonged selection affects stability of resistance to Bacillus thuringiensis in diamondback moth (Lepidoptera: Plutellidae). Journal of Economic Entomology, 88: 219-224.

Tang, J. D. and Shelton, A. M., 1995, Stable resistance to Bacillus thuringiensis in Plutella xylostella. Resistant Pest Management, 7: 8-9.

A. S. Vastrad, S. Lingappa, and K. Basavangoud
Dept. of Agril. Entomology, College of Agriculture
University of Agricultural Sciences, Dharwad

Discriminating Dose Technique for Monitoring Insecticide Resistance in India

INTRODUCTION More than 75 percent of the insecticides used in cotton are being targeted towards *H. armigera*. Of which, synthetic pyrethroids constitute 50 to 70 percent. In India, farmers even apply 36 to 40 rounds of pesticides to the cotton crop of a duration of 150-180 days in a single season *i.e*., one spray for every five days (Banerjee *et al.*, 2000).

Sucking pests at times reduces the crop yield to the extent of 21.2%. Among the sap feeders aphid, *Aphis gossypii* Glover, leaf hopper, *Amrasca devastans* Distant, whitefly, *Bemisia tabaci* Gennadius and thrips, *Thrips tabaci* Lind. are important. Cotton growers in India depend heavily on synthetic pesticides to combat these sucking pests; at least 2-3 sprays are directed against sucking pests (Nagia *et al*., 1989; Lingappa *et al.*, 2001; Anonymous, 1999).

Rice leaffolders generally considered once as pests of minor nature have increased in abundance and have assumed second pest status next to brown planthopper, *Nilaparvata lugens* (Stal.). The loss estimation indicated as high as 75 per cent in some areas. Among the four superficially similar species *viz., C. medinalis, M. patnalis, Marasmia trapezalis* Gn. and *Marasmia ruralis* (Walker), *C. medinalis* and *M. trapezalis* Gn were very common occur together in rice growing areas.

Diamondback moth *Plutella xylostella* (L.) is a serious pest of crucifers especially cabbage, cauliflower and mustard.

Coffee occupies a place of pride among plantation crops grown in India. Coffee green bug, *Coccus viridis* (Green) is a serious pest of coffee and shade trees in coffee plantations.

Tobacco caterpilaar, *Spodoptera litura* (Fabricius) is a poly phagous pest and is a destructive pest of wide ranging crops like, cotton, tomato, chilies, groundnut, cabbage, cauliflower etc. Wide range of insecticides including pyrethroids areused to manage this pest.

Cardamom (*Elettaria cardamomum* Maton) is known as queen of spices.

Considerable damage is done by thrips *Sciothrips cardamomi* (Ramk.), shoot and capsule borer *Conogethes punctiferalis* Guen.

NEED FOR MONITORING In view of the serious nature of above mentioned pests, usage of pesticides on cotton, rice, cole vegetables, cardamom and coffee is both extensive and intensive. The repeated application of pesticides provides ample scope for the development of resistance.

Improved monitoring of resistance would decrease the number of ineffective pesticide applications that are made when a resistance problem exists but has not been diagnosed.

Though field control failure might be due to insecticide resistance, all field control failures need not be due to resistance. It might be due to improper application and spurious material especially in India with marginal holdings and low rate of literacy. To detect the resistance in any case of field control, baseline data on the sociability and method of detection are needed. The information available on base- line data generated for the widely used insecticides in the control of these pests are compiled and presented for ready reference.

MONITORING METHODS The host specific monitoring methods identified will enable everyone to watch for the same signs that resistance problem may be occurring and the severity of the problem.

1. *H.armigera:* In the monitoring programme carried out (Regupathy *et al*., 2004) the discriminating doses for fenvalerate, cypermethrin, quinalphos, profenofos and endosulfan have been fixed using the susceptible cultures available in Australia for topical application method (Forrester and Cahill, 1987; Forrester *et. al*., 1993; Gunning *et al*., 1984).

1.Table Discriminating dose screen for *H. armigera* (After Regupathy *et. al.*, 2004 – Updated)

Chemical	DD µg / µl	Source
Fenvalerate	0.2	(Forrester and Cahill, 1987)
Cypermethrin	0.1	(Gunning *et al* ., 1984)
Deltamethrin	0.0125	
λ - cyhalothrin and β - cyfluthrinl	0.025	
	0.2	
Lambda-cyhalothrin	0.025	(Forrester *et al.*, 1993)
Endosulfan	10	
Quinalphos	0.75	
Profenofos	2	
Methomyl	1.2	
Chlorpyriphos	1	Gowthaman and Regupathy, 2003
Spinosad	10	Personal communication –protocol of CFC-NRI-ICAC- collaborative project
Thiodicarb		Personal communication –protocol of CFC-NRI-ICAC- collaborative project
1.5%W/V		
Piperony butoxide(Pbo)	50	
Propargyloxythalimide	25	
Pongamia oil	50	Gawi Gowda and Regupathy, 1995 & 1996
DEF	20	
Profenofos	0.1	
Emamectin benzoate	0.80ppm	Stanly,2004

The high-tech nature of topical application prevents many field level workers and marginal farmers adopting this due to low literacy rate in India.

In the absence of susceptible strain of *Helicoverpa armigera* in India, indirect method was used to fix discriminating doses (DD) for other methods of bioassay *viz.*, vial, bouquet, spray tower and larval dip for commonly used insecticides *viz.*, endosulfan, quinalphos, chlorpyriphos, fenvalerate and cypermethrin (Gouthaman,1994; Gouthaman and Regupathy, 2003). The DD for other methods were extrapolated by multiplying the DD available for susceptible cultures of NRI, UK and Australia by topical application with the factor of ratio of LD_{99} of topical and other methods. Validation of extrapolated DDs was done by testing on different *H. armigera* populations. The extrapolated DDs inflicted mortality with standard error (SE) varying from 3.9 to 7.1 when batches of 50 insects were used. The variation could be reduced with more number of insects per test.

2. *P. xylostella:* The leaf dip (IRAC method No.7) was used to determine the variability in baseline susceptible response to Bt in DBM field populations. Appropriate discrimination dose of Biobit 50 WP (32000 IU/mg) for third instar was 18 ppm a.i. (99% kill) (Chandrasekaran and Regupathy, 1996 a).

Discriminating doses for carbosulfan, cartap hydrochloride, fenvalerate, monocrotophos and quinalphos were fixed for different methods of bioassay viz., vial, leaf dip, larval dip and spray tower. (Chandrasekaran, 1994; Chandrasekaran and Regupathy, 1996 b,c).

2. Table Discriminating dose screen for *P.xylostella* (III instar, 1.83)

Insecticide	Method	DD	Source
Fenvalerate	vial	115ppm	Chandrasekaran and Regupathy,1996a
	spray tower	170ppm	
	larval dip	130ppm	
Quinalphos	vial	3ppm	
	spray tower	10ppm	
	larval dip	10ppm	
Monocrotophos	vial	140ppm	
	spray tower	170ppm	
	larval dip	150ppm	
Cartaphydrochloride	vial	5ppm	
	spray tower	10ppm	
	larval dip	10ppm	
Carbosulfan	vial	4ppm	
	spray tower	20ppm	
	larval dip	15ppm	
Emamectin	Leaf dip	2ppm	Lavanya, 2004
Spinosad	Leaf dip	12ppm	

The larval dip assay, though easier and economical dose not facilitate treatment in batches as the larvae get clumped up. Therefore, vial assay is suggested for adoption.

3. *S. litura:* The base line susceptibility response to five commonly used insecticides *viz.*, endosulfan, chlorpyrifos, profenofos, fenvalerate and deltamethrin in *Spodoptera litura* (Fabricius) was determined by topical application as per standard *Heliothis* susceptibility test recommended by Entomological Society of America. Third instar larvae weighing about 30-40 mg (approximately 12±0.5 mm length) 8±1d old, were used. The discriminating dose screen was fixed for monitoring the level of insecticide resistance (Niranjankumar, 1999; Niranjankumar and Regupathy, 2001 a,b.

3. Table Discriminating dose screen for *S. litura*

(Early III instar 30-40mg weight, 8 day old, 12 + 0.5 mm length)

Insecticide	Method	DD	
Endosulfan	Topical	2.0µg	Niranjankumar and Regupathy, 2001
Profenofos	Topical	3.0µg	
Chlorpyriphos	Topical	0.15µg	
Fenvalerate	Topical	0.25µg	
Emamectin	S. litura (leaf dip bioassay)	0.40ppm	Stanly,2004
Spinosad	S. litura (leaf dip bioassay)	125ppm	

4. *C. medinalis and M. patnalis:* The bioassay method described by Endo and Masuda (1981b) and Fabeller *et al.* (1988) was followed to determine DD insecticide resistance topical application of insecticides in an

aliquot of 0.5 ul on fourth instar larva weighing 20-30 mg, Based on the susceptibility nature of different populations l of *C. medinalis* and *M. patnalis* s to different insecticides, the DDs arrived for the five different insecticides were: chlorpyriphos 1.00µg, monocrotophos 0.35µg, phosalone 1.90 µg phosphamidon5.50 µg and quinalphos 0.40 µg. (Anandan, 1997; Anbalagan,2001).

5. *C. punctiferalis:* Topical assay by applying insecticide in an aliquot of 1µl on *C. punctiferalis* larvae weighing 18 - 22 mg (length 1.2 cm) . Considering the LD_{95}, the tentative DD is fixed as 0.4µg.

6. *H. antonii:* The technique recommended by the FAO for detecting resistance to insecticides was used (Muhamed and Omar, 1997) with fourth instar nymphs. Considering the LC_{95}, the tentative DD is fixed as 50 ppm (Renuka, 2001).

7. *A. gossypii:* The baseline susceptibility data were generated. (Praveen, 2003; Praveen and Regupathy, 2003 a) adopting IRAC method No. 8. The wingless adults aphids of ca 1.45mm size and weighing ca 0.19mg were used. Based on LC_{95}s of the insecticides, the discriminating doses (ppm) fixed were 10 for thiamethoxam, 20 for imidacloprid, 50 for dimethoate, 400 for methyl demeton, 40 for acephate, and 20 for monocrotophos.

8. *T. tabaci:* Leaf dip bioassay method used by Elbert and Nauen, 1996, was followed using second instar nymphs. (Praveen, 2003; Praveen and Regupathy, 2003 b).

The LC_{95} values obtained for Kumbakonam population were used as (Thanjavur Dist) considering the nil background exposure of thrips population in Kumbakonam and high susceptible nature and the following DD screen is suggested for future resistance monitoring for imidacloprid (0.7 ppm), thiamethoxam (1.0 ppm) and dimethoate (1000.0 ppm).

9. *A. devastans:* The base line data for *A. devastans* population to different insecticides was genereted following IRAC method No. 8. The DD screen was fixed considering F4 of Coimbatore population for thiamethoxam (0.008) and monocrotophos (0.55) and DD) for imidacloprid (0.005), dimethoate (400), methyl demeton (800) and acephate (850) (Jayapradeepa, 2000; Jeyapradeepa and Regupathy, 2002).

10. *B.tabaci.* The resistance frequency of *B. tabaci* population to various insecticides is monitored using DD screens following the method suggested by Elbert and Nauen (1996). The DD screen followed by PAU, Ludhiana (Regupathy *et al.*, 1998) for seven different insecticides *viz.*, thiamethoxam (10), imidacloprid (10), monocrotophos (100), acephate (100), triazophos (10), cypermethrin (50) and endosulfan (5) was used.

11. *C. viridis:* Baseline toxicity of thiamethoxam, imidacloprid and dimethoate to *Coccus viridis* (Green) by conducting acute toxicity studies by leaf dip assay. Discriminating doses of 35, 66 and 500 ppm were fixed for thiamethoxam, imidacloprid and dimethoate respectively (Senthilkumar, 2003: Senthilkumar and Regupathy 2004).

CONCLUSION Traditional monitoring involves comparisons of LD_{50}s, LD_{90}s or the dose response curves between susceptible and field poulations. DD technique is efficient in detecting low frequencies of resistance because all individuals are tested at an appropriate dose and none was wasted at lower doses.

REFERENCES

Anandan, G.K. 1997. Studies on insecticide resistance in rice leaf folder, Cnapholocrocis medinalis Guenee. M.Sc. Thesis, Tamil Nadu Agricultural University, Coimbatore.161p.

Anbalagan. G. 2001. Studies on insecticide resistance in rice leaf folder, Cnaphalocrocis medinalis Guenee. Ph.D. Thesis, Tamil Nadu Agricultural University, Coimbatore.161p.

Anonymous. 1999. Crop Production Guide. Directorate of Agriculture, Chepauk, Chennai-5

Armes, N.J., S.K. Banerjee, K.R. Desouza, D.R. Jadhav, A.B.S. King, K.R. Kranti and A. Regupathy, T. Surilivelu and R. Venugopal Rao. 1994. Insecticide resistance in 3Helicoverpa armigera in India. Recent development. In: Brighton Crop Protection Conference pests and Diseases. British Crop Protection Council, Farmham, Survey, U.K. pp. 437-442.

Banerjee, S.K., K.S.Turkar and R.R. Wanjari. 2000. Evaluation of newer insecticides for the control of bollworms in cotton. Pestology, 24(8): 14-16.

Chandrasekaran, J. 1994. Studies on the insecticide resistance in diamond back moth Plutella xylostella (L.). Ph.D. Thesis, Tamil Nadu Agricultural University, Coimbatore.161p.

Chandrasekaran, J. and A. Regupathy, 1996c. Baseline data for field monitoring of Bacillus thuringiensis resistance in Tamil Nadu (India) on Plutella xylostella (L.) population. In: III International workshop on the management of diamondback moth and other crucifer pests. Oct. 29-Nov.1, 1996, Kuala Lumpur, Malaysia, p. 332-334.

Chandrasekaran, J. and A. Regupathy. 1996a. Insecticide resistance in DBM, Plutella xylostella Lin. in India. Discriminating dose studies In: 20th International Congress of Entomology, Firenze, Italy, Aug. 25-31, Abst. 19-067p.602

Chandrasekaran, J. and A. Regupathy. 1996b. Status of insecticide resistance in field population of diamond back moth (DBM) Plutella xylostella in Tamil Nadu. IPM & sustain Agric-an Ent. Appr. 6: 95-99.

Elbert, A. and R. Nauen. 1996. Bioassays for imidacloprid for resistance monitoring against the whitefly, Bemisia tabaci. In: Brighton Crop Protection Conference: Pests & Diseases - 1996:

Volume 2: Proc. Int. Conf., Brighton, UK, Nov' 18-21, 1996. pp. 731-738.

Endo, S. and T. ~Masuda. 1981b. Evaluation of insecticides applied to rice plant for control of rice leaf roller Cnaphalocrocis medinalis Guenee. Proc. Assoc. Plant Prot., Kyushu. 27: 87-89.

Fabellar, LT., O. Kilin, S. Endo, S. Sivasubramaniam, D.K. Nagia, W. Cen, X. Tian, P. Kenmore, R.P. Basilio and O. Mochida. 1988. Monitoring susceptibility of rice pests to insecticides. IRRN, 13: 24-25.

Forrester, N.W. and M. Cahill. 1987. Management of insecticide resistance in Helicoverpa armigera (Hubner) in Australia. In: Ford, M., D.W. Holloman, B.P.S. Khambay and R.M. Sawicki (eds.) Combating Resistance to Xenobiotics: Biological and Chemical Approaches. Ellis Horwood, U.K., pp. 127 - 137.

Forrester, N.W., M. Cahill, L.J. Bird and J.K. Layland. 1993. Management of pyrethroid and endosulfan resistance in Helicoverpa armigera (Lepidoptera: Noctuidae) in Australia. Bull. Ent. Res. Suppl., No.1: 132 p.

Gavi Gowda and A. Regupathy. 1995. Pyrethroid synergism with vegetable oils. Pod borer Management Newsletter, 6: 7.

Gavi Gowda and A. Regupathy. 1996. Potential use for Pongamia oil as an adjuvant with pyrethroid insecticides. Pod borer Management Newsletter, 7: 7.

Gouthaman, S. 1994. Studies on monitoring technique for detecting insecticide resistance in Helicoverpa (Heliothis) armigera(Hub). M.Sc. Thesis, Tamil Nadu Agricultural University, Coimbatore.161p.

Gouthaman, S. and A. Regupathy. 2003. Monitoring Techniques for Detecting Insecticide Resistance in Helicoverpa armigera (Hubner). Resistant Pest Management Newsletter 13(1.) 38-41. Gouthaman, S. and A. Regupathy.. (Presented and abstract in XIII. International Plant protection Congress, 1995. In: European Journal of Plant Pathology Abs. July 2-7, 1995, The Hague, The Nether lands.)

Gunning, R.V., C.S. Easton, L.R. Greenup and V.E. Edge. 1984. Pyrethroid resistance in Heliothis armigera (Hubner) (Lepidoptera: Noctuidae) in Australia. J. Econ. Entomol., 77: 1283 - 1287.

Jayapradeepa, S. 2000. Studies on insecticide resistance in cotton leaf hopper, Amrasca devastans D. M.Sc. Thesis, Tamil Nadu Agricultural University, Coimbatore.161p.

Jeyapradeepa, S. and A. Regupathy. 2002 Generating base line data for Insecticide Resistance Monitoring in Cotton Leafhopper, Amrasca devastans (Distant) Resistant Pest Management Newsletter 11(2):1-6.

Lavanya, D. 2004. Studeis on the susceptibility of Plutella xylostella (Lepidoptera : Plutellidae) on cabbage the insecticides of new chemistry (special reference to Avermectins. M.Sc. Thesis, Tamil Nadu Agricultural University, Coimbatore, India, 59p.

Lingappa, S., S.S. Udikeri and R.N. Hegde. 2001. Field evaluation of deltamethrin formulations (Decis Tab and 1.8 EC) against cotton insect pests. Pestology, 25(2): 12-14.

Muhamed, R. and D. Omar. 1997. Susceptibility of the cocoa mirid Helopeltis theivora (Water-House) to Gamma-HCH, cypermethrin and deltamethrin. Resist. Pest Mgmt., 9(1): 34-37.

Nagia, D.K., M.L. Saini, S.P. Sharma and Sanjay Kumar. 1989. Bioassay of residual toxicity of a few organophosphorous, organochlorine and synthetic pyrethroids using cotton aphid, Aphis gossypii Glover on cotton. J. Aphidology, 3 (1&2): 143-147.

Niranjan Kumar, B.V. and A. Regupathy. 2000. Generating baseline data for insect resistant monitoring in S. litura (Fab.). Pestic. Res. J. 12(2): 232-234.

Niranjankumar, B.V. 1999. Studies on insecticide resistance in Spodoptera litura Fab. M.Sc. Thesis, Tamil Nadu Agricultural University, Coimbatore.161p.

Niranjankumar, B.V. and A. Regupathy, 2001b. Resistance levels of tobacco cutworm, Spodoptera litura (F.) to different insecticides in south India In: International Conference on Pesticides, Environment, and Food security. 19-23, November, 2001. Society of Pesticide Science India, IARI, New Delhi. p 271-272.

Niranjankumar, B.V. and A. Regupathy. 2001a. Status of Insecticide Resistance in tobacco caterpillar Spodoptera litura (Fab). in Tamil Nadu. Pestic. Res. J., 13(1): 86-89.

Praveen P. M. and A. Regupathy. 2003a. Generating baseline data for insecticide resistance monitoring in cotton aphid, Aphis gossypii Glover Resist. Pest Mgmt.,.12 (2)

Praveen, P.M. and A. Regupathy. 2003b. Insecticide resistance in field Populations of cotton thrips, Thrips tabaci Lindeman in major cotton growing regions of Tamil Nadu. Pestic.Res. J 15(2): 197-200, December, 2003.

Praveen, P.M. 2003. Studies on insecticide resistance In early season sucking pests of cotton in Tamil Nadu. Ph.D. Thesis, Tamil Nadu Agricultural University, Coimbatore.161p

Regupathy, A., D. Yadhav, S.K. Kapoor, D. Singh, K. Kranthi and D. Russel. 1998. Patterns of insecticide resistance across India in Helicoverpa armigera and Bemisia tabaci. In: World Cotton Research Conference - II. Sept' 6-12, 1998, Athens, Greece. Abst. p. 178.

Regupathy, A., Keshav Kranthi, Joginder Singh Arif Iqbal Yidong wu Derrek Russell. 2004. Patterns of Resistance levels in India, Pakistan and China. Proc. World Cotton Research Conference 3, 9-13, March, 2003, Cape Town, South Africa, 1215 -29.

Renuka, S. 2001. Studies on the bioefficacy and dissipation of profenofos applied on cardamom and cashew. Ph.D. Thesis, Tamil Nadu Agricultural University, Coimbatore.131p.

Renuka, S. and A. Regupathy. 1996. Monitoring of insecticide resistance in diamond back moth Plutella xylostella. Pestic. Res. J., 8(2): 168-171.

Senthil Kumar, C. M. 2003. Diversity of Lecanicillium (=Verticillium) lecanii (Zimmermann) Viégas and spiders in coffee ecosystem applied with thiamethoxam. Ph.D. Thesis, Tamil Nadu Agricultural University, Coimbatore.161p.

Senthil Kumar, C.M. and A. Regupathy. 2004. Generating base line data for insecticide resistance monitoring in coffee green scale, Coccus viridis (Green). Resist. Pest Mgmt. 13(5-6).

Stanley, J. 2004. Studies on base line toxicity of Emamectin and Spinosad to Helicoverpa armigera (Hubner) and Spodoptera litura (Fab.) and their bio-efficacy on brinjal fruit borers. M.Sc. Thesis, Tamil Nadu Agricultural University, Coimbatore, India

Sujatha, D. 1994. Studies on the development of resistance to carbofuran, phosphamidon and neem oil in rice BPH, Nilaparvata lugens Stal. M.Sc. (AG). Thesis, Tamil Nadu Agricultural University, Coimbatore.

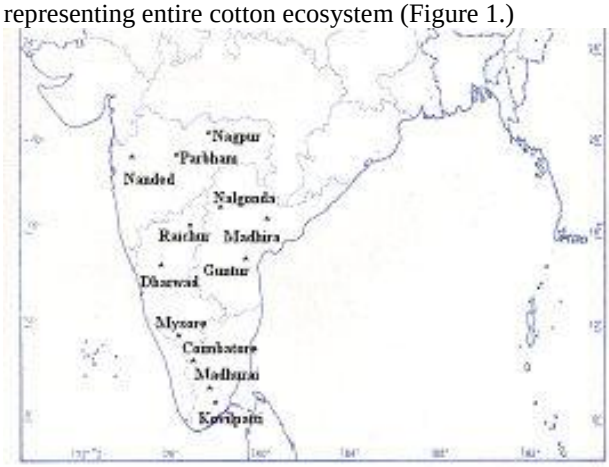
Sujatha. D. and A. Regupathy. 2003. Development of resistance to carbofuran, phosphamidon and neem oil in Rice BHP, Nilaparvata lugens Populations in Tamil Nadu, India. Pesticide Research Journal Vol. 15(2): 194-197, (Paper presented and extended abstract In: International Conference of Integrated approaching to combating resistance, April, 14-16, 1997, Harpenden, Herts UK, P.126.)

R. Ayyasamy and A.. Regupathy
Department of Agricultural Entomology
Tamil Nadu Agricultural University
Coimbatore- 641003

Insecticide Resistance vis-à-vis *Cry*1Ac delta endotoxin Resistance in South Indian Cotton Ecosystem

Insect resistance to insecticide is one of the vexing problems in recent years due to the indiscriminate use of insecticides. About 400 species of insects and Acarina are known to have developed resistance to inorganic compounds, chlorinated hydrocarbons, organophosphate insecticides and carbamates (Brown, 1978). Resistant species are not being controlled at recommended doses and higher doses may end up in uneconomical returns. The Cotton Bollworm, *Helicoverpa armigera*, is a serious, most destructive polyphagous pest attacking several crops including cotton throughout India. In recent years the pest had attained national pest status (#1) in view of severe losses caused by it in crops like cotton, chilli, peanut and tobacco.

Resistance to CHC, OP, carbamates and synthetic pyrethroids has been detected from several parts of the country by several workers (Dhingra et al., 1988; Armes et al., 1996; Mehrotra and Phokela, 1992; Kranthi et al., 2002). Recently we (Fakrudin et al. 2003) indexed resistance levels to almost all commonly used conventional insecticides representing 4 categories of insecticides (carbamates, OP. CHC. And SP) across 11 geographical locations of South India representing entire cotton ecosystem (Figure 1.)



At the time, when resistance to insecticides has become a major problem, many hoped that use of Bt would not follow the same pattern. However insect resistance to Bt toxin has been reported in several populations of cotton bollworm in Australia, USA, and China since the inception of Bt cotton cultivation (Gould et al., 1997; Guhan et al., 2001).

Laboratory studies by several workers (Kranthi et al., 2000,2001; Gujar et al., 2000;) revealed that the cotton bollworm develops resistance to Bt toxins in 6-7 generation of selection pressure. Resistance status of *H. armigera* to *cry* 1Ac toxin in 11 district geographical populations of CBW representing the entire South Indian cotton ecosystem just a year before the actual commercial approval of Bt cotton in India was reported (Fakrudin et al., 2003). Hence, development of resistance to any toxin deployed to control pest is a biological phenomenon, it is only the matter of time and tactics followed to delay.

The Biotechnological approach for the control of *H.armigera*, such as use of a Bt transgenic, generally is not used unless the commonly used chemical insecticides fails to control the pest. Development of resistance to a particular insecticide is not just dependent on the nature of target pest but also on nature of molecule, mode of action, way of deployment, crop husbandry etc. A set of insecticide molecules may have common mode of action where cross-resistance is expected. The cross resistance for molecules not sharing any such relationship also has been reported.

Kinsinger and McGaughey (1979) reported that a malathion resistant strain of the Indian meal moth, *Plodia interpunctella* (Huburer), seemed to be more tolerant to Bt (Berliner) than several malathion susceptible strains. To our knowledge, no methodical study has been conducted to compare the relative susceptibility/resistance with insecticide-resistant and insecticide susceptible insect strains with representative major of entomopathogenic toxins, under field condition.

In the present communication we report the status of relationship in cotton bollworm, *H. armigera* in natural populations across South Indian cotton ecosystem.

MATERIALS AND METHODS The larvae of *Helicoverpa armigera* (second to sixth instar) collected from 12 different geographical locations of South India were reared in the laboratory on semi-synthetic diet to get F1 homogeneous larvae for bioassay (Anonymous 1993). Larvae of 30-40 mg were used for bioassay. Six insecticides viz., monocrotophos, chloropyriphos, endosulfan, carbaryl, cypermethrin and quinalphas, which are extensively used to control bollworm in cotton ecosystems of South India, were used to determine resistance level. Different concentrations of

these selective insecticides (technical grade) were prepared in analytical grade acetone and a Hamilton microapplicator was used to deliver a 1.0 µl drop to the thorasic dorsum of each third instar larva. The larvae of check were treated with acetone alone. The concentrations were varied to obtain 20-80% mortality. Immidiately after exposure to insecticide/acetone the 30 larvae (for each insecticide) were kept individually in 30 ml plastic cup with fresh artificial diet and mortality was assessed 72 hr after treatment. The dose-mortality regressions were computed by using MLP 3.08 software (Ross, 1987). Data pertainimg to toxicity levels of 30-40 mg larvae collected from 11 geographical locations of South India using log dose probit analysis have been presented in Table 1.

Table 1. An inventory of insecticides resistance to xenobiotics (LD50) and Cry 1Ac toxin (LC50) across geographical populations during 2001-02

Location	Carbaryl	Monocrotophos	Endosulfan	Quinalphos	Chlorpyriphos	Cypermethrin	Cry 1Ac
Nagpur	2.922	13.69	4.571	2.84	2.586	1.628	0.927
Nanded	2.149	7.275	3.299	3.32	1.761	2.333	1.095
Guntur	8.728	7.027	13.156	12.564	11.038	10.917	1.044
Nalgonda	9.132	7.291	13.241	20.937	9.479	9.984	1.001
Madhira	1.122	2.14	2.676	2.193	2.11	3.038	0.927
Raichur	13.356	2.233	4.165	4.369	2.345	22.4	0.884
Dharwad	4.838	4.226	4.428	4.385	4.676	4.759	0.191
Mysore	5.151	6.115	2.294	4.155	2.22	4.054	0.26
Coimbatore	1.347	0.777	1.025	1.071	1.128	2.472	0.191
Madurai	0.781	0.452	0.74	0.5	0.36	0.143	0.177
Kovilpatti	0.873	0.308	0.906	0.941	0.463	0.192	0.147

Bioassay for Cry1Ac was carried out using 3rd instar larvae (as there is no discrimination between resistant and susceptible larvae till third day) using leaf dip method. In all 30 larvae in three replicates were tested for each treatment. Assays were performed in the laboratory at 27 + 1ºC and 70% R.H. Median lethal concentration (LC_{50}) presented in Table 1 were derived from log dose probit calculations using MLP 0.38 statistical package.

Comparison was made between the LD_{50} values of insecticides and LC_{50} values of Cry1Ac toxin resistant/susceptible cotton bollworm populations for the detection of cross relationship, if any by developing a correlation matrix using Microsoft Excel Package.

RESULTS

Carbaryl

Raichur population recorded a maximum LD_{50} value to carbaryl (13.36 µg/µL) followed by population from Nalgonda (9.13 µg/µL), Guntur (8.73), Mysore (5.15) and Dharwad (4.84). Lowest LD_{50} value was observed in population from Madurai (0.78µg/µL) followed by Kovilpatti (0.87), Coimbatore (1.35) and Madhira (1.12).

Monocrotophos

Nagpur population recorded a maximum LD_{50} value to monocrotophos (13.690 µg/µL) followed by population from Nalgonda (7.291 µg/µL), Nanded (7.275), Guntur (7.027), Mysore (6.12) and Dharwad (4.22). Lowest LD_{50} value was observed in population from Kovilpatti (0.308) followed by Madurai (0.452 µg/µL), Coimbatore (0.777).

Endosulfan

Nalgonda population recorded a maximum LD_{50} value to endosulfan (13.240 µg/µL) followed by population from Guntur (13.155 µg/µL). Lowest LD_{50} value was observed in population from Madurai (0740 µg/µL) followed by Kovilpatti (0906), Coimbatore (1.025).

Quinalphos

Maximum resistance to quinalphos was observed in Nalgonda population (20.937µg/µL) followed by Guntur population (12.564 µg/µL). Least resistance was noticed in Madurai population (0.5 µg/µL).

Chlorpyriphos

Maximum resistance to chlorpyriphos was recorded in Guntur (11.038 µg/µL) followed by Nalgonda (9.480 µg/µL). Minimum resistance was observed in Madurai population (0.36 µg/µL) followed by Kovilpatti population (0.463) and Coimbatore (1.128).

Cypermethrin

Raichur population recorded a maximum LD_{50} value to cypermethrin (22.40 µg/µL) followed by population from Guntur (10.92), Nalgonda (9.984). Lowest LD_{50} value was observed in population from Madurai (0.143µg/µL) followed by Kovilpatti (0.192), Coimbatore (2.472).

Cry1Ac toxin

Cry 1Ac protein was found to be toxic to all geographic population tested (Table 2). Compared with the others, geographic populations from Nagpur, Nanded, Guntur, Nalgonda, Madhira and Raichur were found most tolerant to the toxin. Mortality of the different populations is presented in Table 1. LC_{50} values for Cry 1Ac ranged from 0.147 to 1.095 µg/ml.

Table2.Correlation matrix showing the relation between xenobiotics and between xenobiotics and Cry 1Ac delta endotoxin

	Carbaryl	Monocrotophos	Endosulfan	Quinalphos	Chlorpyriphos	Cypermethrin	Cry1Ac
Carbaryl	1						
Monocrotophos	0.19	1					
Endosulfan	0.63	0.48	1				
Quinalphos	0.62	0.37	0.93*	1			
Chlorpyriphos	0.59	0.41	0.97*	0.90*	1		
Cypermethrin	0.95*	0.95*	0.47	0.44	0.43	1	
Cry1Ac	0.43	0.58	0.61	0.5	0.5	0.43	1

* Significant at 5% level

CORRELATIONS Correlation studies between resistance levels in terms of LD_{50}/LC_{50} values within xenobiotics and between xenobiotics and cry1Ac toxin indicated positive correlation (non-significant) in all the cases tested. The results are presented in cross-resistance within xenoibiotics is already known and reported by several workers may be due to similarity in mode of action of one xenobiotic with other. Surprisingly Table 2 indicates positive correlation values between the resistance levels of cry1Ac toxin and other xenobiotics.

Among the insecticides tested, maximum correlation value was noticed with endosulfan (r =0.61) followed by monocrotophos (r= 0.58), quinalphos(r= 0.50) and chlorpyriphos (r = 0.50). Correlation with carbaryl (r = 0.43) and cypermethrin (r = 0.43) was positive but non-significant.

How does this happen? The mode of action of Cry toxin is a clear case of interaction between Cry1Ac toxins with the receptor in midgut of the insect. The population studied in the present report was not extensively exposed to Bt toxin through the cultivation of Bt-cotton. But here and there at a very limited extent Bt sprays might have been used. This is insignificant to cause development of resistance in cotton bollworm. On a population scale, presence of alleles conferring resistance to any of the Bt toxins is a biological phenomenon. But differences between geographical populations for extent of resistance must have a reason and a cause. Can resistance to xenobiotics bring about resistance to Bt toxins to some extent? Elevated body metabolism, enzyme activity, body physiology and morphometry to some traits has been reported (Daly and Fitt 1990). It is difficult to rule out body physiology and enzyme activity to impart some amount of extended resistance to Bt toxins too. Systematic laboratory experiments should be done to elucidate cross-resistance relationships of different Bt toxins with selected xenobiotics. Elucidation and comparative analysis of genes involved in both cases would give answer at molecular level.

ACKNOWLEDGEMENTS This research work was supported by the DBT, GOI project grants to Dr. B. Fakrudin. Thanks are also due to Department of Agricultural Entomology, UAS, Dharwad for providing infrastructural facilities and all those scientists who helped us during the visit to different locations across south India for Helicoverpa armigera collection. We sincierly acknowledge the useful discussions and comments from Dr. Chandrashekhar, Dr. A.R.V. Kumar and professor K.N. Ganashaiah, UAS, Bangalore.

REFERENCES

ANONYMOUS,1993, Monitering and Management of Insecticide Resistance in Helicoverpa armigera. Training exercise on bioassay techniques for monitering insecticide resistance, NRI/ICRISAT/ICAR Collaborative Project 1993-95.

ARMES, N.J., JADHAV, D. AND DESAIZA, K.R., 1996, A survey of insecticide resistance in Helicoverpa armigera in the Indian subcontinent. Bulletin of entomological Research, 86: 499-514.

BROWN, A.W.A., 1978, Wiley Inter Science Publication.pp 19-24.

DALY, C.J. AND FITT, G.P., 1990, Monitering for Pyrethroid Resistance in Relation to body weight in Adult Helicoverpa armigera (Hubner) (Lepidoptera: Noctuidae). Journal of Economic Entomology, 83(3): 705-709.

DHINGRA, S., PHOKELA, A. AND MEHROTRA, K.N., 1988, Cypermethrin resistance in the populations of Helicoverpa armigera Hubner. Proceedings of National Academy of Sciences, 58(4) : 123-125.

FAKRUDIN,B.,BADARIPRASAD,P.R.,PRAKASH,S.H.,KRISHNAREDDY,K.B.,PATIL,B.V.AND KURUVINASHETTY,M.S.,2003, Baseline resistance to Cry1Ac toxin in cotton bollworm, Helicoverpa armigera (Hubner) in south Indian cotton ecosystem.84 (10): 1304-1307.

FAKRUDIN, B., BADARIPRASAD, P.R., PRAKASH, S.H., KRISHNAREDDY, K.B., VIJAYKUMAR, PATIL, B.V.AND KURUVINASHETTY, M.S., 2003, Insecticide resistance in cotton bollworm, Helicoverpa armigera (Hubner) in south Indian cotton ecosystem. Resistanr Pest Management Newsletter, 12(2): 13-16.

GOULD, F., ANDERSON, A., JONES, A., SUMMERFORD, D., HECKEL, D. AND LEONARD, R., 1997, Initial frequency of alleles for resistance to Bacillus thuriengiensis in Heliothis virescence. Proceedings of National Academy of Sciences, USA, 94: 3519-3523

GUHAN,L., GOULD,F., HECKEL,D.G., 2001, Identification of a gene associated with Bt resistance in Heliothis virescence. Science, 293(3): 857-859.

GUJAR,G.T., ARCHANA KUMARI, VINAYAKALIA AND CHANDRASHEKHARA,K., 2000, Spatial and temporal variation in susceptibility of the American bollworm, Helicoverpa armigera (Hubner) to Bacillus thuriengiensis Var. Kurstaki in India. Current Science, 78: 995- 1001.

KINSINGER,R.A. AND Mc GAUGHEY,W.H., 1979, Susceptibility of populations of Indian meal moth and Almond moth to Bacillus thuriengiensis. Journal of Economic Entomology, 72 : 346-349.

KRANTHI,K.R.,JADHAV,D.R.,KRANTHI,S.,WANJARI,R.R.,ALI, S.S. AND RUSSEL,D.A., 2002, insecticide resistance in five major insect pests of cotton in India. Crop Protection, 21: 449-460.

KRANTHI, K.R., KRANTHI,S. AND WANJARI,R.R., 2001, Baseline toxicity of Cry1Ac toxins to Helicoverpa armigera (Hubner) (Lepidoptera : Noctuidae) in India. International Journal of Pest Management, 47: 141-145.

KRANTHI, K.R., KRANTHI,S., ALI,S AND BANARGEE,S.K., 2000, Resistance to Cry1Ac/L-endotoxin of Bacillus thuringiensis in a laboratory selected strain of Helicoverpa armigera (Hubner). Current Science,78: 1001.

MEHROTRA,K.N. AND PHOKELA,A., 1992, Pyrethroid resistance in Helicoverpa armigera Hubner. V. Response of populations in Punjab in cotton. Resistance Research Journal,4: 59-61.

ROSS,G.J.S., 1987, Maximum likelihood programme, The Numerical Algorithms group, Rothemsted Experimental Station, Harpenden, UK, 1987.

B. Fakrudin*, Vijaykumar, K. B. Krishnareddy, B. V. Patil, and M.S. Kuruvinashetty

*Correspondance

Morphometric Differences Between Pyrethroid Resistant and Susceptible Populations of Cotton Bollworm, *Helicoverpa armigera*

The cotton bollworm, *Helicoverpa armigera* (Hubner) is a major pest of cotton throughout the world, inflicting yield losses up to 80%. Failure of cotton crop in India has been traced to resistance to commonly used insecticides. The pest management difficulties in several locations of South India involve pyrethroid resistance in *Helicoverpa armigera* (Dhingra et al., 1988; McCaffery et al., 1989). The dynamics of resistance makes it very difficult to formulate IPM strategy for its control; temporal and spatial differences have been well documented (Armes et al., 1992; Singh et al., 1994). Several morphological, biochemical and molecular variations may contribute to this. Thus in the present investigation an attempt was made to know if any relationship exists between larval resistance with morphometry of the population found in the area.

MATERIALS AND METHODS Egg or first instar larvae of *H. armigera* were collected from selected geographical locations of south India cotton ecosystem and reared in laboratory in 36 well plastic trays with a chickpea based artificial diet. After attaining a body weight of 30-40 mg, the larvae were sorted twice daily to test with the lethal doses (LD) of synthetic pyrethroid (cypermethrin). A part of the population from each location was retained and length and weight of final instar larvae was taken after paralyzing the larvae with chloroform. After formation of pupae, the above two parameters were determined with the help of scale and microbalance.

Topical application of chemical was done by means of a Hamilton microapplicator, and corresponding LD_{50} was calculated using MLP 0.38 software package. For each location 30 larvae were tested for each concentration in three replicates.

RESULTS AND DISCUSSION

Insecticide Bioassay

Raichur population recorded a maximum LD_{50} value to cypermethrin (11.309µg/µl) followed by population from Nalgonda (8.281), Guntur (7.920). Lowest LD_{50} value was observed in population from Madurai (1.648µg/µl) followed by Kovilpatti (2.196), Coimbatore (2.889). The resistance ratio (RR) against susceptible strain was found to be highest for population of Raichur (194.98 folds) followed by Nalgonda (142.77), Guntur (136.55). The least resistance ratio was observed in the population of Madurai (28.41) followed by Kovilpatti (37.86). From the bioassay study it is clear that the population from Tamilnadu and part of Karnataka (mysore) are susceptible to pyrethroids, as they recorded lower resistance levels compared to other locations.

Based on bioassay data for cypermethrin resistance (Table 1) Guntur, Nalgonda and Raichur were identified as highly resistant geographic populations and Madurai and Kovilpatti as sensitive populations. Morphometric analysis was done for these populations.

Table 1. Response of geographical population of cotton bollworm for cypermethrin bioassay during 2002-03

Location	Cypermethrin						
	LD_{50}	Fiducial limits		Slope	Chi-square	RR*	RR**
	µg/µl	Lower	Upper		Value		
Parbhani	2.626	1.892	3.603	1.822	2.171	1.59	45.27
Nanded	2.467	1.2857	4.5701	0.952	0.463	1.49	42.53
Guntur	7.92	5.793	11.495	1.807	3.121	4.8	136.55
Nalgonda	8.281	5.6491	13.87	1.413	1.051	5.02	142.77
Madhira	3.99	2.818	6.1938	1.5793	0.667	2.42	68.79
Raichur	11.309	8.7156	15.6764	2.481	3.854	6.86	194.98
Dharwad	3.335	2.414	4.75	1.76	0.551	2.02	57.5
Mysore	4.481	3.0336	7.838	1.382	1.606	2.71	77.25
Coimbatore	2.889	2.063	4.067	1.7125	0.714	1.75	49.81
Madurai	1.648	0.9299	2.4723	1.2745	1.717	1	28.41
Kovilpatti	2.196	1.5385	3.0192	1.7355	0.318	1.33	37.86

* Susceptible Madurai population
**Susceptiblestrain

Larval Length and Weight

The mean larval length was found to vary between 2.15 to 2.70. The lengthiest larva was found in Raichur (2.70 cm) followed by Guntur (2.60), Nalgonda (2.55). The mean larval weight was found to vary between 0.48 - 0.51 g across location. Heaviest larva was found in Guntur (0.51g) followed by Raichur, Nalgonda. Widest range was observed in Nanded (0.40 to 0.60 g) followed by Guntur (0.44 - 0.61 g), whereas, both larval length and weight found to be lowest for

southern most states (Tamilnadu) (Table 2).

Table 2. Range and mean of the larval weight (g) and length (cm) across the geographical populations of south Indian cotton ecosystem

	Larval weight (g)		Larval length (cm)	
Location	Range	Mean	Range	Mean
Guntur	0.44-0.61	0.51	2.20-2.90	2.6
Nalgonda	0.43-0.55	0.5	2.10-2.70	2.55
Raichur	0.45-0.61	0.5	2.00-3.20	2.7
Madurai	0.42-0.57	0.48	2.00-2.60	2.2
Kovilpatti	0.45-0.57	0.5	1.90-2.80	2.29

Pupal Length and Weight

The mean pupal length of Raichur population was found to be highest (1.75 cm) followed by Guntur population (1.74 cm). Mean pupal weight of Raichur and Guntur populations was found to be highest (0.28g) followed by Nalgonda (0.27g). Among the populations, Kovilpatti strain was lightest (0.24g) (Table 3).

Table 3. Range and mean of the pupal weight (g) and length (cm) across the geographical populations of south Indian cotton ecosystem

	Pupal length (cm)		Pupal weight (g)	
Location	Range	Mean	Range	Mean
Guntur	1.5-2.0	1.74	0.18-0.34	0.28
Nalgonda	1.5-1.9	1.64	0.20-0.36	0.27
Raichur	1.5-2.0	1.75	0.20-0.38	0.28
Madurai	1.4-1.9	1.64	0.18-0.35	0.25
Kovilpatti	1.5-1.9	1.63	0.18-0.30	0.24

Correlation Studies

Correlation between morphometric parameters and cypermethrin resistance levels showed positive and significant correlation with larval length and pupal weight (r= 0.9855 and 0.9255 respectively) and positive and non-significant correlation with larval weight and pupal length (Table 4).

Table 4. Correlation (r values) between morphometric parameters and cypermethrin resistance

	LARVA		PUPA	
	Weight	length	weight	length
Resistance	0.5938	0.9855*	0.9255*	0.7615

* Significant at 5% level

CONCLUSION It was clear that cypermetyhrin resistance levels varied greatly from location to location and it is evident that populations from northern part of south India cotton ecosystem recorded higher resistance folds for cypermethrin as well as higher phenotypic attributes when compare to southern part of south Indian cotton ecosystem. It is very difficult to say if resistance to xenobiotics is responsible for better phenotypic attributes. Higher phenotypic attributes in terms of higher larval and pupal length and weight might have strengthened the body physiology with increased enzymatic activity enabling larvae to tolerate higher doses of insecticide.

ACKNOWLEDGEMENTS This research work was supported by the DBT, GOI project grants to Dr. B. Fakrudin. Thanks are also due to Department of Agricultural Entomology, UAS, Dharwad for providing infrastructural facilities and all those scientists who helped us during the visit to different locations across South India for Helicoverpa armigera collection.

REFERENCES

ARMES, N.J., BOND, G.S. AND COOTER, R.J., 1992, The laboratory culture and development of Helicoverpa armigera. Natural Resources Institute Bulletin 57, Natural Resources Institute, Chatham, UK, 1992.

DHINGRA, S., PHOKELA, A. AND MEHROTRA, K.N., 1988, Cypermethrin resistance in the populations of Helicoverpa armigera Hubner. Proceedings of National Academy of Sciences, 58(4): 123-125.

Mc CAFFERY, A.R., KING, A.B.S., WALKER, A.J. AND EL-NAYIR, H., 1989, Resistance t synthetic pyrethroids in the bollworm, Heliothis armigera from Andhra Pradesh, India. Pesticide Science, 27: 65-76.

SINGH, H.N., JOSE ROSE. AND SINGH, H.K., 1994, Helicoverpa armigera insecticide resistance management at Varanasi. Pod borer Management Newsletter No, 4: 3.

B. Fakrudin*, Vijaykumar, K. B. Krishnareddy, B. V. Patil, and M.S. Kuruvinashetty

* For correspondence

Susceptibility of *Cyclocephala comata* Bates (Coleoptera: Sacarabaeidae) to Different Biopesticides

ABSTRACT In Mexico, the control of *C. comata* is mainly based on chemical products. The excessive use of pesticides has caused a high level of *C. comata* resistance. It is necessary to evaluate biopesticides in order to find the ones to control the pest properly. The objective of the present research is to determine the LD_{50} of plant extracts such as garlic, onion and Neem and also, entomopathogenic agents such as mushrooms (*Metarhizium anisopliae*) and nematodes (*Steinernema* and *Heterorabditis*); these results allowed us to determine the *C. comata* susceptibility grade to different biopesticides. The products were dissolved in distilled water and bioassays were carried out, one for each biopesticide. Topic application technique was used. Each biopesticide was evaluated 5 ranks per dosage on 20 larvae. Mortality was corrected by Abbot. Results were analyzed by Probit Analisis for dosage response. Results were graphed on logarithmic scale to obtain a slope. Garlic, onion and Neem treatments didn`t have control effect on larvae. *C. comata* population was very susceptible to the pathogenic mushroom *Metarrhizium anisopliae*, which LD_{50} was $2.1X10^{11}$ conidias/larva. With the *Steinernema* nematode the LD50 was 209 nematodes/larva and with *Heterorhabditis* LD50 was 42 nematodes/larva.

KEY WORDS *Cyclocephala*, *Metarhizium*, Nematodes, Biopesticide, Susceptibility.

INTRODUCTION In Mexico, soil pests represent one of the most important problems to corn crops. Jalisco, main representative state at national level, has an infested surface closely to 200,000 ha, affecting mainly the center zone, including weather areas.

In the genus of white grubs, larvae of *C. comata* are one of the main plagues affecting the soil phytosanitary characteristic of the agricultural fields in Jalisco, harming different crops, mainly maize, and consequently affecting about 29 families of plants and approximately 100 different crops. They can attack almost any crop in its different stages of growth, this attack being more critical during the 60 first days, when the larva requires ingesting of 45 to 65 times its body weight. Furthermore, as a consequence of the radicular system hurting, the incidence of soil pathogens increase, causing diseases in the crops (Moron, 1998 and 2001).

Nowadays, biological and chemical controls are the main tools used by the agriculturist to face the plague, being the ultimate method more commonly applied. Chlorates were the insecticides used at the beginning, although today there are no more in market. In the present time, the current chemical compounds are phosphorates, carbamates, and recently pyretroids. Some of them have been in the market since 15 years ago, showing high resistance levels since 1993. Today the insecticide dosages have been increased from 15 kg to 60 kg by hectare (Felix, 1990).

Bisset et al. (2000) have reported results with other different plagues that showed that after certain periods of time, by using insecticides of the same toxicological group, the insects develop some metabolic pathways that allow to them to unfold the molecules, and by doing so the insecticides become more innocuous for the insect, obtaining the denominated resistance effect. By the other side, Brogdon et al. (2000) indicate that the main metabolic routes used by insects are enzymatic ways, such as: esterases, mix function microsomic oxidases (MFO) and glutatione-S-transferases, besides piretroid compounds showed an additional mechanism called demolished resistance, and finally organophosphorate insecticides became insensible to the acetilcolinesterase.

Because today *C. comata* larvae have become more resistant to traditional insecticides (Posos, et al 1995), it is necessary to evaluate a new type of bioinsecticides. These biologic insecticides shall allow obtaining an adapted plague control, so the objective of the present work was to evaluate the biologic effectiveness of different natural agents, such as entomophatogens fungi, nematodes, and plant extracts as an alternative to control *C. comata*.

MATERIALS AND METHODS Present study was carried out with a population of *C. comata*, collected in San Martín Hidalgo, Jalisco, México from commercial crops during the cycles S/S 2001-2002. Once collected, larvae were carried to laboratories at the Centro de Investigación y Graduados Agropecuarios, CIGA-ITA26, sited in Tlajomulco de Zúñiga, Jalisco. The larvae collected were put and fed in plastic boxes with organic and ground material. Homogenized samples were obtained by selecting larvae in the third instar and according to larvae size. Immediately, they were weighed and classified in groups of 20 larvae, which were put in disposable polypropylene boxes filled with organic and ground material. In order to calculate LD_{50} of *C. comata*, bioassays were carried out using the topical application technique, proposed by FAO (Lagunes and Vázquez, 1994). In this research the treatments evaluated were: garlic extract at 500,000 ppm concentration, onion extract at 500,000 ppm concentration, neem extract at 3000 ppm concentration, *Metarhizium anisopliae* at $5x10^9$ conidias/g, *Steinernema carpocasae* $10x10^6$ nematodes/vial and *Heterorhabditis bacteriophora* $10x10^6$ nematodes/vial.

For each bioassay, were proved 5 discriminatory doses against the control test. Twenty larvae for each dosage were used, and when mortality was present in the control check, percent of mortality was calculated by Abbot's formula (1925). Results were analysed by means of the method Probit-Analysis, by Finley (1971), (cited by Lagunes and Vázquez, 1994). The digital analysis was made by using the software SPSS V. 10.0 (2001).

RESULTS AND DISCUSSION Table 1 show that assays with onion, garlic and Neem extracts do not have control on white grub. Same results were observed with *C. comata*; on neither case they cause poisoning symptoms in the larvae. In the case with entomopathogen fungi, *Metarrhizium anisopliae*, the mean lethal dose obtained was close to $2.1X10^{11}$ conidias per larva. These results are similar to those obtained by Shanon (1993) and Hidalgo (2001), where they carried out similar assays for controlling Phyllophaga, obtaining good results (Table 1).

Table 1. Lethal dosages and y fiducial limits of biopesticides in white grub *Cyclocephala comata* Bates en San Martin, Hidalgo, Jalisco, Mexico.

Treatment	Regression Equation	LD50*	Fiducials Limits 95%	LD95*	X^2	r^2
Garlic extract		No effect				
Onion extract		No effect				
Neem extract		No effect				
Metarhizium anisopliae	Y=0.0008X+4.885	2.1X10^11*	(17.5x10^10-2.5x10^11)	19.3x10^11	0.0534	0.91
Steinernema carpocasae	Y=0.0312X+38.11	209.45**	(138.97-329.46)	135509.48	0.0048	0.86
Heterorhabditis bacteriophora	Y=0.309X+66.047	41.98	(27.45-57.10)	1518.88	0.0454	0.75

*Dosages in conidias per larva

**Dosages in nematodes per larva

C. comata larvae were highly susceptible to biologic control with *Metarhizium anisopliae*. Very low doses of this agent caused a high mortality of larvae, which must allow the insect return to susceptibility.

With entomopathogenic nematodes, *C. comata* populations were very susceptible to entomopathogenic nematodes as well as with entomopathogen fungi. However, mean lethal dose in young instars of *S. carpocapsea* was estimated in 209 juveniles nematodes by larvae, as observed in the susceptibility of *C. comata* larvae with *H. bacteriophora* nematodes was higher than that obtained for *S. carpocapsea*, since only 42 juvenile nematodes by larvae were necessary as mean lethal dose. The results can be considered similar to results found by Cheng et al. (1998) and Mahr (1999), who evaluate the nematodes *H. bacteriophora* and *S. carpocapsea* in the control of *Heliothis sp.* larvae, observing a very similar behaviour to the obtained with *C. comata*.

In the table is shown regression line and confidence limits, at the 5% significant level, as a response of *C. comata* population to *Steinernema*. It can be observed that larvae population had a lower susceptibility with *Steinernema* than *Heterorhabditis*, other entomo-nematode proved, since in that case was required almost four times the number of nematodes by larva to obtain similar mortality level. However, the nematode *Steinernema* represents a good biological control alternative, mainly to insect populations that have been treated with a chemical control since a lot of years, as *C.comata* case.

The mode of action of chemical insecticides makes the insect modify or change its biochemical systems for surviving. In effect the individuals are able to develop metabolic routes for unfolding the insecticides and later, by changing to the biological control, population could be more susceptible, and thus be easier to achieve the population control. All this results in a environmental aspect better to sustainable production.

REFERENCES

Abbott, W. S. 1925. A method for computing the effectiveness of an insecticide. J. Econ. Entomology. 18: 265-267.

Bisset, A.J., Rodríguez M., Díaz C. y Soca A. 2000. Evolución de la resistencia a insecticidas en Culex quinquefasciatus (Diptera: Culicidae) en un área de la Habana, Revista cubana de Medicina tropical, 52 (3) 180-185.

Brogdon, W. G and Janet C. McAllister. 2000. Insecticide resistance and vector control. Center for Disease Control and Prevention. Atlanta, Georgia, USA. 4, (4): 605.

Cheng C. C, Tang L.C; Hou R. F. 1998. Efficacy of the entomopatogenic nematode, Steinernema carpocapsae (Rabditidae: Steinernematidae), against the Asian Corn Borer, Ostrinia furnacalis (Lepidoptera: Pyralidae). Chinese J. Entomol. 18: 51-60.

Félix, E. 1990. Plagas rizófagas de cultivos básicos de Jalisco. Dirección General de Sanidad Vegetal. SARH. Delegación Jalisco. Boletín Técnico. Pp. 2-3.

Hidalgo E. 2001. Uso de Microorganismos para el control de Phyllophaga spp. Revista ManejoIntegrado de Plagas y Agroecología. CATIE Costa Rica. 60 (2) 4-16

Lagunes, T. A. y Vázquez N. 1994. El bioensayo en el manejo de insecticidas y acaricidas. Colegio de Postgraduados. 159 pp.

Mahr S. 1999. Special Entomopathogenic Nematode Issue. In Midwest Biological Control News on line. University of Wisconsin. Vol. VI No. 11.

Morón, M. A. 1990. Los escarabajos y las plantas cultivadas. En: Conacyt Revista de Información Científica y Tecnológica. 164 (12): 48-53 p.

Morón M. A. 2001. Larvas de escarabajos del suelo en México (Coleoptera: Melolonthidae) en Acta Zoológica Mexicana. Nueva Serie (85) 45-67.

Posos, P .P., Corrales R. J., Rodríguez G. E. 1995. Laboratory Bioassay in corn In: Arthropods Management Tests. Published by Entomological Society of America. (20) 329-330.

Shanon, PJ; Smith, SM; Hidalgo E. 1993. Evaluación en el laboratorio de aislamientos costarricenses y exóticos de Metarrizhum spp. y Bauveria spp. contra larvas de Phyllophaga spp. (Coleoptera: Scarabaeidae). En Diversidad y Manejo de Plagas Subterráneas. Veracruz, México. Sociedad Mexicana de Entomología. P. 203-215.

SPSS 2001. Statistical Package for the Social Sciences. Version 10.0 Written by Gil Einstein and Ken Abernethy.

Pedro Posos Ponce
José Luis Martínez Ramírez
Centro Universitarios de Ciencias Biológicas y Agropecuarias
Universidad de Guadalajara
Calzada San Juan No. 1621

Colonia Ciudad Granja
Zapopan, Jalisco. México. 45010

Javier Carreon Amaya
Juan Carlos Serratos Arévalo
Fernando A. Peraza Luna
Intituto Tecnológico Agropecuario de Jalisco
Tlajomulco de Zúñiga , Jalisco
México

Eugenio Guerrero Rodríguez
Departamento de Parasitologia Agrícola
Universidad Autoinoma Agraria Antonio Narro
Buenavista, Saltillo, Coahuila
México

Monitoring Insecticide Resistance in Diamondback Moth, *Plutella xylostella* (L.) in Karnataka, India

ABSTRACT Insecticide resistance in diamondback moth was monitored throughout the year using diagnostic doses at different locations of Karnataka during 1998-99. The resistance level at different periods at all locations fluctuated within a narrow range. Resistance levels for endosulfan, monocrotophos, methomyl, fenvalerate, cartap hydrochloride and *Bacillus thuringiensis* Berliner was 69.40, 78.42, 71.24, 71.19, 11.89 and 3.80 per cent in Dharwad population, 74.38, 81.75, 76.58, 81.64, 10.87 and 3.35 per cent in Belgaum population, 79.96, 83.90, 82.43, 87.36, 11.89 and 5.74 in Haveri population and 61.23, 81.43, 79.18, 81.55, 20.63 and 7.17 per cent in Bidar population, respectively. All the DBM populations monitored continuously at three locations and randomly at 12 locations indicated low to moderate level of resistance to cartap hydrochloride and high level of resistance to monocrotophos, fenvalerate methomyl and endosulfan. Resistance to conventional insecticides was relatively high during winter whereas seasonal variation in resistance to *B. thuringiensis* and cartap hydrochloride was not discernible.

METHODOLOGY Larvae and pupae were collected (300-400) from fields around Dharwad, Belgaum Haveri, Bidar, Gadad and Davanagere during 1998-99 (Figure 1). A composite of larvae collected from 2-4 fields within 2-3 km served as the sample for each location. Larvae thus collected were reared on mustard seedlings and cabbage leaves following the method described by Liu and Sun (1984). Diagnostic doses (LD/LC_{99} of F_{50} laboratory population) of endosulfan (2213.01 mg ai/g), monocrotophos (1602.73 mg ai/g), fenvalerate (49.17 mg ai/g), methomyl (374.78 mg ai/g), cartap hydrochloride 2000.26 mg ai/ml) and *Bacillus thuringiensis* var. *kurstaki* (99.55 mg ai/ml) (Sannaveerappanavar, 1995) were used for monitoring. For diagnostic dose tests with endosulfan (Thiodan 35 EC), monocrotophos (Nuvacron 36 SL), methomyl (Lannate 12.5 L) and fenvalerate (Tatafen 20 EC), required doses were prepared using commercial grade insecticide with analytical grade acetone (99.5 per cent purity). Topical application method (FAO method no. 21) as outlined by Busvine (1980) was adopted. A minimum of one hundred, field collected (F_1) third instar (0.5±0.15 cm; 1.65±0.20 mg) larvae were treated in batches of ten each. Diagnostic dose @ 0.25 ml was applied manually on the dorsal region of the larvae with the help of Burkard micro-applicator.

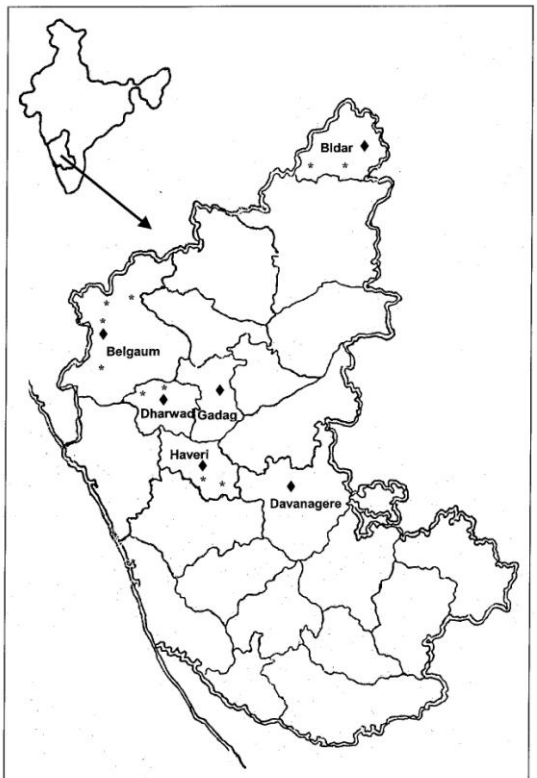


Fig.1: Karnataka map showing the areas monitored for insecticide resistance

Controls were treated with acetone alone. Treated larvae were transferred to plastic petri-plates (10x2.5 cm) containing cabbage leaf disc (6 cm dia). Mortality counts were taken up to 48 hr at an interval of 24 hr. In the case of cartap hydrochloride (Padan 50 WP) and *Bacillus thuringiensis* var. *kurstaki* (Biobit 50 HPWP),

leaf dip method described by Tabashnik and Cushing (1987) was used. Treated leaves were placed in plastic container (6 cm diameter and 8 cm height) over a moistened filter paper and ten third instar larvae were released in each petriplate. For each treatment 10 replications were maintained. Whenever the control mortality exceeded 20 per cent the data was rejected and fresh batch of larvae were used for the treatment. Survival percent was calculated using the formula outlined in NRI Manual (Anonymous, 1993-95).

ACKNOWLEDGEMENTS Authors are grateful to the Indian Council of Agricultural Research, New Delhi for the financial help.

REFERENCES

Anonymous, (1993-95) Monitoring and management of insecticide resistance in Helicoverpa armigera. NRI Manual, p. 33.

Busvine, J. R. (1980) Recommended methods for the detection and measurement of resistance of agricultural pests to pesticides: Method for the diamondback moth (Plutella xylostella). FAO Plant Protection Bulletin 28, 44-46.

Tabashnik, B. E. & Cushing, N. L. (1987) Leaf residue vs topical bioassays for assessing insecticide resistance in the diamondback moth, Plutella xylostella L. FAO Plant Protection Bulletin 35, 11-14.

FIGURES AND TABLES

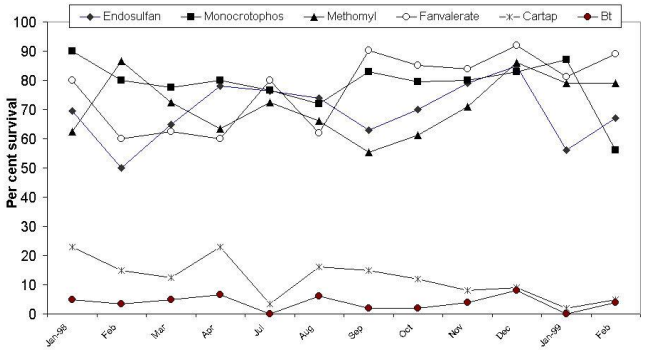


Fig.2: Dynamics of insecticide resistance in DBM at Dharwad

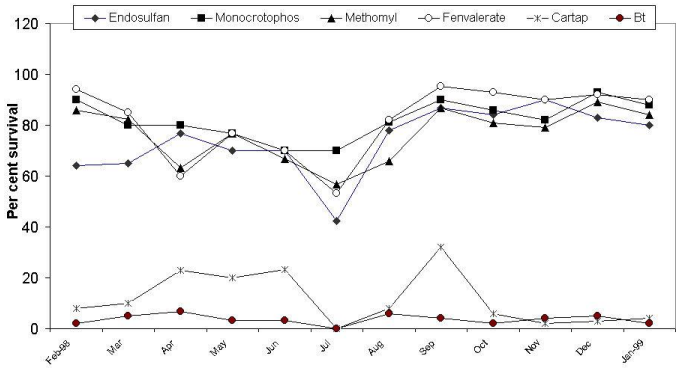


Fig.3: Dynamics of insecticide resistance in DBM at Belgaum

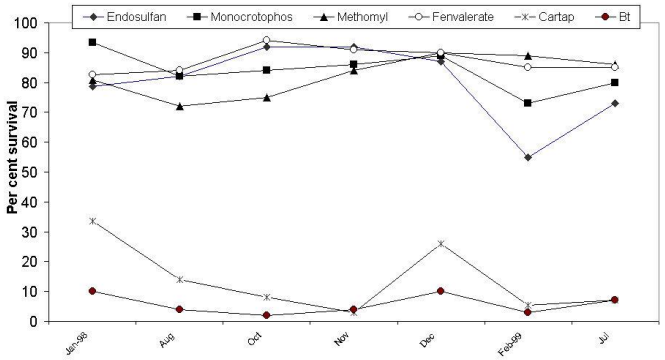


Fig.4: Dynamics of insecticide resistance in DBM at Haveri

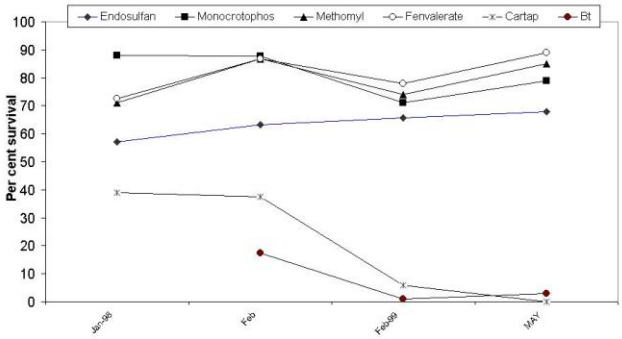


Fig.5: Dynamics of insecticide resistance in DBM at Bidar

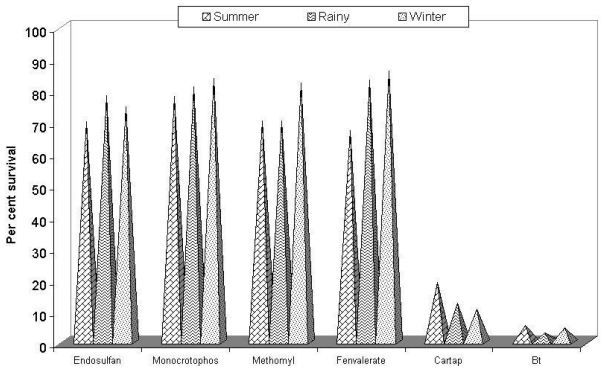


Fig.6: Seasonal variations in resistnace levels in DBM

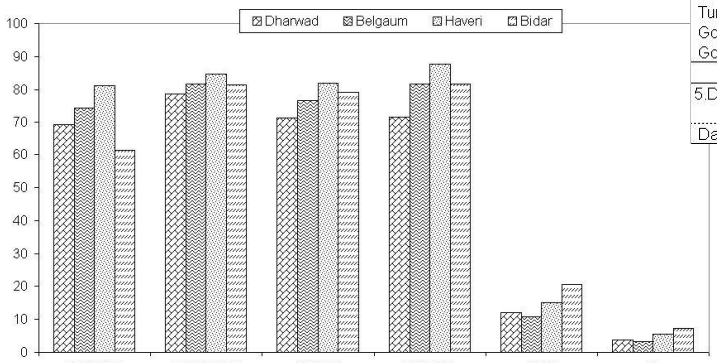


Fig.7: Resistance levels in DBM at different locations

Table 1: Survival of DBM larvae collected from different locations

District/Village	Month	Endosulfan	Monocrotophos	Methomyl	Fenvalerate	Cartap	Bt
1. DHARWAD							
Bidnal	Jan-98	30.0 ± 2.8	90.0 ± 0.0	62.5 ± 2.1	80.0 ± 1.4	23.0 ± 5.2	4.75 ± 0.9
Malanur	Jan-98	41.9 ± 1.8	97.4 ± 3.6	78.3 ± 2.4	81.7 ± 2.4	19.6 ± 4.5	12.3 ± 0.3
Shibargatti	Dec-98	88.0 ± 9.8	90.0 ± 8.9	88.0 ± 4.0	85.0 ± 5.0	5.0 ± 0.5	3.0 ± 0.5
Garag	Jan-99	75.0 ± 8.2	63.0 ± 7.8	74.0 ± 6.6	89.0 ± 8.4	3.0 ± 0.6	2.0 ± 0.4
Garag	Feb-99	68.0 ± 6.0	64.0 ± 6.6	65.0 ± 8.1	81.0 ± 7.0	4.0 ± 0.2	6.0 ± 0.5
2. GADAG							
Gadag	Jan-98	74.6 ± 4.5	96.0 ± 0.0	96.0 ± 0.0	97.5 ± 4.3	39.8 ± 4.8	5.6 ± 1.0
3. BIDAR							
Basavakalyan	Feb-98	45.0 ±5.0	90.0 ± 0.0	76.7 ± 2.5	87.5 ± 4.3	27.5 ± 4.8	7.5 ± 2.5
Humnabad	Feb-98	52.0 ± 4.2	73.3 ± 4.7	70.0 ± 4.2	90.0 ± 0.0	20.0 ± 4.3	10.0 ± 1.3
4. BELGAUM							
Bailhongal	Apr-98	53.3 ± 2.4	73.3 ± 4.4	70.0 ± 5.0	73.3 ± 4.7	20.0 ± 1.4	16.7 ± 2.5
Hirebagewadi	Jun-98	60.0 ± 8.2	86.7 ± 4.7	86.7 ± 4.7	83.3 ± 7.4	3.3 ± 0.7	3.3 ± 0.7
Tumbergaddi	Jun-98	73.3 ± 4.7	86.7 ± 4.7	80.0 ± 8.2	90.0 ± 0.0	6.7 ± 4.7	3.3 ± 0.7
Gokak	Jul-98	30.0 ± 5.0	68.3 ± 7.6	40.0 ± 5.0	73.3 ± 5.8	3.0 ± 0.5	0.0 ± 0.0
Gokak	Dec-98	64.0 ± 5.5	82.00 ± 9.0	80.0 ± 8.7	94.0 ± 4.9	3.0 ± 0.4	5.0 ± 0.5
5.DAVANGERE							
Davangere	Aug-98	66.0 ± 2.0	84.0 ± 8.0	78.0 ± 7.5	92.0 ± 4.0	5.0 ± 1.0	2.0 ± 0.4

Vastrad, A. S*, Lingappa, S. and Basavanagoud, K.
Department of Agricultural Entomology
University of Agricultural Sciences
Dharwad-580 005, Karnataka, India

*For further information, email: vastrad_v@yahoo.com or vastrad_a@rediffmail.com

Resistance in *Plutella xylostella* (Lepidoptera: Plutellidae) to the Insecticidal Crystal Toxins Produced by *Bacillus thuringiensis*

ABSTRACT The bioassays were carried out to determine the median lethal doses of Cry 1Aa, Cry 1B and Cry 2A to *P. xylostella* (L.). The results showed that Cry 1Aa was more toxic followed by Cry 1B and Cry 2A. The selection experiment from F_4 generation onwards resulted in the resistance ratio of 2.1,4.7 and 2.8 to Cry 1Aa, Cry 1B and Cry 2A respectively. To evaluate the dominance resistance mass crosses were made between susceptible and resistance population and the LC_{50} values for the F_1 hybrid was calculated. The results indicated the incompletely dominance of resistance in *P.xylostella* to these Bt toxins.

RESULTS AND DISSCUSION The results in Table 1 indicate that Cry 1Aa was most potent among the Bt toxins screened (1.25 ng/ cm^2), where as the Cry 1B and Cry 2A recorded 30.62 and 31.34 ng/cm^2 respectively. These findings support the results of Tang et al (1996) who reported that Cry 1Aa was more toxic followed by Cry 1Ac, Cry 1Ab and Cry 1C. Mandaokar et al.1998 and Ferrere et al. 1991 reported that Cry 1B was more toxic to *P.xylostella*, where as in the present investigation it ranked second. This variation in toxicity may be due to genetic variation in susceptibility to Bt. The genetic variation among the geographical populations of *P. xylostella* and minor difference in the aminoacid sequences of same toxin produced by different Bt strain may be cause for this variation in toxicity. (Tang et al. 1997 & Crickmore et

al.1998).

Table 1 : Toxicity of Bt ICPs to of *P. xylostella* (F4 generation)

δ-endotoxin	LC_{50}	LC_{90}	LC_{95}	Slope (± SEd)	Fiducial limits		Chi-square values
	ng/cm^2				Lower	Upper	
Cry1A	1.25	6.7	0.78	1.76 (± 0.21)	1.06	1.76	6.59
Cry1B	30.62	114.76	166.92	2.23 (±0.28)	25.76	36.41	7.5
Cry2A	31.34	76.91	99.21	3.28 (±0.41)	28.44	34.53	2.02

The selected *P. xylostella* populations resulted in the resistance ratio of 2.1,4.7 and 2.8 fold respectively for Cry 1Aa, Cry 1B and Cry 2A. The resistance ratios were low compared to the data reported in the earlier investigations. (Sayyed et al. 2000 and 2001). The low-level resistance probably reflects less exposure of the population under study to any of the Bt formulations. The rate of increase in toxicity in each generation (Table 3) indicated that there is slow and gradual increase in the tolerance to the Bt toxins.

Table 3: Response of Resistant and Susceptible *P. xylostella* and their hybrid F1 (RXS) to different ICP' s

Toxin /ICP	Population	Generation	LC_{50}	Fiducial limit at LC_{95}	Slope	RR[a]	D[b]	h[c]	n[d]
	Cry1A – SEL	F_9	2.06	3.71-6.04	4.54	2.11	--	--	240
Cry1A	UNSEL	F_9	0.97	2.07 – 3.69	2.82	--	--	--	240
	F_1(Cry1A R X S)	F_1	1.92	4.09 – 8.15	3.45	--	0.817	0.908	240
	Cry1B – SEL	F_9	114.54	272.88 – 496.45	3.24	4.71	--	--	240
	UNSEL	F_9	24.27	81.63 –299.77	2.03	--	--	--	240
Cry1B	F_1(Cry1B R X S)	F_1	56.64	89.83 –153.10	5.2	--	0.1	0.55	240
	Cry2A – SEL	F_9	58.86	94.50 – 119.83	6.39	2.85	--	--	280
Cry2A	UNSEL	F_9	20.62	44.49 – 80.67	3.55	--	--	--	240
	F_1(Cry 2A R X S)	F_1	43.71	73.84 – 116.49	5.03	--	0.432	0.7116	240

a - resistant ratio

b - degree of dominance

c - estimate of dominance, if the value of estimate of dominance is ' 0' it shows complete recessive and if it is ' 1' shows complete dominance of resistance

d – number of larvae

Bioassays of F_1 progeny from mass crosses between the selected sub population and unselected subpopulation expressed that LC_{50}s of F_1 progeny yielded a degree of dominance "D" values of 0.81,0.1 and 0.43 for Cry 1Aa, Cry 1B and Cry2A respectively. The estimate of dominance indicates the incomplete dominance of these toxins to *P. xylostella* . Contrarily, Tabashnik et al.1997 recessive nature of Cry 1Aa resistance in *P. xylostella*.

As per Sayyed et al. (2000) at least two different gene interactions occur, if complete or partial dominance of resistance exists in the population. To confirm the dominance of resistance it is necessary to undertake investigations at molecular level, which shall give clear indications on these finer aspects.

ACKNOWLDGEMENTS We thank R.K. Bhatnagar and R.Rajagopal for the supply of the recombinant *E.coli* strains having Bt genes. The authors are also thankful to Board of Research in Nuclear Science (BRNS) for funding this project.

REFRENCES

Crickmore, N., Zeigler, D.R., Feitelson, J., Schnepf, E., VanRie, J., Lereclus, D., Baum, J. and Dean D.H. (1998). Revision of nomenclature for the Bacillus thuringiensis pesticidal crystal proteins. Microbiol. Molecular Biol. Rev. 807 - 813.

Fletcher (1914). Some South Indian insects. Supd. Govt. Press Madras: 565.

Gujar, G.T. (1999). Farmer's fight against diamondback moth. Pestic. World. 64 - 65.

Lenin, K., Udayasuriyan, V. and Kannaiyan, S. (2001). Bacillus thuringiensis and its use in agriculture. Madras Agri. J. 88(4-6): 187 - 197.

Mandaokar, A., Chakrabarti, S.K. Rao, N.G.V., Ananda Kumar, P. and Sharma, R.P. (1998). Short communications: A fusion gene coding for two different d-endotoxins of Bacillus thuringiensis toxic to P. xylostella and useful for resistance management. World J. Microbiol. and Biotech. 4(1): 1- 3.

McGaughey, W.H. and Beeman, R.W. (1988). Resistance to Bacillus thuringinesis in colonies of Indian meal moth and Almond moth. (Lepidoptera: Pyralidae). J. Econ. Entomol. 81(1): 28 - 33.

Sayyed, A.H. and Wright, D.J. (2001). Cross resistance and inheritance of resistance to B. thuringiensis toxin Cry1Ac in Diamondback moth from low land Malyasia. Pest. Mgt. Sci. 57(5): 413 - 421.

Sayyed, A.H., Haward, R., Herrero, S., Ferre, J. and Wright, D.J. (2000). Genetic and Biochemical approach for characterization of resistance to B. thuringiensis toxin Cry1Ac in a field population of the diamondback moth, P. xylostella. App. Environ. Microbiol. 66(4) : 1509 - 1516.

Sidlyarerich, V. and Lameko, V. (2000). Some ecological peculiarities of the diamondback moth (P. maculepennis Curt.) population development. Vegetable Crop Res. Bull. 52: 31 - 38.

Suresh Ramchandran, G., Buntin, G.D., All J.N. Tabashnik, B.E., Raymer, P.L. Adang, M.J., Pulliam, D.A. and Stewart, JR. C.N. (1998). Survival, Development and oviposition of resistant Diamondback moth (L:P) on transgenic Canola producing a B. thuringiensis toxin. J. Econ. Entomol. 91(6) : 1239 -1244.

Tabashnik, B.E., Liu, Y.B., Malvar, T., Heckel, D.G. Masson, L., Bellester, V., Granero, F., Mensua, T.L. and Ferre, J. (1997). Global variation in the genetic and biochemical basis of diamondback moth resistance to B. thuringiensis. Proc. Natl. Acad. Sci. 94(24): 12750- 12785.

Tabashnik, B.E., Finson, N., Chilautt, C.F., Cushing, N.L. and Johnson, M.W. (1993): Increasing efficiency of bioassays: Evaluating resistance to B. thuringiensis in diamondback moth. (L: P). J. Econ. Entomol. 86(3): 635 - 644.

Tabashnik, B.E., Liu, Y.B. Finson, N., Masson, L. and Heckel, D.G. (1997). One gene in Diamondback moth confers resistance to four B. thuringiensis toxins. Proc. Natl. Acad. Sci. 94(5): 1640 - 1644.

Talker, N.S. (1992). Management of diamondback moth and other crucifers pests. Proceedings of the second international workshop. AVRDC. Taiwan. 603.

Tang, D.J., Collins, H.L., Roush R.T., Metx, T.D., Earle, E.D. and Shelton, A.M. (1999). Survival, weight gain and oviposition of resistant and susceptible P. xylostella (L: P) on Broccoli expressing Cry1Ac toxin of B. thuringiensis. J. Econ. Entomol. 92(1): 47 - 55.

Tang, J.D., Gilboa, S., Roush, R.T. and Shelton, A.M. (1997). Inheritance stability and lack of fitness costs of field selected resistance to B. thuringiensis in diamondback moth (L: P) from Florida. J. Econ. Entomol. 90(3): 732 - 741.

FIGURES AND TABLES

Table 2: Selection Response of *Bt* toxins over generations

Toxin	Generation	Dose ng/cm^2	Survival percentage	'n'
Cry1A	F_4	2.61	22	200
	F_5	3.058	29.5	200
	F_6	3.494	36.5	200
	F_7	3.93	41	200
	F_8	4.36	43	200
Cry1B	F_4	46.69	38	200
	F_5	54.48	45.5	200
	F_6	62.22	47	200
	F_7	70.04	52.2	200
	F_8	93.39	59.5	200
Cry2A	F_4	38.26	35	200
	F_5	42.09	42	200
	F_6	45.91	54	200
	F_7	53.57	51.5	200
	F_8	57.39	57.5	200

n – Number of larvae treated.

Shanmugam P.S.*, S.N. Tikar*, T.K. Dongre** and N.G.V.Rao*

* Insect Biotechnology Laboratory
Department of Entomology, Post Graduate Institute
Dr. Panjabrao Deshmukh Agricultural University
Akola (MS) -444 104, INDIA

**Bhaba Atomic Research Center
Trombay, Mumbai, India

*For more information, email psshanmugam@hotmail.com

Organophosphorus Resistance in *Helicoverpa armigera* and Synergism with Synthetic Pyrethroids in Central India

ABSTRACT The responses of field population of *H. armigera* to OP's during each month of the cropping season during 1999-2000 showed that resistance was wide spread but the frequency was overall moderate to low, profenofos being the best, exhibiting negligible resistance in *H. armigera*. The synergism studies showed that better synergism of organophosphorus resistance could be achieved with the use of DEM implied the role of GST as possible metabolic mechanism. Whereas the combination studies along with cypermethrin indicated that profenofos 0.1 µg and profenofos 0.1 µg + cypermethrin 0.1 µg exhibited cent percent mortality throughout the season with no resistance followed by monocrotophos 10 µg + cypermethrin 0.1 µg.

MATERIALS AND METHODS The larvae of *H. armigera* were reared on semi-synthetic diet (Armes et al., 1992) and 3rd instar stage (35-45 mg) were used for discriminating dose assays. The basis of resistance monitoring is careful calibration of the discriminating doses of insecticides. During present investigation, discriminating doses:

1. Quinalphos 0.75 µg/larva
2. Monocrotophos 1.0 µg/larva
3. Chlorpyriphos 1.0 µg/larva
4. Profenofos 0.1 µg/larva

were calibrated for 35-45 mg *H. armigera* larvae from susceptible population, which have been reported earlier. Synergists like PBO, TPP, DEM and DEF were applied 15-20 minutes prior to application of insecticide. At least 48 larvae were dosed with each insecticide solution at regular intervals. Equal numbers of larvae were simultaneously dosed with 1 µl of acetone alone to check for control mortality. Larval mortality was assessed after every 24 hours up to 168 hours. Moribund larvae giving no response to probing were treated as dead. From the fortnightly observations, monthly frequencies of resistance were worked out.

Synergistic suppression of quinalphos resistance in *H. armigera* at Akola location was studied during 1999-2000 using different synergists such as PBO 50 µg, DEF 20 µg, DEM 50 µg, and TPP 50 µg, along with quinalphos 0.75 µg/larva. Mortality was recorded up to 7 days and the levels of resistance were calculated. The difference between resistance of quinalphos and quinalphos with synergists were recorded as percent resistance suppression. Similarly, toxicity studies of organophosphates with pyrethroid and some synergists were conducted by conducting bioassays. The values of median lethal dose (LD_{50}) for each insecticide with and without synergists were worked out month wise by subjecting the mortality data to Probit analysis of (Finney, 1977). The resistance level for each insecticides and synergists was

calculated by comparing the LD_{50} of organophosphates with LD_{50} of synergist with organophosphates.

FIGURES AND TABLES

Table 1: Organophosphate Resistance Frequencies in *H. armigera*

Months	Per cent Resistance to			
	Quinalphos	Monocrotophos 1.0 µg/larva,	Chlorpyriphos	Profenofos
	0.75 µg/larva		1.0 µg/larva	0.1 µg/larva
Aug. 99	14.01	41.66	26.24	0
Sept. 99	2.96	44.16	22.12	0
Oct. 99	13.81	28.75	19.23	0
Nov. 99	1.04	30.83	16.27	0
Dec. 99	13.58	26.03	24.48	0
Jan. 2000	14.77	24.16	23.95	0
Feb. 2000	10.41	32.19	29.93	0
Mar. 2000	6.73	36.26	30.42	0

Table 2: Quinalphos Resistance Suppression by Synergists in *H. armigera*

Months	Per cent Resistance to Quinalphos 0.75 µg/larva			
	PBO 50 µg	TPP 50 µg	DEM 50 µg	DEF 20 µg
Aug. 99	-6.82	-15.15	14.01	9.85
Sept. 99	-38.7	-17.87	2.96	-5.37
Oct. 99	-19.52	-15.35	13.81	5.48
Nov. 99	-23.95	-15.61	1.04	-3.11
Dec. 99	-23.92	-11.42	13.58	1.08
Jan. 2000	-10.23	-6.06	14.77	6.44
Feb. 2000	-18.75	-6.25	10.41	6.25
Mar. 2000	-14.1	-5.77	6.73	-1.6

Table 3: Organophosphorus Resistance suppression in combination with cypermethrin

Months	OP resistance frequencies alone				In combination with cypermethrin				Per cent resistance suppression		
	Quinal 0.75 µg/larva	Mono 1.0 µg/larva	Chlor 1.0 µg/larva	Prof 0.1 µg/larva	Quinal + Cyper 0.1	Mono + Cyper 0.1	Chlor + Cyper 0.1	Prof + Cyper 0.1	Quinal – (Quinal + Cyper 0.1)	Mono -- (Mono + Cyper 0.1)	Chlor - (Chlor + Cyper 0.1)
Aug. 99	14.01	41.66	26.24	0	1.09	22.23	24.37	0	12.92	19.43	1.87
Sept. 99	2.96	44.16	22.12	0	1.04	21.45	19.27	0	1.92	22.71	2.85
Oct. 99	13.81	28.75	19.23	0	2.5	22.56	10.14	0	11.31	6.19	9.09
Nov. 99	1.04	30.83	16.27	0	0.8	21.87	15.25	0	0.24	8.96	1.02
Dec. 99	13.58	26.03	24.48	0	1.04	15.65	17.22	0	12.54	10.38	7.26
Jan. 2000	14.77	24.16	23.95	0	0	14.77	22.28	0	14.77	9.39	1.67
Feb. 2000	10.41	32.19	29.93	0	3.12	23.48	24.49	0	7.29	8.71	7.44
Mar. 2000	6.73	36.26	30.42	0	2.17	17.13	27.17	0	4.56	19.13	3.25

REFERENCES

Abbot, W. S. (1925): A method of computing the effectiveness of an insecticide. J. Econ. Ent. 18: 265-267.

Armes N. J.; G.S. Bond and R. J. Cooter (1992): The laboratory culture and development of Helicoverpa armigera. Natural Resources Institute Bulletin 57, NRI, Chatham, UK

Armes, N.J.; D.R. Jadhav and K.R. DeSouza (1996): A survey of insecticide resistance in H. armigera in the Indian subcontinent. Bull. Entomol. Res. 86: 499-514

Dhingra, S.; A. Phokela and K. N. Mehrotra (1988): Cypermethrin resistance in population of Heliothis armigera (Hubner). Nat. Acad. Sci. Letters. 11: 123-125

Finney, D. J. (1952): Probit analysis. Cambridge University Press. 2nd Edn. Pp. 318.

Gunning, R. V.; C. S. Easton; M. E. Balfe and I. G. Ferris (1991): Pyrethroid resistance mechanism in Australian Helicoverpa armigera. Pesti. Sci. 33(4): 473-490.

Kranthi K. R., N. J. Armes; N. G. V. Rao; SheoRaj and V. T. Sundarmurthy (1997): Seasonal dynamics of metabolic mechanisms mediating resistance in Helicoverpa armigera in central India. Pestic. Sci. 50: 91-98.

McCaffery, A. R.; A. B. S. King; A. J. Walker and H. Nayir (1989) : Resistance to synthetic pyrethroids in the bollworm H. armigera from Andhra Pradesh, India. Pestic. Sci. 27: 65-76.

Mehrotra, K. N. and Amrita Phokela (1992): Pyrethroid resistance in Heliothis armigera Hubner. Response of pyrethroid in Punjab. Pesti. Res. J., 4: 59-61.

Pawar, C. S. (1998): Helicoverpa - A national problem which needs a national policy and commitment for its management. Pestology 22(7): 51-59.

Pawar, S. A. and A. K. Mali (1992): Effect of insecticide mixtures against Helicoverpa armigera Hubner. Pestology. 21(3): 36-38.

Peter, A. J., K. Srinivasan and S. Chelliah (1997): Laboratory evaluation of insecticides and their combinations against bollworms, Helicoverpa armigera (Hub.). Pestology. 21 (11): 19-21.

Price, N. R (1991): Insect resistance to insecticides: mechanisms and disgnosis. Comp. Biochem. Physiol. 100: 319-326.

Reed, W. and C. S. Pawar (1982): Heliothis: a global problem. Proc. International Workshorp on Heliothis Management ICRISAT, Patanchern, A.P.(Indin). pp: 9.

Regupathy, A. and G. A. Ashokan (1996): Helicoverpa armigera insecticide resistance management research in India, PodborerManagement Newsletter: 7.

Satpute, U. S.; D. N. Sarnaik; B. S. Rajurkar and P. B. Bhalerao (1986): Assessment of losses due to sucking pests and bollworms of cotton. P K V Res. J. 10: 75-76.

Vadodaria, M. P.; R. B. Patel; I. M. Maisuria; U. G. Patel and R. H. Patel (1998) Bioefficacy of Dipel ES as biopesticides for the management of bollworms of cotton. Pestology, 23(12): 13-15.

Y. T. Jadhav, N.G.V. Rao*, N.S. Satpute, S. N. Tikar, M. P. Moharil and S. A. Nimbalkar

Insect Biotech Laboratory, Department of Entomology
Dr. Panjabrao Deshmukh Agricultural University
Akola 444104 (MS), India

*For more information, email ngvrao@pdkv.mah.nic.in

Fungicide Resistance

In vitro Evaluation of Fungicides Against Stem-end Rot Causing Pathogens in Citrus

Citrus cultivars are of major economic importance and rank next to grapes in world food production. The Punjab state of India is well known throughout the country for production of high quality citrus fruit, but production is rather low during last few years due to preharvest stem-end rot in orchards. In spite of fungicidal application by farmers, pathological fruit rot in the months of September and October is causing great economic losses. In the recent study, stem-end rot of Kinnow (a hybrid of king mandarin and willow) was observed to be due to *C. gloeosporioides* isolates Cg-1 and Cg-2, *Gloeosporium limetticola* and *Diplodia natalensis* in Punjab (Kaur, 2000). The great economic loss occurred even after the application of recommended fungicides against these pathogens, thus an effort was made to observe the efficacy of different fungicides against the pathogens under *in vitro* conditions.

MATERIALS AND METHODS Diseased fruits were collected during October and November from New Orchard, Department of Horticulture, Punjab Agricultural University, Ludhiana, Punjab, India. Isolations of pathogens were done on potato dextrose agar medium. Efficacy of five commercial fungicides aureofungin (antibiotic), Bavistin (carbendazim), Blitox (cu-oxychloride), Indofil M-45 (mancozeb) and Kavach (cholorothalonils) in inhibiting mycelial growth of stem-end rot causing pathogens was tested by employing poisoned food technique. Potato dextrose agar was amended with fungicide concentrations of 10, 50, 100, 200, 500 and 1000 µg/ml, on active ingredient basis.

RESULTS

D. natalensis

Carbendazim caused complete inhibition of mycelial growth of *D. natalensis* at 10 µg/ml concentration (Figure 1). ED_{50} for aureofungin and chlorothalonils was 25.22 µg/ml and 2.66 µg/ml respectively (Table 1). The comparison of relative potency of fungicides revealed that carbendazim has maximum relative potency in comparison to cu-oxychloride and mancozeb for the mycelial growth inhibition of *D. natalensis* (Table 2). The goodness of fit tests (p-values = 0.00, 0.00; d.f 24) and the probability plot suggested that the Weibull distribution did not fit the data adequately. Since the test for equal slopes was significant (p = 0.00, d.f 4), the comparison of different fungicides against *D. natalensis* will not be similar regardless of concentration level. Carbendazim was found to be the most potent fungicide against *D. natalensis* as the survival probability associated with it was very less (0.00 %) followed by chlorothalonils, aureofungin, mancozeb and cu-oxychloride (Table 3).

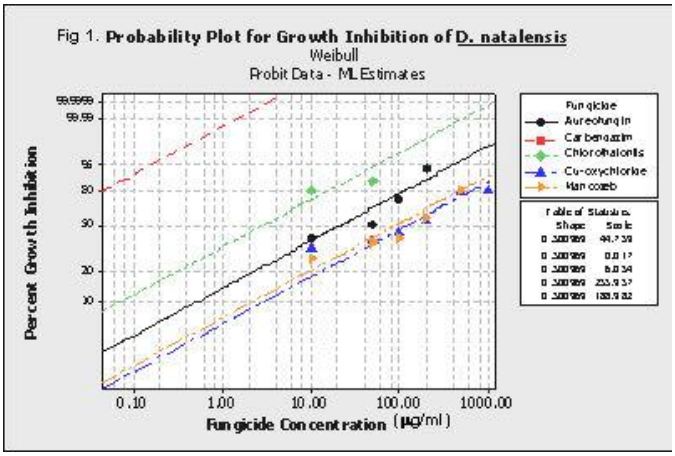


Fig 1. **Probability Plot for Growth Inhibition of D. natalensis**

Table 1. ED50 value for different fungicides against stem-end rot causing pathogens in citrus

Fungicides	ED_{50} values (µg/ml)			
	D. natalensis	Cg-1[a]	Cg-2[b]	*G. limetticola*
Aureofungin	25.22	10	0.53	-*
Carbendazim	-*	8.78	12.63	31
Cu-oxychloride	87.31	59.18	58585	10297.4
Mancozeb	82.68	0.34	17.4	117.27
Chlorothalonils	2.66	177658.4	2205.2	500.6

* Complete growth inhibition at all the concentrations

a slow growing and b fast growing isolates of *C. gloeosporioides*

Table 2. Relative potency of different fungicides in inhibiting mycelial growth of stem-end rot causing pathogens

Comparison of fungicides	Relative Potency			
	D. natalensis	Cg-1[a]	Cg-2[b]	*G. limetticola*
Aureofungin vs carbendazim	0	0.51	8.55	15293
Aureofungin vs chlorothalonils	0.1	3170.96	955.7	213742
Aureofungin vs cu-oxychloride	5.7	21.45	1496.16	1193832
Aureofungin vs mancozeb	4.2	5.22	12.18	84920
Carbendazim vs chlorothalonils	352.6	6177.7	111.72	14
Carbendazim vs cu-oxychloride	14908.3	41.79	174.9	78
Carbendazim vs mancozeb	11007.4	10.18	1.42	6
Chlorothalonils vs cu-oxychloride	42.3	0.01	1.57	6
Chlorothalonils vs mancozeb	31.2	0	0.01	0
cu-oxychloride vs mancozeb	0.7	0.24	0.01	0

a slow growing and b fast growing isolates of *C. gloeosporioides*

Table 3 Survival probabilities of stem-end rot causing pathogens when confronted with different fungicides

Fungicides	Survival probability			
	D. natalensis	Cg-1[a]	Cg-2[b]	*G. limetticola*
Aureofungin	0.35	0.08	0.06	0
Carbendazim	0	0.03	0.23	0.7
Cu-oxychloride	0.64	0.51	0.74	0.84
Mancozeb	0.6	0.29	0.26	0.6
Chlorothalonils	0.06	0.93	0.7	0.7

* Complete growth inhibition at all the concentrations

a slow growing and b fast growing isolates of *C. gloeosporioides*

C. gloeosporioides Isolate Cg-1

Aureofungin and carbendazim at concentration of 50 µg/ml were most effective in inhibiting mycelial growth of *C. gloeosporioides* Isolate Cg-1 and inhibited 100 per cent mycelial growth at this concentration (Figure 2). Mancozeb was also effective having ED_{50} value 0.34 µg/ml (Table 1). Relative potency of carbendazim verses chlorothalonils was followed by aureofungin verses chlorothalonils (Table 2). The data for mycelial growth inhibition of Cg-1 after treatment with different fungicides suggested that it did not follow the Weibull distribution (p-values = 0.00, 0.00, d.f. 24). The test for equal slopes (p = 0.00, d.f.4) was found to be significant; therefore, the fungicides differed in their action against Cg-1 isolate of *C. gloeosporioides* regardless of the concentration level. On the basis of survival probability, carbendazim was found to be the most potent fungicide against Cg-1 isolate of *C. gloeosporioides* as it was very less (0.03 %) followed by aureofungin, mancozeb, cu-oxychloride and chlorothalonils (Table 3).

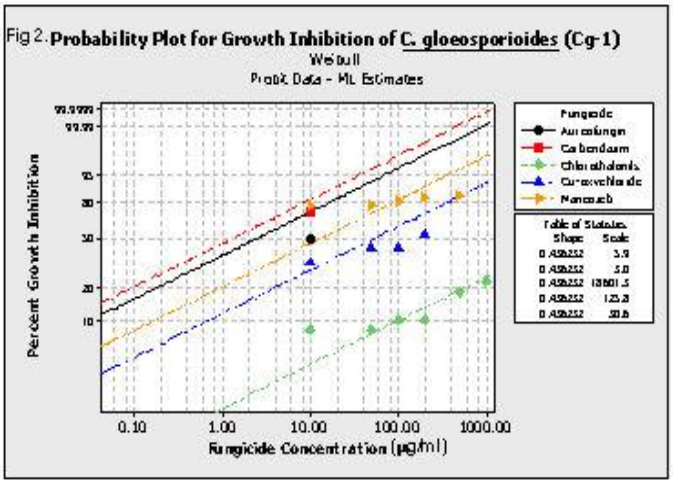


Fig 2. Probability Plot for Growth Inhibition of C. gloeosporioides (Cg-1)

C. gloeosporioides Isolate Cg-2

Aureofungin and mancozeb at concentration of 200 µg/ml were effective, in inhibiting mycelial growth of Cg-2 while carbendazim was effective at 500µg/ml cu-oxychloride and chlorothalonils were ineffective even at 1000 µg/ml concentration (Figure 3). Relative potency values (Table 2) showed that aureofungin had maximum potency verses cu-oxychloride. The test of goodness of fit (p = 0.00, 0.00 d.f. 24) and probability plot indicated that the data for mycelial growth inhibition of isolate Cg-2 isolate of *C. gloeosporioides* due to different fungicides did not fit in the Weibull distribution adequately. The comparison of different fungicides for their potency in inhibiting the mycelial growth showed that they were significantly different in their action. Since the tests for equal slopes was significant (p = 0.00, d.f. 4). As survival probability rate associated with aureofungin was least in comparison to other fungicides so it was considered most potent against Cg-2 (Table 3).

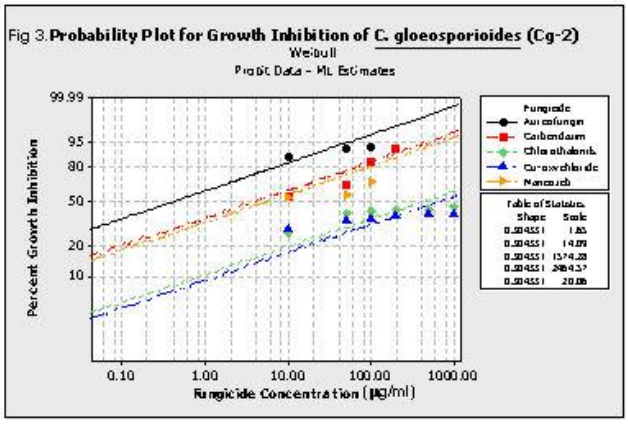
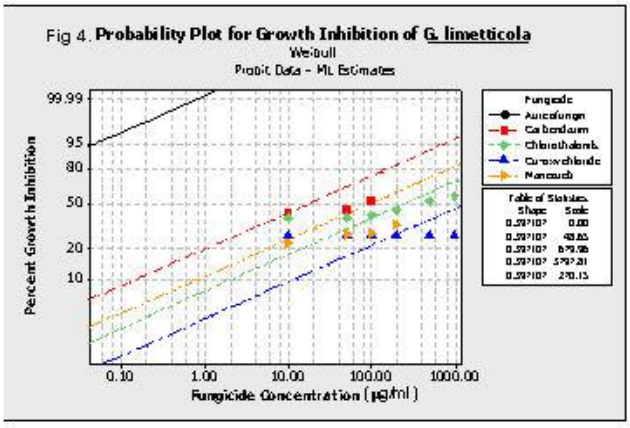


Fig 3. Probability Plot for Growth Inhibition of C. gloeosporioides (Cg-2)

G. limetticola

The growth of *G. limetticola* was inhibited by aureofungin at 10 µg/ml while carbendazim and mancozeb were effective at 200 µg/ml and 500 µg/ml, respectively (Figure 4). This is further substantiated by the maximum relative potency values for aureofungin vs cu-oxychloride. The Weibull distribution did not fit on the data for inhibition of mycelial growth of *G. limetticola* (p = 0.00, 0.00, d.f 24). Aureofungin was appeared to be the most potent fungicide for inhibition of *G. limetticola* as value for survival probability for it was the lowest (Table 3).



DISCUSSION *In vitro* evaluation of fungicides against stem-end rot pathogens revealed that aureofungin was most effective against *G. limetticola*, *C. gloeosporioides* isolate Cg-1 and Cg-2 but less effective on *D. natalensis*. Carbendazim was effective on *D. natalensis* and Cg-1 at <10 µg/ml and 8.78 µg/ml concentrations. Brown (1984) and Das & Dubey (1987) found carbendazim most effective against stem-end rot of citrus due to *D. natalensis* and similar results were obtained during present studies. Mancozeb and cu-oxychloride were found ineffective in inhibiting mycelial growth of *D. natalensis*. Balasubramaniam (1991) suggested that pre-harvest stem-end rot of *Citrus aurantifolia* could be controlled by monthly spray of Carbendazim (1%) or Mancozeb (0.2%). Ramanjulu and Reddy (1989) reported stem-end rot of *C. sinensis* (L.) Osb. due to *G. limetticola* can be controlled by three sprays of 0.1 per cent carbendazim 50 WP applied in July- August and September and cu-oxychloride @ 0.3 per cent was not effective but Aureofungin @ 0.05 per cent gave statistical better control over untreated fruits. Mancozeb was effective against Cg-1 and Cg-2 having ED50 value 0.34 and 17.40 µg/ml. Chlorothalonils was effective against only *D. natalensis* having ED_{50} value 2.66 µg/ml. Cu-oxychloride was effective against Cg-1 at 500 µg/ml and on the other pathogens it was not able to inhibit mycelial growth even at 1000 µg/ml. Botelho et al (2000) tested different calcium chloride (CaCl2.2H2O) concentrations in post-harvest treatments of guavas (*Psidium guajava* L.) 'Branca de Kumagai' by the temperature differential method (fruits at 26ºC and solution at 5ºC by 2 hours) in order to study its effect on control of anthracnose decay (*Colletotrichum gloeosporioides* Penz.). It was verified that calcium chloride stimulated the development of this fungus up to a certain concentration; above that, there was an inhibitory effect. Peres, et al (2002) developed a fungicide application decision (FAD) support system for *C. gloeosporioides* causing post bloom fruit drop of citrus (PFD). The PFD-FAD system considers previous history of PFD in the grove, susceptibility of the citrus species, the stage of the bloom as well as rainfall, duration of leaf wetness following the rain, and the current inoculum levels in the grove. It predicts the need for a fungicide application based on these factors and the time since the last application. The PFD-FAD system is easy to use and minimizes the need for scouting of groves and acquisition of exact weather information, and is more widely applicable to other regions. All programs reduced counts of persistent calyces by about 50% and increased fruit counts by about 20%. Cost savings with the use of PFD-FAD was about $47/ha. Cu-oxychloride presently most sought out fungicide in Punjab for the control of stem-end rot of citrus was found effective only against Cg-1 and on other pathogens it was not able to inhibit mycelial growth showing maximum survival probabilities (50 - 84 %) for all pathogens as compared to rest of the four selected fungicides. Therefore, it was found to be the least effective in inhibiting the mycelial growth of stem end rot causing pathogens. Since, presently farmers are depend more on copper fungicides, it may be the reason of aggravated problem of stem-end rot being faced in the state. In conclusion, although extremely high levels of resistance were attained for these fungicides, yet these differences may not necessarily translate to the reduction or loss of field performance of them. We must exercise caution in directly extrapolating results from laboratory experiments to the field situations. Pathogen isolates found in the fields are usually more heterogeneous and their responses to fungicide pressures are more complex and diverse. Field responses would be the result of the interactions of environment, population structure and selection intensity. The alteration of fungicides and immigration of susceptible isolates from other crops could delay the evolution of resistance in the field. Many of the fungicides used to control stem end rot in citrus are rendered ineffective more easily because of the occurrence of cross and multiple resistance.

REFERENCES

Balasubramaniam, P. (1991). Efficacy of fungicides on pre-harvest stem-end rot of acid lime caused by Colletotrichum gloeospoirioides. Penz. Madras Agric. J. 78: 129-130.

Botelho, R.V., Souza, N.L. de and Peres, N.A.R. (2000). Effect of the postharvest treatment with calcium chloride by the

temperature differential method on the control of Colletotrichum gloeosporioides in guavas 'Branca de Kumagai'. Summa Phytopathologica 26, 268-271.
Brown, G. E. (1984). Efficacy of citrus post harvest fungicides applied in water or resin solution waster wax. Pl. Dis. 68: 415-418.
Das, B. C. and Dubey, L. N. (1987). Effect of some chemicals on pre-harvest stem-end rot of Khasi manadarin in Assam. Indian. J. Mycol. Pl. Path. 17: 207.
Finney, D. J. (1971). Probit analysis, 3rd ed. Cambridge University Press, Cambridge, England.
Kaur, R. (2000). Studies on stem end rot in Kinnow orchards. M.Sc. thesis, Punjab Agricultural University, Ludhiana, Punjab, India. pp-80.
Le Ora Software. (1987). Polo - PC: a user's guide to probit or logit analysis. LeOra Software, Berkeley, CA.
Penzig, O. (1982). Fungi agrumicoli, contribuzione allo studio dev funghi parasiti . Degli Agrumi: pp 124.
Peres, N. A. R., Kim, S., Beck, H. W., Souza, N. L., Timmer, L. W. (2002). A fungicide application decision (FAD) support system for postbloom fruit drop of citrus (PFD). Online. Plant Health Progress doi: 10.1094 PHP-2002-0731-01-RV.
Ramanjulu, V. and Reddy, M. R. S. (1989). Preharvest stem end rot of satgudi sweet oranges caused by Gloeosporium limetticola. Indian Phytopath 42: 108-09.
Reddy, M. R. S., Naidu, B. C and Raju, D. G. (1985). Pre-harvest stem-end rot of Satgudi sweet orange caused by Gloeosporium limetticola. Indian Phytopath 38 : 169-70.
Srivastava, M. P. and Tandon, R. N. (1971). Efficacy of certain fungcides and an antibiotic against four isolates of Botryodiplodia theobromae. Indian Phytopath. 24: 396-97

Ramandeep Kaur and H S Rewa1
Department of Plant Pathology
Punjab Agricultural University
Ludhiana, Punjab, India-141004

Amit Sethi
Department of Entomology and Nematology
University of Florida
P.O.Box 110620
Gainesville, FL, 32611-0620

Herbicide Resistance

Occurrence of Resistant *Chrysanthemum coronarium* to ALS Inhibiting Herbicides in Israel

INTRODUCTION *Chrysanthemum coronarium* L., an annual weed, belongs to the Compositae, is native in the Mediterranean and most abounded in the Middle East countries. In Israel, it is considered a serious weed of rainfed cereals and pulses and at high infestation rate it competes vigorously with the crops and reduces their economic yields. Sulfonylureas (SU) herbicides such as chlorsulfuron and tribenuron were introduced in the mid 1980's and subsequently were used efficiently and intensively to control broad-leaf weeds in wheat. In 2001 the first biotype of *C. coronarium* with suspected resistance to SU herbicides was identified in Gilat experimental farm, and later a second biotype was reported in Beeri, Israel (Figure 1).

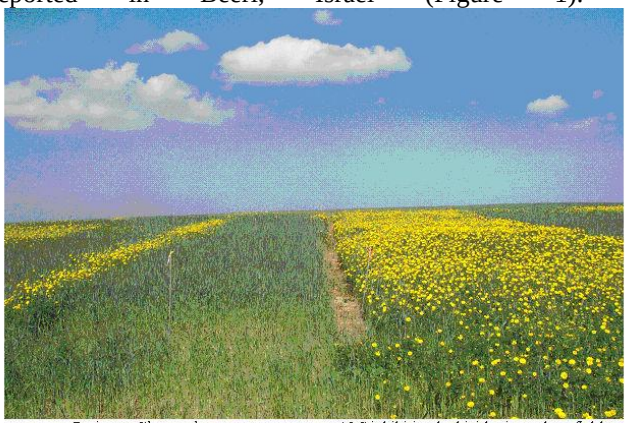


FIGURE 1: Resistant *Chrysanthemum coronarium* to ALS inhibiting herbicides in a wheat field near Beeri, Israel. The right and left plots were treated post-emergence with 22.5 g a.i/ha tribenuron and 480 g a.i/ha bromoxynil, respectively.

Today, there are more than 50 commercial acetolactate synthase (ALS; EC 4.1.3.18) inhibiting compounds grouped into five chemically dissimilar classes: the SU, the imidazolinones (IMI), the triazolopyrimidines (TP), the pyrimidinylthiobenzoates (PTB) and the sulfonylaminocarbonyltriazolinones (SCT). Unfortunately, the frequent use of these herbicides rapidly select resistant weed biotypes, and now, there are more than 80 weed species that evolved resistance to ALS inhibiting herbicides worldwide (Heap 2004). The objectives of the present study were to 1) examine and verify under controlled conditions the resistance of *C. coronarium* on both whole-plant and enzyme levels, and 2) identify differences in the nucleotide and amino acid sequences of ALS between the resistant (R) and susceptible (S) biotypes.

MATERIALS AND METHODS In the study we used two resistant *C. coronarium* populations, Gilat and Beeri, which were discovered in southern Israel as a result of failure of SU herbicides to control weeds in wheat. Susceptible populations were collected from nearby organic fields that have never been treated with herbicides. Seeds were planted in 200-ml plastic pots and grown in a net-house under the Israeli winter conditions. To assess herbicide sensitivity, seedlings at the three- to four-leaf stage of development were sprayed with different commercial herbicides, using motorized laboratory sprayer equipped with a flat-fan nozzle (8001E) calibrated to deliver 300 litre/ha at 245 kPa. Herbicides were applied at increasing rates of the recommended field dose. Three weeks after treatment, the plants were cut at the soil surface and their fresh weight was determined. ALS crude enzyme was extracted from R and S seedlings and assayed as described by Sibony et al. (2001). The effective dose of herbicide causing 50% reduction in shoot fresh weight (ED_{50}) and the herbicide concentration required to

decrease ALS activity by 50% (IC_{50}) were calculated using SlideWrite Plus® software capable of nonlinear regression analysis (Logistic Dose Response). Total genomic DNA from a single seedling was extracted using the Puregene® kit (Gentra systems, USA) according to the manufacturer's instructions. The forward and reverse primers CH1for: 5'GAAGCCCCTGGAACGTGAAGGT'3 and CH1rev: 5'CGACCCGACCTCGCTAAAAAA'3 for Region 1 and CH2for: 5'ATGAAYGTKCAAGAGYTRGC'3 and CH2rev: 5'CCTTCKGTDATSATCYTTGAA'3 for Region 2 were designed from sequences of species in GenBank to encode 423 and 367-bp fragments of *ALS* gene, in which a point mutations associated with herbicide resistance were previously reported (Tranel and Wright 2002). PCR methods were employed to amplify the corresponding regions of *C. coronarium ALS* gene. PCR product was sequenced either directly or after cloned using pGEM® -T easy vector system (Promega, USA) following the manufacturer's instructions.

RESULTS AND DISCUSSION Dose-response experiments at the whole-plant level have revealed that the resistant (R) biotype from Gilat was highly resistant to tribenuron (>729 fold) relative to the susceptible wild-type (Table 1). Other SU herbicides such as iodosulfuron, chlosulfuron and sulfometuron have also exhibited R/S>100 (results not shown). However, moderate and variable resistance (4 to 48 fold) have shown to herbicides from other ALS inhibiting groups: imazethapyr, flumetsulam, pyrithiobac-Na and propoxycarbazone-Na (Table 1). Comparison of ALS sensitivity between enzyme extracted from R and S biotypes have confirmed the whole-plant observations (Figure 2), indicating that the resistance is due to an alteration in the target site (ALS). Region 1 and 2 of the ALS gene known to vary in ALS-resistant biotypes (Tranel and Wright 2002) were amplified and sequenced in *C. coronarium*. Two amino acid substitutions were found in region 1 of resistant *C. coronarium*. One was found in Gilat biotype, a change from proline197 (numbering based on *Arabidopsis thaliana* ALS) to threonine, and the second in Beeri biotype from proline197 to serine. Multiple substitutions in proline197 have also been reported in *Kochia scoparia* (Guttieri et al. 1995), *Raphanus raphanistrum* (Tan and Medd 2002) and *Lindernia spp*. (Uchino and Watanabe 2002), and their significance is not completely understood.

TABLE 1. Effect of ALS inhibiting herbicides on ED50 values of shoot fresh weight of susceptible (S) and resistant (R) *Chrysanthemum coronarium* biotypes from Gilat.

Herbicide	Class	ED_{50} (g a.i/ha)[a]		R/S
		S	R	
Tribenuron	SU	2.5	>1822	>729
Imazethapyr	IMI	21	192	9
Flumetsulam	TP	36	>160	>4
Pyrithiobac-Na	PTB	271	3381	12
Propoxycarbazone-Na	SCT	56	>2688	>48

a ED50 is the effective dose (g a.i/ha) required to reduce the fresh weight by 50%.

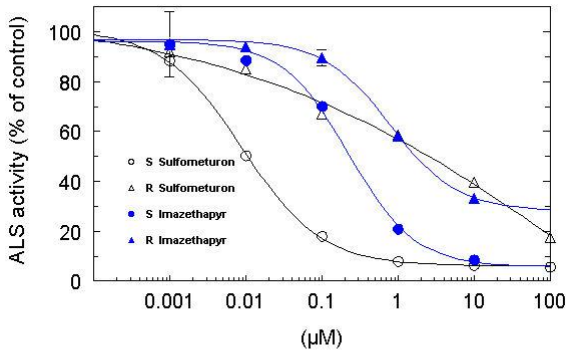


FIGURE 2: Inhibition of ALS extracted from S and R biotypes of *C. Coronarium* from Gilat by sulfometuron and imazethapyr.

CONCLUSIONS The results confirm that resistance to ALS inhibiting herbicides evolved in *C. coronarium* in Israel is due to an alteration in the target site (ALS) and associated with the substitutions in the proline197 in the *ALS* gene. The agricultural implications for these results are that ALS inhibiting herbicides cannot be used solely in fields were resistance has established and should be either replaced or combined with herbicides having different mode of action.

REFERENCES

Guttieri, M. J., C. V. Eberlein and D. C. Thill (1995). Diverse mutations in the acetolactate synthase gene confer chlorsulfuron resistance in kochia (Kochia scoparia) biotypes. Weed Sci. 43:175-178.

Heap, I. The International Survey of Herbicide Resistant Weeds. Online. Internet. March 18, 2004. Available www.weedscience.com

Sibony, M., A. Michel, H. U. Haas, B. Rubin and K. Hurle (2001). Sulfometuron-resistant Amaranthus retroflexus: cross-resistance and molecular basis for resistance to acetolactate synthase-inhibiting herbicides. Weed Res. 41:509-522.

Tan, M. K. and R.W. Medd (2002). Characterisation of the acetolactate synthase (ALS) gene of Raphanus raphanistrum L. and the molecular assay of mutations associated with herbicide resistance. Plant Sci. 163:195-205.

Tranel, P. J. and T. R. Wright (2002). Resistance of weeds to ALS-inhibiting herbicides: what have we learned? Weed Sci. 50:700-712.

Uchino, A., and H. Watanabe (2002). Mutations in the acetolactate synthase genes of sulfonylurea-resistant biotypes of Lindernia spp. Weed Biology and Management, 2:104-109.

Avi Tal and Baruch Rubin
The Institute of Plant Science and Genetics

Faculty of Agriculture, Food and Environmental Sciences
The Hebrew University of Jerusalem
P. O. Box 12, Rehovot 76100, Israel

Management and Factors Affecting White Rust Development in Indian Mustard (*Brassica juncea* L.)

ABSTRACT Indian mustard [*Brassica juncea* (L.) Czern & Coss.] is an important oilseed crop grown during *Rabi* (post-monsoon) season. White rust caused by *Albugo candida* (Pers. Ex. Lev.) Kuntze is an important and endemic disease of the crop. Zoosporangial concentration of 10-20 zoosporangia per microscopic field of 100X magnification was optimum to produce disease. Disease severity increased with the number of inoculations. A minimum leaf wetness period of 6 h was found essential for initiating the disease. Sucrose solution (0.2%) supported maximum zoosporangial germination. Early and normal planted crop escaped the disease whereas, the late sown crop suffered more damage under field conditions. Under detached leaf culture technique the disease appeared after 7-9 days of incubation. Younger leaves of host and upper surface of leaves were found to be more prone to infection. Disease intensity in crop sown in first fortnight of October was less compared to the one sown in mid-November. Metalaxyl+Mancozeb (Ridomil MZ) sprayed leaves did not show any disease development while *Azadirachta indica* leaf extract was more effective among plant extracts in checking the disease.

KEY WORDS White rust, *Albugo candida*, *Brassica juncea*

RESULTS AND DISCUSSION

Factors Affecting Germination of Zoosporangium

Type of water and sucrose solution: Zoosporangial germination was best in 0.2 per cent sucrose solution (75.0%) followed by 0.1 per cent sucrose solution (70.1%), sterilized distilled water (69.6%), distilled water (66.6%) and minimum in tap water (65.0%). Variation in pH and carbon source of these waters and mineral composition could be the reason for varied response of water on zoosporangial germination. Mishra and Chona (1963) observed good germination of *A. candida* in double distilled, tap and rainwater. Mathur (1989) also reported similar results using distilled, double distilled, tap and rain water.

Factors Affecting Disease Development

Leaf position: Experiments were carried out on leaves collected from upper, middle and lower position of 35-40 day old plants. The number of lesions that developed on each leaf was observed. The highest per cent disease index was observed in leaves collected from upper position of the plant after 7 days of inoculation whereas, the middle and lower leaves showed less disease index (Table 1)

Table -1. Effect of leaf position and leaf surface on development of white rust on detached leaves of mustard

Leaf position/ surface	Incubation Period (days)	Percent Disease Index*
Lower (old) leaf	8	31.1 (26.7)
Middle (medium-aged) leaf	8	35.5 (33.7)
Upper (young) leaf	7	50.8 (60.0)
Upper (adaxial) surface	7	50.8 (60.0)
Lower (abaxial) surface	8	31.1 (6.7
S Em+/-		0.8
CD (P<0.05)		4.7

*Average of three replications (3 leaves per replication).
Figures in parantheses are actual percent disease index and others are arc sine transformed values

.

Leaf surface: Leaves collected from 35-40 day old plant were inoculated on upper and lower surfaces separately. The inoculation of upper surface of leaves exhibited highest percent disease index (60.0%) after 7 days of incubation as compared to lower surface of leaves (26.7%) even at 8 days of incubation (Table 1). Kumar et al. (1995) reported abaxial surface of lower leaves to be more susceptible. In the present study, the lower (old) leaves were found less susceptible.

Inoculum density: Inoculation with concentrations of 1-10 to 41-50 zoosporangia per microscopic field (100X) revealed that highest percent disease index (48.7%) was observed with the inoculum 11-20 zoosporangia density (Table 2), followed by 21-30, 31-40 and 41-50 zoosporangia per microscopic field (100X). However, lowest percent disease index (21.3) was recorded in the

treatment having 10 zoosporangia density.

Table -2. Effect of concentration in zoosporangial on development of white rust on detached leaves of mustard

Zoosporangial concentration in 100x microscopic field	Incubation Period (days)	Percent Disease Index*
0-10	9	27.5 (21.3)
11-20	8	44.2 (48.7)
21-30	8	43.5 (47.3)
31-40	7	42.3 (45.3)
41-50	7	40.0 (41.3)
S Em+/-		0.8
CD (P<0.05)		2.6

*Average of three replications (3 leaves per replication)

Figures in parantheses are actual percent disease index and others are arc sine transformed values

Number of inoculations: Highest percent disease index (55.3%) was recorded with 6 inoculations (Table 3). The percent disease index or severity gradually increased as the number of inoculations increased.

Table -3. Effect of number of inoculations on development of white rust on detached leaves of mustard

Number of inoculations	Incubation Period (days)	Percent Disease Index*
1	8	27.5 (21.3)
2	8	38.0 (38.0)
3	8	39.6 (40.7)
4	7	40.8 (42.7)
5	7	45.0 (50.0)
6	7	48.0 (55.3)
S Em+/-		1.0
CD (P<0.05)		3.0

*Average of three replications (3 leaves per replication)

Figures in parantheses are actual percent disease index and others are arc sine transformed values

Quality of light: The quality of light had significant influence on disease development. However, highest disease index was recorded in yellow light (49.3%) among different colours of lights (Table 4) used though it was less than natural conditions.

Table -4. Effect of different qualities of light on development of white rust on detached leaves of mustard

Treatment	Incubation Period (days)	Percent Disease Index*
Yellow	7	44.6 (49.3)
Red	8	38.8 (39.3)
Blue	8	32.8 (29.3)
Black	8	27.0 (20.7)
Green	8	38.8 (39.3)
Control	7	46.1 (52.0)
S Em+/-		0.5
CD (P<0.05)		1.6

*Average of three replications (3 leaves per replication)

Figures in parantheses are actual percent disease index and others are arc sine transformed values

There is no literature available for any previous work done on effect of leaf position, inoculum density, number of inoculations and quality of light on severity of white rust disease in mustard.

Effect of wetness: The perusal of data showed that the percent disease index increased with the increase in duration of leaf wetness. The minimum requirement of leaf wetness was 6 h after inoculation for disease development. Percent disease index was 18.7 percent for 6 h of leaf wetness (Table 5). The zoosporangia required free film of water for infecting the host surface. Chauhan and Singh (1994) reported 6 h leaf wetness for pea rust (*Uromyces viciae-fabae*) development. Similarly, Butler and Jadhav (1991) also observed a period of 6h leaf wetness for groundnut rust (*Puccinia arachidis*) development.

Table -5. Effect of different duration of wetness on development of white rust on detached leaves of mustard

Duration of wetness (h)	Incubation Period (days)	Percent Disease Index*
2	-	4.0 (0.0)
4	-	4.0 (0.0)
6	9	25.6 (18.7)
8	9	28.0 (22.0)
10	8	33.6 (30.7)
12	8	38.0 (38.0)
14	7	45.0 (50.0)
S Em+/-		0.6
CD (P<0.05)		1.8

*Average of three replications (3 leaves per replication)

Figures in parantheses are actual percent disease index and others are arc sine transformed values

Effect of sowing dates: White rust in all cruciferous crop is much affected by agronomic management practices. In early sowing (October 1 and October 15), the disease intensity was less whereas, the late

(November 15) sown crop suffered more damage (Table 6). Severity of white rust increased significantly as the dates of sowing were delayed except November 30 sown crop. Mathur (1989) also advocated that early sowing escaped damage due to white rust in rapeseed-mustard. These studies indicated, suitable sowing time to avoid losses from white rust in mustard would be around early October.

Table -6. Effect of different dates of sowing on white rust severity on mustard

Date of sowing		Percent Disease Index*
Oct. 01		27.0 (20.7)
Oct. 15		34.4 (32.0)
Nov. 01		40.0 (41.3)
Nov. 15		48.1 (55.3)
Nov. 30		42.3 (45.3)
S Em+/-		1.4
CD (P<0.05)		4.3

*Average of three replications (3 leaves per replication)

Figures in parantheses are actual percent disease index and others are arc sine transformed values

Management of the disease: Metalaxyl + Mancozeb (Ridomil-MZ) treated leaves did not show any disease development. This was followed by Mancozeb, which showed 22.0 per cent disease index (Table 7). The other fungicides that proved effective in suppressing the disease were Blitox-50 (29.3), Baynate (34.7) and Sulfex (40.0). Leaves sprayed with *A. indica* extract did not show any infection (Table 8). Leaf extract of *O. sanctum* was also found effective followed by *D. stramonium* and *A. sativum*. These plant extracts have been found to be effective against some diseases in other crops (Chattopadhyay, 1999). Metalaxyl + Mancozeb (Ridomil-MZ) and Blitox-50 were effective in controlling the disease followed by Mancozeb and Antracol (Table 9). Metalaxyl, a phenolamide fungicide specific to oomycetous fungi has been reported effective against the white rust pathogen (Mathur and Bhatnagar, 1991). In the present study, Metalaxyl + Mancozeb (Ridomil-MZ) was also found to be the best fungicide in controlling white rust under both field and detached leaf culture technique. Apart from Metalaxyl + Mancozeb (Ridomil-MZ), Blitox-50 and Mancozeb were effective in controlling white rust (Srivastava and Verma, 1989; Sokhi et al., 1994). However, the fungicide Mancozeb proved less effective under field condition than on detached leaves. Leaf extract of *A. indica* was found highly effective under detached leaf culture technique followed by *O. sanctum* and *D. stramonium* leaf extract.

Table -7. Effect of fungicides on development of white rust on detached leaves of mustard

Fungicide	PDI*	Percent disease control
Metalaxyl + Mancozeb (Ridomil-MZ)	4.0 (0.0)	100.0
Mancozeb	22.0 (27.9)	56.5
Baynate	34.7 (36.1)	31.5
Blitox-50	29.3 (32.8)	42.1
Sulfex	40.0 (39.2)	21.0
Antracol	45.3 (42.3)	10.5
Aliette	39.3 (38.8)	22.4
Control	50.7 (45.4)	-
S Em+/-	1.0	
CD (P<0.05)	4.0	

*Average of three replications (3 leaves per replication)

*Figures in parantheses are actual percent white rust severity and others are arc sine transformed values

Table -8. Effect of plant products on white rust development on detached leaves of mustard

Plant product	PDI*	Percent disease control
Azadirachta indica leaf extract	4.0 (0.0)	100.0
Ocimum sanctum leaf extract	18.7 (25.6)	62.7
Datura stramonium leaf extract	30.0 (33.2)	40.0
Allium sativum bulb extract	34.0 (35.7)	32.0
Ovis	38.0 (38.1)	24.0
Zetron	38.7 (38.5)	22.7
Control	50.0 (45.4)	-
S Em+/-	0.7	
CD (P<0.05)	3.2	

*Average of three replications (3 leaves per replication)

*Figures in parantheses are actual percent white rust severity and others are arc sine transformed values

Table -9. Effect of sprays of fungicides and plant products on white rust in mustard development under field condition

Fungicide/Plant product	PDI*	Percent disease control
Metalaxyl + Mancozeb (Ridomil-MZ)	20.7 (27.0)	61.7
Blitox-50	21.3 (27.5)	60.5
Mancozeb	43.3 (40.0)	19.7
Antracol	47.3 (43.5)	12.3
Zetron	42.7 (40.8)	21.0
Ovis	44.0 (41.5)	18.5
Control	54.0 (47.1)	-
S Em+/-	2.1	
CD (P<0.05)	6.4	

*Average of three replications (3 leaves per replication)

*Figures in parantheses are actual percent white rust severity and others are arc sine transformed values

REFERENCES

Butler, D.R. and Jadhav, D.R. 1991. Response of leaf wetness and temperature for infection of groundnut by rust. Pl. Pathol. 40:395-400.

Chattopadhyay, C. 1999. Yield losses attributable to Alternaria blight of sunflower (Helianthus annuus L.) in India and some potentially effective control measures. International Journal of Pest Management 44: 1-7.

Chauhan, R.S. and Singh, B.M. 1994. Effect of different duration of leaf wetness on pea rust development. Pl. Dis. Res. 9:200-201.

Kolte, S.J. 1985. Diseases of Annual Edible Oilseed Crops. Vol II, CRC Press Inc. 135 pp.

Kumar, V., Kaushik, C.D. and Gupta, P.P. 1995. Role of various factors in development of white rust disease of rapeseed-mustard. Indian J. Mycol. Pl. Pathol. 25:145-148.

Mathur, S. 1989. Studies on epidemiology and control of white rust in mustard (Brassica juncea Cass.) caused by Albugo candida (Pers.) Kuntze. Ph.D. Thesis, RAU, Bikaner (Raj.) pp.89.

Mathur, S. and Bhatnagar, M.K. 1991. Comparative tolerance of Albugo candida and Peronospora parasitica to Metalaxyl. International Journal of Tropical Plant Desease 9: 192-199.

Meena, P.D., Chattopadhyay, C., Fateh Singh, Bhoori Singh and Ajit Gupta. 2002. Yield loss in Indian mustard due to white rust and effect of some cultural practices on Alternaria blight and white rust severity. Brassica 4: 18-24.

Mishra, M.D. and Chona, B.L. 1963. Factors affecting sporangial germination and epidemiology of white rust of Amaranthus [Albugo bliti (Biv.) Kzn.]. Indian Phytopath. 16:333-343.

Naresh Mehta and Saharan, G.S. 1998. Effect of planting dates on infection and development of white rust and downy mildew disease complex in mustard. J. Mycol. Plant Pathol. 28: 259-265.

Saharan, G.S., Kaushik, C.D., Gupta, P.P. and Tripathi, N.M. 1984. Assesment of losses and control of white rust of mustard. Indian Phytopath. 37: 397

Sokhi, S.S., Grewal, R.K. and Munshi, G.D. 1994. Development of spray schedule for the control of white rust of Raya. Plant Disease Research 9: 225-226.

Srivastava, L.S. and Verma, R.N. 1989. Fungicidal control of white rust of mustard in Sikkim. Indian Phytopathology 42: 84-86.

Verma, P.R. and Petrie, G.A. 1978. A detached leaf culture technique for the study of white rust disease of Brassica species. Can. J. Plant Sci. 58:69-73

Yadava, J.S., Meena, S.S., Rajbir Yadav and Singh, N.B. 2000. Rapeseed-Mustard Production Advancements - a Critical Analysis. All India Coordinated Research Project on Rapeseed-Mustard, Project Coordinating Unit, National Research Centre on Rapeseed-Mustard, Sewar, Bharatpur 321303 (Rajasthan), 107 pp.

R.L. Meena*, P. D. Meena, and C. Chattopadhyay
National Research Centre on Rapeseed-Mustard
Sewar, Bharatpur-321303

*For more information, email pdmeena@yahoo.com

Research in Resistance Management

Seasonal Dynamics of Resistance to QoI and DMI Fungicides in *Podosphaera xanthii* and Impact on Control of Cucurbit Powdery Mildew

ABSTRACT Resistance to QoI (strobilurin) fungicides in *Podosphaera xanthii* was first detected in the US in 2002. A seedling bioassay was used in 2003 to monitor QoI resistance in cucurbit fields at 10 commercial farms and 1 research facility in Suffolk Co., NY. Not all sites were included in each bioassay. Squash seedlings were dipped in solutions of QoI fungicide (50 mg/L trifloxystrobin formulated as Flint), DMI fungicide (20 mg/L myclobutanil; Nova), or both. About 12 hrs later they were put with nontreated seedlings in fields for up to 1 day, then kept in a greenhouse until severity was rated. On 27 Jul, when mildew was first seen in the area, QoI resistant strains were found in 1 of 5 commercial and research fields with early plantings of summer squash or pumpkin not QoI-treated. On 19-21 Aug, mildew severity in 4 commercial pumpkin fields where QoIs were used averaged 1% on upper leaf surfaces and 11% on lower surfaces. On 31 Aug, QoI resistance was common in these fields, 2 other pumpkin fields, and a winter squash experiment (61 to 100% frequency). Nova but no QoIs was used in 1 field. Moderate DMI insensitivity occurred in all fields (12 to 56%). Severity then exceeded 50% on most lower leaf surfaces. On 25 Sep, QoI resistance was detected in 3 commercial pumpkin fields where no QoIs or DMIs were used (2, 38, and 56%) and in fields where QoIs and/or DMIs were used (88 to 97%). In summary, QoI resistant

strains were present at mildew onset, their frequency increased greatly during the season, efficacy was affected, and they occurred in crops not treated with QoIs.

INTRODUCTION Powdery mildew is the most common disease of cucurbit crops throughout the world and fungicides with systemic or translaminar activity plays a critical role in managing this disease. These fungicides are better than contact fungicides for controlling powdery mildew because they are effective on the underside of leaves. Conditions are more favorable for disease development on the underside than on upper leaf surfaces (Figure 1).

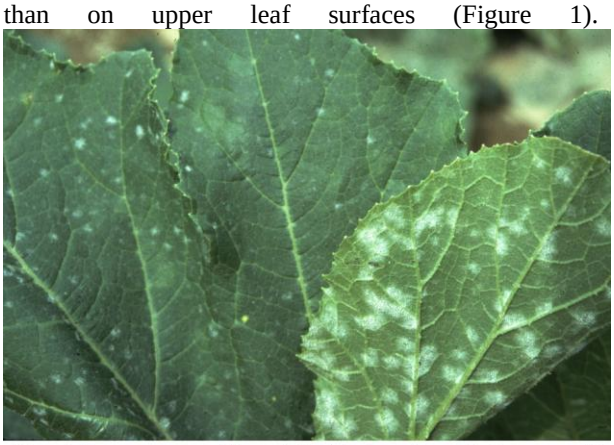


Figure 1. Because powdery mildew colonies typically are larger with denser sporulatio on lower leaf surfaces than on upper surfaces, control is very important on lower surfaces (right section of this leaf which is folded over).

Fungicide resistance is a major concern with cucurbit powdery mildew. Unfortunately, most systemic and translaminar fungicides are at risk for resistance development because they have a single-site mode of action. Three chemical classes of this type are registered for this disease in the US: benzimidazoles, quinone outside inhibitors (QoIs, a.k.a. strobilurins), and demethylation inhibitors (DMIs). The cucurbit powdery mildew fungus, *Podosphaera xanthii*, has demonstrated a high potential for developing resistance (McGrath 2001). Presence of resistant strains has been associated with control failure. In the US and in many other areas of the world, this pathogen has developed resistance to all three chemical classes. Most recently, resistance to QoI fungicides was detected in 2002 (McGrath and Shishkoff 2003). Cross-resistance has been documented among QoI fungicides.

As long as QoI-resistant strains of *P. xanthii* are not common, QoI fungicides will continue to be important tools for managing both cucurbit powdery mildew and resistance to other groups of fungicides with high resistance risk. Resistance has already developed to the benzimidazoles and DMIs. Resistance to the benzimidazoles and the QoIs is qualitative. Thus isolates of the cucurbit powdery mildew fungus are typically either sensitive or highly resistant. Highly resistant strains cannot be effectively controlled with the fungicide. Benzimidazoles are rarely being used for cucurbit powdery mildew in the US due to resistance and to the introduction of more effective DMIs and QoIs.

Resistance to DMIs is quantitative. With this type of resistance, strains of the pathogen exhibit a range in sensitive to the effects of the fungicide. Strains with low sensitivity can often be controlled, as well as fully sensitive strains, by applying a DMI fungicide at high rate and/or short interval, or by selecting an inherently more active DMI fungicide. In the US, resistance to DMIs in the cucurbit powdery mildew pathogen is such that the first active ingredient registered, triadimefon, is no longer effective, while myclobutanil and triflumizole are still effective at high rates. DMIs should not be used exclusively when QoI fungicides are effective, as this will put an undesirable amount of pressure on the powdery mildew pathogen population. Exclusive use of one mode of action will select for strains with a higher level of resistance.

If growers are to manage fungicides wisely, they need information from their area on the proportion of the pathogen population that is resistant before they make the first application. Because early spring-planted summer squash typically becomes infected with powdery mildew before main-season crops in the same area, the early crops can be used to determine the composition of the pathogen population for later planted pumpkin, gourd, winter squash and melon. If QoI-resistant strains are found to be uncommon in these early crops, then these fungicides will be recommended for use with other fungicides in a resistance management program.

The recommended weekly fungicide program for 2003 was a QoI fungicide plus a contact fungicide applied in alternation with a DMI fungicide plus a contact. The threshold level for starting applications was one infected leaf of 50 old leaves examined. The program has two strategies for managing resistance:

1. Alternation among systemic/translaminar fungicides in at least two chemical classes, and
2. Inclusion of contact multi-site mode of action fungicides.

When and if the monitoring in main-season crops after 1 to 2 applications of QoIs revealed that the frequency of QoI-resistant strains remained below about 50%, then another application would be warranted. An in-field seedling bioassay is an inexpensive means for obtaining estimates of the frequency of resistance in just 7 to 10 days (McGrath and Shishkoff 2001). Laboratory assays with individual isolates would take 14 to 20 days.

MATERIALS AND METHODS An in-field seedling bioassay was used in 2003 to determine the fungicide sensitivity of the powdery mildew fungal pathogen

population in cucurbit fields at 10 commercial farms and at the Cornell University Long Island Horticultural Research and Extension Center (LIHREC) in Suffolk County, NY. Suffolk is in the eastern portion of Long Island. Not all sites were included in each bioassay. Three early crops of zucchini and yellow summer squash that had not been sprayed with QoI or DMI fungicides were identified for the first bioassay. Two early plantings of pumpkin that had not been treated with fungicides were also selected for this bioassay because powdery mildew was observed in these plants in late July at the same time symptoms were first observed in the squash plants. The first bioassay was conducted at these five sites on 27 July (Table 1). Another bioassay was conducted on 31 Aug in six commercial pumpkin fields and a winter squash experiment at LIHREC after QoI and/or DMI fungicides were used in these fields (Table 2). Powdery mildew severity was assessed in some of these fields before the bioassay was conducted and again at the time of the bioassay. A third assay conducted on 25 Sep included pumpkin fields where no QoI or DMI fungicides were used (Table 2).

Table 1. Proportion of cucurbit powdery mildew fungal population estimated to be moderately insensitive to DMI fungicides and proportion resistant to QoI fungicides based on results from a fungicide sensitivity seedling bioassay.

	DMI moderately insensitive isolates (%)			Strobilurin (QoI) resistant isolates (%)		
Site	**7/27**	**8/31**	**9/25**	**7/27**	**8/31**	**9/25**
1		33			61	
2	1	13		0	100	
Cornell LIHREC	16	56		0	100	
3 (no QoIs)		44	5		83	88
4	6	56		61	91	
5		12	1		89	89
6	25			0		
7		35	1		97	97
8 (Organic)			1			2
9 (Organic)			1			56
10 (no QoIs or DMIs)	17		1	0		38

z blank indicates bioassay not conducted at that site on that date.

Table 2. Crop, field size, approximate distance from field at site 1, and fungicides applied to crop where resistance to QoI and DMI fungicides was examined.

Site and crop	Field size	Distance (miles)	Fungicide[z] and rate per A (application date or interval)
1 pumpkin	45 A	--	Equus DF 1 lb + Kocide 1.5 lb (7/25), Equus DF 1 lb + Quadris 12 oz + Kocide 1 lb (8/10), Stylet-oil 1% (8/11), Kumulus DF 4 lb + Phostrol 2 pt (8/17), Nova 5 oz + Manex 1 qt + Stylet-oil 1% (8/22), Kumulus 5 lb + Nova 5 oz + Phostrol 4 pt + Equus 1 lb + Kocide 1 lb (8/29), Kocide 1.5 lb, Nova 5 oz, Equus 1.5 lb, Phostrol 4 pt (9/6), Kocide 1 lb + Nova 5 oz + Equus 1.5 lb (9/12), Flint 2 oz + Bravo 1.4 lb + Kocide 1 lb, Phostrol 1.5 pt, Kocide 2.5 lb (9/22)
2 pumpkin	60 A	5.4	Bravo and Ridomil/Copper (late July), Quadris alternated with Nova + contact fungicides (7 to 10 day program)
Cornell butternut squash	0.3A	9.3	Flint 2 oz + Bravo 2.7 lb/A (7/31, 8/14, 8/26), Procure 6 oz + Microthiol Disperss 4 lb (8/7, 8/20, 9/6)
3 pumpkin	5A	10.8	Equus or Bravo and Kocide (about twice before symptoms seen); Nova + Kocide; Bravo; Nova + Stylet-oil; Bravo; Nova + Stylet-oil (7-10-day schedule)
4	6A	14.7	Bravo + Kocide (late July), Quadris once, Bravo + Nova (10-day schedule)
5 pumpkin	26 A	15.1	Quadris 14 oz + Equus 2.7 lb + Kocide 2000 1.5 lb (8/13), Nova 5 oz + Equus 2.7 lb + Kocide 2000 1.5 lb + Phostrol 5 pt (8/19, 8/25, 8/31, 9/6), Topsin 8 oz (8/31, 9/6), Kocide 2000 1.5 lb + Phostrol 5 pt + Topsin 1 lb (9/13, 9/20)
7 pumpkin	30 A	17.5	Alternated among Bravo, copper, and Stylet-oil on a weekly schedule. 1 –2 applications of Quadris and Nova.
8 pumpkin	2A	18.3	None: organically-produced crop
9 pumpkin	1A	24.5	None: organically-produced crop
10 pumpkin	20 A	31.3	Manex 1 qt every 10 days

z QoI fungicides: Quadris, Flint. DMI fungicides: Nova, Procure. Benzimidazole fungicide: Topsin. Contact fungicides: Bravo (chlorothalonil), Equus (chlorothalonil), Kocide (copper), Kumulus (Sulfur), Microthiol Disperss (Sulfur), Stylet-oil, Fungicides labeled for other diseases: Manex, Phostrol.

The seedling bioassay entailed placing fungicide-treated seedlings in a field of cucurbits with powdery mildew (Figure 2). Summer squash seedlings were grown in a growth chamber. Their growing point and unexpanded leaves were removed just before treatment. Seedlings varied in size from 1 to 9 true leaves. Treatments were no fungicide, QoI fungicide (50 mg/L trifloxystrobin formulated as Flint), DMI fungicide (20 mg/L myclobutanil; Nova), and a combination of the QoI and DMI fungicides. Seedlings were dipped in the fungicide solutions, and then allowed to dry overnight

before setting in a cucurbit crop in groups of four plants with the four treatments. There were 2 to 7 groups per field. After being in fields for 4 to 22 hours, seedlings were kept in a greenhouse until symptoms of powdery mildew were visible, which took at least one week. Then severity (percent tissue with symptoms) was visually estimated for each leaf on a 0 to 100% continuous scale. Frequency of resistant pathogen strains in a field was estimated by calculating the ratio of severity on fungicide-treated plants relative to non-treated plants for each group, then determining the field average.

Figure 2. Fungicide sensitivity bioassay seedlings in a commercial planting of zucchini on 27 July 2003. Seedlings had been dipped in fungicide solution or left nontreated.

The fungicide concentrations used were found to be good discriminating concentrations in previous studies (McGrath et, al, 1996, McGrath and Shishkoff 2003). Isolates able to tolerate 50 mg/L trifloxystrobin are considered to be resistant to QoI fungicides. These isolates were common in fungicide efficacy experiments where QoI fungicides were not as effective as in previous experiments at the same site. Isolates able to tolerate 20 mg/L myclobutanil are considered to be moderately insensitive to DMI fungicides because under field conditions these isolates have been associated with ineffective control with triadimefon and good control with myclobutanil applied at a high label rate.

Powdery mildew occurrence was monitored in 4 of the commercial pumpkin fields (sites 1, 2, 4, and 5). Severity was assessed on upper and lower leaf surfaces of 24 leaves in each field on 19-21 Aug and on 31 Aug.

RESULTS AND DISCUSSION QoI resistance was detected on 27 July in 1 of 5 fields with early plantings of summer squash and pumpkin (Table 1). A high proportion (61%) of the cucurbit powdery mildew fungus population in the field at site 4 was estimated to be resistant based on results from the seedling bioassay. No powdery mildew developed on QoI-treated seedlings placed in the other 4 fields. A low level of moderate DMI insensitivity was detected in all fields (Table 1). Thus QoI-resistant strains of *Podosphaera xanthii* and strains moderately insensitive to DMIs were present at a detectable level before these fungicides are known to have been applied in Suffolk County, NY, in 2003.

Powdery mildew was first observed on 29 July in the winter squash experiment at LIHREC and on 7-8 Aug in the 4 commercial pumpkin fields examined. Powdery mildew in these pumpkin fields became more severe on the lower surface of leaves than expected based on performance of QoIs in previous fungicide efficacy experiments (McGrath and Shishkoff 1999). Average severity on upper leaf surfaces on 19-21 Aug was 0.1%, 0%. 4%, and 0%, respectively, in the 4 pumpkin fields; whereas on lower leaf surfaces severity was 5%, 11%, 11%, and 18%. Good control on upper leaf surfaces indicates application timing was good. Contact fungicides (e.g. chlorothalonil, copper) only work where deposited, which is mostly the upper surface. Control on lower surfaces is provided by systemic/translaminar fungicides. Severity on 31 Aug exceeded 50% on most lower surfaces while there remained few symptoms on upper surfaces (0-5%). Several leaves died by 31 Aug, likely due to poor control of powdery mildew (Figure 3). In a fungicide efficacy experiment conducted on pumpkin at LIHREC in 2003, level of control on lower surfaces provided by programs with QoI and DMI fungicides was inferior to that provided by a new fungicide, Quintec (McGrath 2004).

Figure 3. Fungicide-treated squash plants for a fungicide sensitivity bioassay in a commercial pumpkin field on 31 August 2003. Powdery mildew is more severe than expected in this fungicide-treated field, with several dead pumpkin leaves and those remaining severely infected, suggesting that fungicide resistance might be affecting control.

QoI resistance was detected in all 7 fields where the second bioassay was conducted on 31 Aug, including one field where Nova was used but not QoIs (Tables 1 and 2, Figure 4). The proportion of the pathogen population estimated to be resistant was 61 - 100%. Moderate DMI insensitivity was detected in all fields as well (12 - 56%). Nontreated seedlings became severely infected, with some leaves completely white due to powdery mildew developing after infection,

which revealed the large quantity of spores in the air.

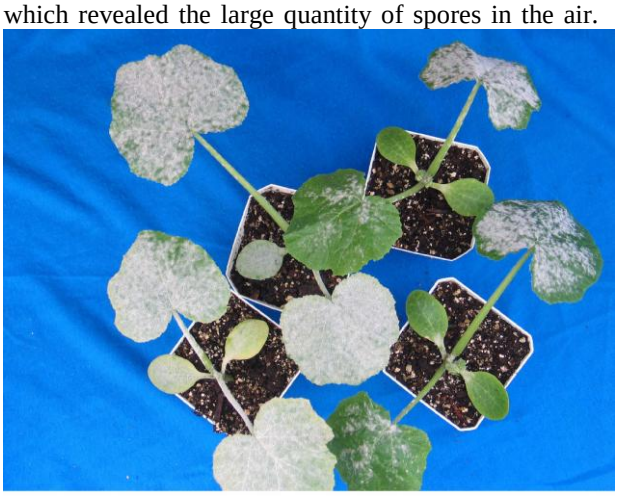


Figure 4. Squash plants 10 days after they were placed in a commercial pumpkin field on 31 August 2003. Treatments starting with the lower left plant and progressing clockwise are nontreated, QoI fungicide, DMI fungicide, and a combination of the QoI and DMI fungicides.

A third bioassay was conducted on 25 Sep to determine if resistant strains were sufficiently widespread in Suffolk County to be present where no QoI or DMI fungicides were used. Two of these 3 fields were being organically managed (Table 2). QoI resistance was detected in these fields (2 - 56%) and also in the fields included in this bioassay where QoI and/or DMI fungicides had been used (88 - 97%) (Table 1).

Powdery mildew severity on seedlings treated with Nova generally was similar to severity on seedlings treated with both Nova and Flint for each field, which suggests that most isolates moderately insensitive to DMIs were also resistant to QoIs. Almost all individual isolates tested in 2002 using a laboratory assay were either sensitive to both chemical groups or insensitive to DMIs and also resistant to QoIs.

QoI resistant strains were present at the start of powdery mildew development in 2003 and their frequency increased greatly during the season, efficacy was affected, and they occurred in crops not treated with QoIs. Information from the first two fungicide sensitivity seedling bioassays, along with recommendations on how to modify fungicide programs, was provided to growers as soon as the results were known through newsletter articles. Where resistance had developed, growers were able to avoid unnecessary applications of an expensive fungicide during the second half of the epidemic when QoI resistant strains were sufficiently common that QoI fungicides were unlikely to have been effective.

REFERENCES

McGrath, M. T. 2001. Fungicide resistance in cucurbit powdery mildew: Experiences and challenges. Plant Disease 85(3): 236-245.

McGrath, M.T., and Shishkoff, N. 1999. Evaluation of fungicide programs for managing powdery mildew of pumpkin, 1998. Fungicide and Nematicide Tests 54:230-231.

McGrath, M. T., and Shishkoff, N. 2001. Resistance to triadimefon and benomyl: dynamics and impact on control of cucurbit powdery mildew. Plant Disease 85(2):147-154.

McGrath, M. T. 2004. Evaluation of fungicide programs for managing powdery mildew of pumpkin, 2003. Fungicide and Nematicide Tests 59:V056.

McGrath, M. T., and Shishkoff, N. 2003. Resistance to strobilurin fungicides in Podosphaera xanthii associated with reduced control of cucurbit powdery mildew in research fields in the Eastern United States. Resistant Pest Management 12(2). (on line at http://whalonlab.msu.edu/rpmnews/vol.12_no.2/globe/rpm_g_mcgrath_shishkoff_2_.htm

McGrath, M. T, Staniszewska, H., Shishkoff, N., and Casella, G. 1996. Fungicide sensitivity of Sphaerotheca fuliginea populations in the United States. Plant Disease 80:697-703.

Margaret T. McGrath
Department of Plant Pathology, Cornell University
Long Island Horticultural Research and Extension Center
3059 Sound Avenue, Riverhead, New York 11901-1098

Resistance Management News

Cardiff Resistance Monitoring Service

The launch of a UK-based resistance monitoring service is attracting a great deal of interest from the pest control industry. Cardiff Resistance Monitoring Service (CARMS) brings together the facilities and expertise of the Pest Management & Ecotoxicology Centre, a Centre of Excellence at Cardiff University's School of Biosciences, and I2L (Insect Investigations Ltd), a leading UK product testing and development centre for the pest control industry. It provides pest control companies with a comprehensive, high quality pesticide resistance diagnosis and monitoring service. Using this service has the added advantage from the perspective of the end-users that monitoring of resistance in arthropod pests to pesticides is independent of the companies marketing them.

Staff engaged in the delivery of CARMS have a combined expertise of many decades in the diagnosis and characterisation of pesticide resistance in pests of public health, animal health and agricultural importance. The range of expertise available offers CARMS the flexibility to exactly tailor its service to match its client's needs.

Specialist facilities available through this service include:

- Pest rearing
- Pesticide susceptibility assays
- Resistance diagnosis by biochemical and molecular assays
- Biochemical and molecular characterisation of pesticide resistance mechanisms
- Pesticide metabolism studies with analysis by thin-layer chromatography, HPLC, LC-MS, and GC-MS

For further information about CARMS please follow this link: http://www.insect-investigations.com/resistance.html

Dr Graham Small
Pest Management & Ecotoxicology Centre
Cardiff School of Biosciences
Cardiff University, Main College
Park Place, PO Box 915, Cardiff, CF10 3TL
Wales, UK
www.pestecotox.cf.ac.uk

Dr Peter McEwen
Insect Investigations Ltd
Capital Business Park, Wentlooog
Cardiff, CF3 2PX
Wales, UK
www.insect-investigations.com

Abstracts

Preliminary Studies on Resistance of *Fulvia fulva* to Flusilazole in Tomato

Fifty *Fulvia fulva* single-spore isolates were collected in 2002 and 2003 from protected fields in several regions of Liaoning Province. Sensitivity of these isolates to flusilazole was determined by the methods of mycelial growth inhibition. The results showed that the percentage of moderate and high resistant isolates was 15.69% and 11.76% respectively. Significant differences in mycelial growth, fresh weight and osmotic sensitivity were observed between sensitive and high resistant isolates. This means the fitness of the high resistant isolate decreased significantly. There was no cross-resistance between flusilazole and procymidone as well as azoxystrobin, whereas, there was cross-resistance between flusilazole and myclobutanil as well as triadimefon.

KEY WORDS: tomato, *Fulvia fulva*, flusilazole, fungicide resistance

Zhang Chi, Ji Mingshan, Wang Yingzi, Qu Changming, Cheng Genwu, Qi Zhiqiu, Gu Zumin, and Li Lina
Department of Pesticide Science, College of Plant Protection, Shenyang Agricultural University, Shenyang 110161

Announcements and Submission Deadlines

Thank you to those who contributed to this issue - you have really made the newsletter a worthwhile reading experience! Our contributors truely increase the newsletter's success at sharing resistance information worldwide.

We encourage all of our readers to submit articles, abstracts, opinions, etc (see the newsletter online at http://whalonlab.msu.edu/rpmnews/general/rpm_submission.htm for submission information).

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

The next two **submission deadlines** are:

Monday, September 20th, 2004
Monday, March 21st, 2005

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

Libraries that wish to receive a printed version may send a request to:

Newsletter Coordinator
Resistant Pest Management Newsletter
Center for Integrated Plant Systems
Michigan State University
East Lansing, MI 48824-1311
USA

Please visit us online today at **http://whalonlab.msu.edu/rpmnews/**

Header not on first page