

# Resistant Pest Management Newsletter

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

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## Letter from the 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).



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## 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 neonicotinoid 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. Hadjistyli 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 occurring 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 a susceptible, 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.

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### 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.

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## 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<sub>50</sub> of thiamethoxam, imidacloprid and dimethoate to first generation of *C. viridis* was 1.4287, 3.2278 and 11.4155 ppm respectively. The LC<sub>50</sub> 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 (1 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<sub>50</sub> and LC<sub>95</sub> obtained for the final generation of *C. viridis*, maintained without insecticide exposure in the glass house (Regupathy and Dhamu, 2001).

$$\text{Susceptibility index} = \frac{\text{LC}_{50} \text{ of F1}}{\text{LC}_{50} \text{ of Fn}} \quad \text{or} \quad \frac{\text{LC}_{95} \text{ of F1}}{\text{LC}_{95} \text{ of Fn}}$$

The rate resistance decline (R) used to quantify the rate of changing LC<sub>50</sub> when the selection pressure is stopped was estimated by the formula;

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

Where,

- n is the number of generations not exposed to insecticide
- Final LC<sub>50</sub> is the LC<sub>50</sub> after n generations without selection, and
- Initial LC<sub>50</sub> is the LC<sub>50</sub> of the parental generation before n generations of selection.

The number of generations (G) required for ten-fold decrease in the LC<sub>50</sub> value was calculated using the formula.

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

**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<sub>50</sub>) 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<sub>95</sub> 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

Thiamethoxam										
Generation	Regression equation	χ <sup>2</sup>	LC <sub>50</sub> (ppm)	Fiducial limits			LC <sub>95</sub>	Fiducial limits		
				LL		UL		LL		UL
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<sub>50</sub> and LC<sub>95</sub> values for all the insecticides tested. The susceptibility index varied between 1.13 and 1.15 based on LC<sub>50</sub> and

it was in the range of 1.14 to 1.27 based on LC<sub>95</sub> 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<sub>50</sub>, 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<sub>95</sub>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		Rate of Resistance Decline	
	LC <sub>50</sub> (ppm)	LC <sub>95</sub> (ppm)	R	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<sub>50</sub> 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.

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## Arthropod Resistance

### Status of Resistance to Acaricides in Mexican Strains of the Southern Cattle Tick *Boophilus microplus* (Acari: Ixodidae)

**ABSTRACT** This paper reviews the current status and mechanisms of resistance to commonly used acaricides in Mexican strains of the southern cattle tick, *Boophilus microplus* (Canestrini). Resistance to organophosphates (OPs) and pyrethroids was measured using the FAO Laval Packet Test (LPT), and a modification of the FAO-LPT was used to measure amitraz resistance. The resistance ratios to pyrethroids range from 10 to >1000, depending on the mechanisms involved. The highest resistance ratios to organophosphate acaricides coumaphos and diazinon were 10 and 35, respectively. A survey of 15 field strains of *B. microplus* from Mexico revealed low levels of resistance to amitraz, with resistance ratios ranging from 2 to 5. Many tick populations in Mexico demonstrated multiple resistances to all three classes of acaricides. Resistance to pyrethroids was conferred by either a sodium channel mutation or enhanced activity of a carboxyesterase, *CzEst9*. Resistance to

organophosphate acaricides was found to be conferred by insensitive AChE and/or enhanced activity of mixed function oxidases. Resistance to amitraz is considered to be mainly by target site insensitivity, although there was also evidence for the involvement of metabolic enzymes.

**INTRODUCTION** The southern cattle tick, *Boophilus microplus* (Canestrini), is one of the most damaging ectoparasites of cattle in some tropical and subtropical regions of the world, including Mexico, Central and South America, Australia and Africa. Damages to the host are inflicted by both direct blood feeding and by bovine babesiosis (or cattle fever disease) caused by protozoan agents (*Babesia spp.*) that ticks transmit (Friedhoff and Smith, 1981). This tick-transmitted disease caused great loss to the cattle industry in the southern part of the United States at the beginning of the 20th century. There was an intensive cattle tick

eradication program that started in 1907 to eradicate this pest and disease vector from the United States. Intensive efforts, including dipping treatments in acaricides and pasture evacuation, over a period of 50 years led to the successful eradication of *B. microplus* from the southern states of the U.S., except in several counties in Texas along the U.S.-Mexican border (Graham and Hourigan, 1977). *B. microplus* is endemic in Mexico and continues to cause severe economic losses to Mexican ranchers. The U.S. imports over one million head of cattle from Mexico each year, and there is a risk for cattle ticks to be re-introduced back to the U.S. via cattle imported from Mexico. To prevent this economically damaging pest from re-entering of the U.S. through cattle importation from Mexico, the USDA has maintained an active Cattle Fever Tick Eradication Program (CFTEP) for the past 50 years. All Mexican cattle presented at the ports of entry for importation are inspected for tick infestation. If cattle inspectors find a single tick, the whole group of cattle will be rejected. The cattle that pass inspection will then be dipped in the organophosphate acaricide coumaphos (0.3% active ingredient) to eliminate any larva that may have escaped inspection (Bram et al., 2002). This practice has been so far very successful. However, potential problems have emerged in recent years as *B. microplus* ticks in Mexico have been found to have developed resistance to various acaricides, including coumaphos (Santamaria et al., 1999).

Coumaphos is the only registered acaricide that can be used in the dipping treatment at the ports of entry for cattle importation. Resistance to this acaricide may allow resistant ticks to survive the dipping treatment, therefore, raising the potential of jeopardizing the continued success of the CFTEP. In order to develop diagnostic tools for resistance detection, and to develop new strategies for the control and eradication of resistant *B. microplus* ticks, the USDA, ARS, Knippling-Bushland U.S. Livestock Insects Research Laboratory (KBUSLIRL), Kerrville, Texas has conducted research in recent years to determine the levels of resistance in Mexican strains of *B. microplus* and to investigate the mechanisms of resistance to several major classes of acaricides. This paper is intended to give a brief account on the history of tick control and acaricide resistance in *B. microplus* in Mexico, the bioassay techniques used in resistance evaluation, and the mechanisms of resistance to three major classes of acaricides.

**ACARICIDE USE AND DEVELOPMENT OF RESISTANCE**  
Organophosphate (OP) acaricides were heavily used in the national tick eradication program between 1975-1985 in Mexico (Trapapa, 1989). The OPs used during that period include coumaphos, chlorpyrifos, chlorfenvinfos, diazinon and ethion. The first case of OP resistance was detected in *B. microplus* ticks from a

ranch near Tuxpan in the state of Veracruz in 1983, and the tick strain established from ticks collected from this location demonstrated 10- to 14-fold resistance to coumaphos, chlorpyrifos and ethion. (Aguirre et al., 1986). Resistance to OPs soon became widespread in central and eastern Mexico. Pyrethroid acaricides were then introduced into Mexico in 1986 in order to alleviate OP resistance problems. Resistance to pyrethroids was first detected in 1993 and soon became extensive (Ortiz et al. 1994; Fragoso et al., 1995). The levels of resistance to pyrethroids were generally in the range of 10- to 350-fold, with exception of two tick samples in which more than 1000-fold resistance was detected (Miller et al. 1999; Santamaria et al. 1999). Different mechanisms of resistance were suspected (Miller et al., 1999). As a result of intense selection pressure from the use of OPs and pyrethroids, *B. microplus* were found to have developed resistance to both classes of the acaricides in at least 15 states of Mexico (Santamaria et al. 1999). Our primary concern is resistance to coumaphos, the only acaricide registered for use in dipping vats at cattle importation facilities. The most resistant tick strains we obtained from Mexico in recent years demonstrated approximately 10-fold resistance to coumaphos (Davey and George, 1999; Li et al., 2003a). Efficacy trials using the coumaphos-resistant larvae from the Tuxpan strain resulted in 86.3% control after a single dip in 0.279% coumaphos (Davey and George, 1999). Efficacy trials with another OP-resistant strain, the San Roman strain, resulted in only 46.8% control following a single dip in 0.3% coumaphos. (Davey et al., 2004). A second dip in the same concentration of coumaphos 7 days after the first dip increased the efficacy of control to 92.9% (Davey et al., 2004), which is still lower than the standard of 100% control set for the CFTEP. These results highlight the potential risk of resistant *B. microplus* being brought to the U.S. on imported Mexican cattle.

Amitraz was introduced to control OP-resistant ticks at the same time the pyrethroids were introduced in 1986, but its use was initially limited due to a higher cost. The use of amitraz became more frequent after 1993 when pyrethroid resistance problems started to hinder the tick control efforts in Mexico. The first case of amitraz resistance was detected in the San Alfonso strain of *B. microplus* collected from a ranch in the state of Tabasco (Soberances et al., 2002). This strain demonstrated 42-fold resistance to amitraz, and moderate resistance (1.5- to 6.9-fold) to OPs, and high degree of resistance to pyrethroids. Results of a recent study on a Brazilian amitraz-resistant strain also revealed resistance to OPs and pyrethroids (Li et al., 2004). The findings of *B. microplus* resistant to all three major classes of acaricides in Mexico underscore the seriousness of the resistance situation and the importance of having a resistance management strategy



in Mexico. Amitraz was considered to have the potential to be used for the dipping treatment of cattle at the ports of entry for the CFTEP (George et al., 1998). However, the emergence of amitraz resistance in Mexican *B. microplus* diminishes the possibility of using this acaricide as an alternative to coumaphos in the dipping vats at USDA, APHIS's cattle importation facility for the CFTEP.

**BIOASSAY TECHNIQUES** The larval packet test (LPT) is the standard bioassay technique recommended by the Food and Agriculture Organization (FAO) for testing acaricide susceptibility in *B. microplus* (FAO, 1971). LPT was described originally by Stone and Haydock (1962) in Australia, where serious problems of acaricide resistance arose in the 1970s (Kunz and Kemp, 1994). Briefly, technical grade acaricide dissolved in a mixture of trichloroethylene and olive oil (2:1 ratio) is used to treat filter papers that are then set for 2 h in a fume hood to allow trichloroethylene to evaporate before being folded into packets using bulldog clips. Approximately 100 *B. microplus* larvae are added into the treated filter paper packets, which is then sealed with additional bulldog clips and placed in an incubator (27 °C and 90% R.H.) for 24 h. before numbers of live and dead larvae are counted.

The FAO-LPT has been successfully used for organophosphate (OP) and pyrethroid acaricides (Miller et al., 1999; Li et al. 2003a), but it is not applicable to amitraz. Amitraz shows a slower killing of the tick larvae, and it is also difficult to differentiate the dead from the alive. Results from such bioassays are often useless since the slopes of amitraz concentration-mortality response are flat (Kemp et al. 1998). Recent modification of the FAO-LPT has allowed successful evaluation of susceptibility/resistance to amitraz in *B. microplus* (Miller et al. 2002). The key modifications include using nylon fabric instead of filter paper as substrate and using formulated amitraz instead of technical grade amitraz. Other bioassay techniques for evaluating acaricide susceptibility in *Boophilus* ticks include the larval immersion test (LIT) and the adult immersion test (AIT) (Jonsson et al., 2003). For the LIT, *B. microplus* larvae are immersed in serial water dilutions of the formulated acaricides for a certain period of time (10 min). Then, the treated larvae are transferred to clean filter paper packets, and are kept in an incubator (27 °C, 90% R.H.) for 72 h before mortality is determined. A modified version of the Shaw's LIT has been successfully used at the Mexican National Laboratory of Parasitology in Jiutepect, Morelos, Mexico to detect the first case of amitraz resistance in Mexico (Soberances et al., 2002). Recent studies revealed that the modified Shaw's LIT had a higher sensitivity in detecting amitraz resistance, thus both the modified Shaw's LIT and the Miller's nylon-LPT offer the much

needed diagnostic tool that allows the detection and characterization of amitraz resistance in *B. microplus* (Li et al., 2003b, 2004; Miller et al., 2003). The AIT involves immersing engorged female ticks, within 2 d of dropping from host, in water dilutions of commercial acaricides for 30 min, and holding the treated females ventral side up with double-sided sticky tape in Petri dishes at room temperature for 7 days. The mortality was determined based on the numbers of treated females that did not lay eggs. Although the AIT has been used in Mexico to evaluate efficacy of acaricides against the cattle tick, and to detect resistance to various acaricides (Santamaria et al., 1999; Soberances et al., 2002), results from a recent study on amitraz susceptibility using the AIT indicated a poor fit of the bioassay data with the probit model (Jonsson et al., 2003). Significant differences were also found between data obtained from susceptible reference tick strains at two different laboratories (Jonsson et al., 2003). This makes it hard to compare amitraz AIT bioassay results from different countries or laboratories.

**MECHANISMS OF RESISTANCE** It has been well documented in many insect pests that resistance is conferred primarily by two major physiological mechanisms: insensitive target site and enhanced activity of metabolic enzymes, such as esterases, mixed function oxidases, and glutathion-S-transferases (Devonshire et al., 1999; Siegfried and Scharf, 2001). The targets of pyrethroids and organophosphates are the sodium channel and acetylcholinesterase (AChE), respectively. Synergist chemicals, which inhibit specific classes of metabolic enzymes, were often used as a tool to determine the contribution of various metabolic detoxification enzymes.

Miller et al. (1999) used synergist bioassays to characterize mechanisms of resistance to pyrethroid acaricides. Triphenylphosphate (TPP) synergism ratio was found to be significantly higher in a pyrethroid resistant strain (Cz), with 166-fold resistance to permethrin, than in the reference susceptible strain, suggesting an enhanced esterase activity. Biochemical studies resulted in the isolation and identification of an over-expressed esterase, *CzEst9*, which hydrolyzes pyrethroids (Pruett et al., 2002). Further molecular study also indicated that the transcript of *CzEst9* gene was more abundantly expressed in the Cz strain than any other tick strains (Guerrero et al., 2002a; Hernandez et al., 2002). In contrast, synergists failed to synergize pyrethroid toxicity in two Mexican strains of *B. microplus* that had more than 1000-fold resistance to pyrethroids, suggesting the existence of a target site insensitivity mechanism. Such speculation was strengthened by the observation of cross-resistance between pyrethroids and DDT in those strains (Miller et al., 1999), concurring with previous observations on

pyrethroid resistance in insect species. The para-type sodium channel gene was sequenced from *B. microplus*, and a point mutation of the para-type sodium channel gene was subsequently identified in these highly resistant tick strains (He et al., 1999a, b). A PCR assay has been developed to detect the *kdr*-like gene mutation (Guerrero et al., 2001). The ability of tick larvae to survive pyrethroid treatment was directly linked to the genotype composition as determined by the PCR assay (Guerrero et al., 2002b).

Synergists were also used to characterize metabolic resistance to coumaphos and diazinon in a number of Mexican strains of *B. microplus* (Li et al., 2003a). The bioassay results provide evidence that suggests an involvement of mixed function oxidases in coumaphos resistance, in addition to the mechanism of insensitive AChE (Pruett, 2002). Furthermore, such an oxidative mechanism seems to be specific toward coroxon, the active metabolite of coumaphos. It had no effect on detoxification of diazinon, the active metabolite of which is diazoxon (Li et al., 2003a). Much less is understood about the mode of action, as well as the mechanism of resistance to amitraz. Synergist bioassays on several amitraz-resistant strains from Mexico and one Brazilian strain of *B. microplus* indicated some involvement of esterase and glutathion-S-transferase (Li et al., 2004). However, the major mechanism of resistance to amitraz is speculated to be insensitive target site, presumably the octopamine receptor. The possible target site mechanisms, particularly the octopamine receptor, are being investigated at KBUSLIRL.

**RAPID DETECTION OF RESISTANCE** Molecular and biochemical assays have become increasingly important in resistance detection (Sangster et al., 2002). This is particularly true for resistance studies of ticks. The traditional bioassay technique requires a large number of larvae, and may take several weeks before such larvae become available for bioassays. It is often the case that only a small number of partially or fully engorged females may be obtained from tick inspectors at the cattle importation facilities. The molecular and biochemical assays require only a small sample of ticks, whether it be larvae, nymphs, or adults. Such molecular techniques not only allow detection of the presence of resistance genes, but also allow determination of the genotype of individual ticks (Guerrero et al., 2002b). Information on genotype frequency would help define the risk of resistance. The current molecular and biochemical assays are highly specific and effective only for the known mechanisms of resistance. Such techniques may not allow detection of resistance conferred by emerging and new mechanisms yet to be identified.

**PERSPECTIVES** Because of its high efficacy in killing ticks and its low toxicity to cattle, coumaphos has been

the only registered acaricide for use in dipping vats to eliminate any tick on cattle headed for importation to the U.S. from Mexico. Resistance to this and other OP acaricides in the Mexican populations of *B. microplus* poses a major threat to the continued success of the CFTEP. The emergence of *B. microplus* resistance to amitraz in Mexico diminishes the hope of using amitraz in the dipping vats at the ports of entry for cattle importation. The newer classes of acaricides, such as fipronil and spinosad, have been evaluated for their use in the control of cattle ticks (Davey et al., 1998, 2001). The highest dose (0.15% a.i.) of spinosad used as a single whole-body spray resulted in only 87.9% control, far below the standard 100% control required by the CFTEP (Davey et al., 2001). Due to the lack of 100% control from a single treatment, spinosad cannot

be used at U.S. ports of entry to treat cattle imported from Mexico. However, it may be used to eradicate ticks on the premises in tick-infested area of the U.S. if repeated treatments were applied (Davey et al., 2001). Although a near 100% control was achieved with a single pour-on treatment of fipronil (1% a.i.), the long withdrawal period required for this material prevents its use at U.S. ports of entry to treat cattle imported from Mexico. With no other options immediately available to replace coumaphos, it is critical to find new strategies that may help mitigate resistance problems in Mexico. The development and use of molecular and/or biochemical resistance detection techniques would allow us to detect and monitor acaricide resistance situation in Mexico in a timely manner. Successful management of acaricide resistance problems in *B. microplus* in Mexico would reduce the possibility of resistant ticks surviving dipping treatment in coumaphos at the ports of entry to the U.S. Close collaboration between the U.S. and Mexico is essential to maintain our ability to prevent the cattle fever ticks from reinfesting the U.S.

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### Status of Insecticide Resistance in Geographical Populations of Cotton Bollworm, *Helicoverpa armigera* in South Indian Cotton Ecosystem During 2002-03

The polyphagous *Helicoverpa armigera* has been a concern in several countries, including India. It has been detected for high degree of resistance to pyrethroids. In India it has been a major problem causing crop failure (Dhingra et al., 1988). Afterwards this phenomenon was reported to almost all the conventional insecticides used in cotton crop production in North Indian state of Punjab (Mehrotra and Phokela, 1992) and southern India (Patil 1998). There is very less insecticide use in innocent regions like Varanasi in Eastern Uttar Pradesh (Singh et al., 1994). The complex and dynamic nature of resistance makes it difficult even in IPM strategy. Insect resistance to insecticides is found to vary over space and time (Armes et al., 1992; Singh et al., 1994). In India, cotton consumes over half the total insecticides, although it occupies only 5% of the cropped area. Andhra Pradesh alone consumes more than 33% of all insecticides used in the country with over 54% of this on cotton (Puri et al., 1995). Over the past 30 years, the cost of insecticides has constituted 30-40% of the growing costs of cotton in Andhra Pradesh and is now around 44% in India as a whole. Continuous monitoring of status of resistance to most commonly used insecticides is a prerequisite in an active IRM strategy. In the present communication we report the situation of resistance to 6 insecticides over 11 geographical populations of *H.armigera* representing the entire south Indian cotton ecosystem.

**MATERIALS AND METHODS** As a primary step in the process of developing an insecticide resistance management strategy, identification of baseline resistance to each of the insecticides used in the region to control bollworm is indispensable and same was undertaken at the University of Agricultural Sciences Dharwad Karnataka state, India.

The larvae of *Helicoverpa armigera* (second to sixth instar) collected from 11 different geographical

locations of South India



were reared in the laboratory on semi-synthetic diet to get F1 homogeneous larvae for bioassay. Larvae of 30-40 mg were used for bioassay. Six insecticides viz., monocrotophos, chloropyrifos, endosulfan, carbaryl, cypermethrin and quinalphos, which are extensively used to control bollworm in cotton ecosystems of South India, were used to determine baseline resistance. 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 thoracic dorsum of each third instar larva. The larvae of check were treated with acetone alone. The concentrations were varied to obtain 20-80% mortality. Immediately 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 72hr after treatment. The dose-mortality regressions were computed by using MLP 3.08 software (Ross, 1987).

Data pertaining to toxicity levels of 30-40 mg larvae collected from 12 geographical locations of South India using log dose probit analysis have been presented in the Tables 1, 2, 3, 4, 5, and 6 insecticide

wise.

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

Location	Carbaryl						
	LD <sub>50</sub>	Fiducial limits		Slope	Chi-square Value	RR*	RR**
		Lower	Upper				
	µg/µl						
Parbhani	5.329	3.8239	8.5316	1.758	0.064	2.5	26.64
Nanded	4.192	3.077	6.0517	1.893	0.968	1.97	20.96
Gunur	11.07	8.5717	15.0441	2.624	0.406	5.2	55.35
Nalgonda	6.022	4.4303	9.3401	2.004	1.155	2.83	30.11
Madhira	2.324	1.5759	3.3142	1.547	0.474	1.09	11.62
Raichur	9.38	6.8939	13.8133	1.932	1.917	4.14	46.9
Dharwad	5.375	3.9843	7.1711	2.05	1.586	2.52	26.87
Mysore	5.07	3.8319	6.574	2.333	7.307	2.38	25.35
Coimbatore	2.126	1.6301	2.6907	2.622	3.146	1	10.63
Madurai	2.348	1.7863	3.0074	2.462	5.136	1.1	11.74
Kovilpatti	2.535	1.9159	3.287	2.333	7.307	1.19	12.67

\* Susceptible Coimbatore population  
 \*\* Susceptible strain

Table 2. Response of geographical population of cotton bollworm for monocrotophos bioassay during 2002-03

Location	Monocrotophos						
	LD <sub>50</sub>	Fiducial limits		Slope	Chi-square Value	RR*	RR**
		Lower	Upper				
	µg/µl						
Parbhani	11.857	9.1945	16.2627	2.702	0.243	9.81	59.58
Nanded	9.329	6.8995	13.5466	1.976	0.085	7.72	46.87
Gunur	8.317	6.1407	11.9022	1.923	0.506	6.88	41.79
Nalgonda	8.26	5.8293	12.8647	1.586	0.297	6.83	41.5
Madhira	4.458	3.3749	5.731	2.379	5.865	3.69	22.4
Raichur	5.9	4.3348	8.0577	1.904	1.498	4.88	29.64
Dharwad	6.006	4.518	7.9798	2.125	0.925	4.97	30.18
Mysore	5.35	4.0148	7.0482	2.192	3.679	4.42	26.88
Coimbatore	1.57	0.8566	2.2309	1.863	1.751	1.29	7.88
Madurai	1.208	0.4365	1.9409	1.466	3.293	1	6.07
Kovilpatti	3.731	2.5097	5.1715	1.655	3.988	3.08	18.74

\* Susceptible Madurai strain  
 \*\* Susceptible strain

Table 3. Response of geographical population of cotton bollworm for endosulfan bioassay during 2002-03

Location	Endosulfan						
	LD <sub>50</sub>	Fiducial limits		Slope	Chi-square value	RR*	RR**
		Lower	Upper				
	µg/µl						
Parbhani	4.386	3.3652	5.563	2.562	0.898	3.75	20.02
Nanded	3.17	2.1671	4.2735	1.848	7.049	2.71	14.47
Gunur	9.99	7.6026	13.866	2.332	0.636	8.54	45.61
Nalgonda	10.673	8.0676	15.1594	2.28	0.243	9.13	48.73
Madhira	4.802	3.5243	6.4016	2.009	3.567	4.1	21.92
Raichur	8.614	6.3269	12.5744	1.858	1.097	7.36	39.33
Dharwad	4.002	2.9899	5.1112	2.285	5.229	3.42	18.27
Mysore	2.01	1.0894	2.9201	1.56	5.354	1.71	9.17
Coimbatore	1.459	0.6962	2.1624	1.671	3.523	1.24	6.66
Madurai	1.169	0.5434	1.7161	1.986	1.102	1	5.33
Kovilpatti	1.452	0.7808	2.0602	1.947	1.121	1.24	6.63

\* Susceptible Madurai strain  
 \*\* Susceptible strain

Table 4. Response of geographical population of cotton bollworm for quinalphos bioassay during 2002-03

Location	Quinalphos						
	LD <sub>50</sub>	Fiducial limits		Slope	Chi-square Value	RR*	RR**
		Lower	Upper				
	µg/µl						
Parbhani	3.406	2.3956	5.0827	1.577	0.875	2.34	20.03
Nanded	2.88	2.063	4.067	1.712	0.714	1.98	16.94
Gunur	10.87	8.306	14.6891	2.102	2.263	7.48	63.94
Nalgonda	7.92	5.7928	11.4949	1.807	3.121	5.45	46.58
Madhira	3.997	2.8181	6.1938	1.579	0.667	2.75	23.51
Raichur	3.335	2.414	4.75	1.76	0.551	2.29	19.61
Dharwad	2.62	1.892	3.603	1.822	2.171	1.8	15.41
Mysore	3.335	2.4139	4.7502	1.76	0.551	2.29	19.61
Coimbatore	2.32	1.7086	3.3906	1.946	0.093	1.59	13.64
Madurai	2.19	1.5385	3.0192	1.735	0.318	1.5	12.88
Kovilpatti	1.452	0.7808	2.0602	1.947	1.121	1	8.54

\* Susceptible Kovilpatti strain  
 \*\* Susceptible strain

Table 5. Response of geographical population of cotton bollworm for chlorpyrifos bioassay during 2002-03

Location	Chlorpyrifos						
	LD <sub>50</sub>	Fiducial limits		Slope	Chi-square Value	RR*	RR**
		Lower	Upper				
	µg/µl						
Parbhani	2.27	1.6604	3.0163	2.022	3.492	1.94	16.33
Nanded	2.729	1.8672	3.9974	1.51	0.364	2.33	19.63
Gunur	7.956	5.6015	12.2755	1.602	3.019	6.8	57.23
Nalgonda	6.331	4.304	9.1955	1.651	4.251	5.41	45.54
Madhira	2.824	1.9221	4.1998	1.473	0.507	2.41	20.31
Raichur	4.08	2.9802	5.9331	1.819	2.568	3.49	29.35
Dharwad	2.478	1.8477	3.2578	2.145	0.544	2.11	17.82
Mysore	2.2	1.5385	3.0192	1.735	0.318	1.88	15.82
Coimbatore	1.57	0.8566	2.2309	1.863	1.751	1.34	11.29
Madurai	1.169	0.5434	1.7161	1.986	1.102	1	8.41
Kovilpatti	1.208	0.4365	1.9409	1.466	3.293	1.033	8.69

\* Susceptible Madurai strain  
 \*\* Susceptible strain

Table 6. Response of geographical population of cotton bollworm for cypermethrin bioassay during 2002-03

Location	Cypermethrin						
	LD <sub>50</sub>	Fiducial limits		Slope	Chi-square Value	RR*	RR**
		Lower	Upper				
	µg/µl						
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
Gunur	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 strain  
 \*\* Susceptible strain

### Insecticide Bioassay

Log probit assay was carried out for carbaryl, monocrotophos, endosulfan, quinalphos, chlorpyrifos and cypermethrin across geographical populations representing cotton ecosystem of south India. The assay was carried out on larvae weighing 30 - 40 mg. The results are presented in Tables 1, 2, 3, 4, 5, and 6 insecticide-wise for the year 2002-03. The resistance

factor have been worked out considering the lowest LD<sub>50</sub> value from among the results of present study and by considering the LD<sub>50</sub> value of a susceptible strain.

## RESULTS

### *Carbaryl*

Guntur population recorded a maximum LD<sub>50</sub> value to carbaryl (11.07 µg/µl) followed by population from Raichur (9.38 µg/µl), Nalgonda (6.02), Dharwad (5.37) and Parbhani (5.32). Lowest LD<sub>50</sub> value was observed in population from Coimbatore (2.12µg/µl) followed by Madhira (2.32) and Madurai (2.34). The resistance ratio (RR) against susceptible strain was found to be highest for population of Guntur (55.35 folds) followed by Raichur (46.90), Nalgonda (30.11), Dharwad (26.87), Parbhani (26.64), Coimbatore (10.63). The least resistance ratio was observed in the population of Coimbatore (10.63) followed by Madhira and Madurai

### *Monocrotophos*

Parbhani population recorded a maximum LD<sub>50</sub> value to monocrotophos (11.857 µg/µl) followed by population from Nanded (9.329 µg/µl), Guntur (8.317), Nalgonda (8.260), Dharwad (6.006) and Raichur (5.900). Lowest LD<sub>50</sub> value was observed in population from Madurai (1.208) followed by Coimbatore (1.570 µg/µl), Kovilpatti (3.731). The resistance ratio (RR) against susceptible strain was found to be highest for population of Parbhani (59.58 folds) followed by Nanded (46.87), Guntur (41.79), Nalgonda (41.50), Dharwad (30.18), Raichur (29.64). The least resistance ratio was observed in the population of Madurai (6.07) followed by Coimbatore (7.88) and Kovilpatti (18.74).

### *Endosulfan*

Nalgonda population recorded a maximum LD<sub>50</sub> value to endosulfan (10.673 µg/µl) followed by population from Guntur (9.999µg/µl). Lowest LD<sub>50</sub> value was observed in population from Madurai (1.169µg/µl) followed by Kovilpatti (1.452), Coimbatore (1.459). The resistance ratio (RR) against susceptible strain was found to be highest for population of Nalgonda (48.73 folds) followed by Guntur (45.61). The least resistance ratio was observed in the population of Madurai (5.33) followed by Kovilpatti (6.63) and Coimbatore (6.66).

### *Quinalphos*

Maximum resistance to quinalphos was observed in Guntur population (10.870µg/µl) followed by Nalgonda population (7.92 µg/µl). Least resistance was noticed in Kovilpatti population (1.452 µg/µl). High resistance to quinalphos was recorded by population from Guntur (63.94 folds) followed by Nalgonda population (46.58 folds) as against susceptible population.

### *Chlorpyrifos*

Maximum resistance to chlorpyrifos was recorded in Guntur (7.956µg/µl) followed by Nalgonda (6.33µg/µl). Minimum resistance was observed in Kovilpatti population (1.169 µg/µl) followed by Madurai population (1.208) and Coimbatore (1.570). The resistance ratio against susceptible strain was found to be highest for population of Guntur (57.23 folds) followed by Nalgonda (45.54) and Raichur (29.35). Least ratio was recorded in population from Kovilpatti (8.41) and Madurai (8.60).

### *Cypermethrin*

Raichur population recorded a maximum LD<sub>50</sub> value to cypermethrin (11.309µg/µl) followed by population from Nalgonda (8.281), Guntur (7.920). Lowest LD<sub>50</sub> 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).

It is clear from the results on resistance ratio that cotton bollworm, *Helicoverpa armigera*, has virtually developed resistance to almost all the insecticides as the LD<sub>50</sub> values recorded were far higher compared to recommended dosages indicating existence of resistance as was already reported by earlier (Armes et al., 1992). However, there is a great deal of variation in insecticide resistance from location to location within South Indian cotton ecosystems. The resistance levels in Guntur, Nalgonda and Raichur region (heavy insecticide usage area) is due to heavy dependence on insecticides. This clearly explains that resistance levels were proportionate with the usage of pesticides. The study conducted by Forrester (1990) also clearly revealed that resistance levels rose when pyrethroids were used but fell significantly when they were withheld. Thus the pesticides were creating very high selection pressure for resistant genotypes. This suggests that indiscriminate use and heavy dependence on pesticide will further complicate the already worsened situation and this hints at aiming for insecticide resistance management strategies.

Thus, *H.armigera* insecticide-resistance issue in India is becoming ever more acute. Pyrethroid resistance is widespread in populations in almost all geographic populations. It is likely that few refugia of susceptible populations remain to dilute the build-up of resistant populations. Resistant management strategies appropriate for the region should be implemented immediately. These should include greater control over insecticide application and use, particularly on cotton. Unless this happens, the areas affected by resistant

*H.armigera* populations will continue to increase and could ultimately result in the abandonment of cotton growing in large areas of South India. By some strategy, the allelic frequency for major insecticide resistance genes (say cypermethrin) need to be diluted, though it is difficult to field release of laboratory reared highly susceptible strains of *H.armigera* may do this. This has to be tested over experimental basis before embarking. As on date we don't have patterns of mobility of *H.armigera* in this region. It is very difficult to do but molecular markers can aid this. In an effort we have developed population fingerprints for all the 12 geographic populations of south Indian cotton ecosystem (Report to DBT, 2004. Manuscript under preparation). These population DNA fingerprints will be extremely useful in pinpointing precise population fluxing patterns over time and space. Outcome of such efforts should be integrated with results reported in this paper for contingent planning to mitigate problem of insecticide resistance.

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### Insecticide Resistance vis-à-vis *Cry1Ac* 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* (Hubner), 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, chloropyrifos, 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 thoracic dorsum of each third instar larva. The larvae of check were treated with acetone alone. The

concentrations were varied to obtain 20-80% mortality. Immediately 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 pertaining 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	Quinalphas	Chloropyrifos	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 + 1°C and 70% R.H. Median lethal concentration (LC<sub>50</sub>) presented in Table 1 were derived from log dose probit calculations using MLP 0.38 statistical package.

Comparison was made between the LD<sub>50</sub> values of insecticides and LC<sub>50</sub> 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<sub>50</sub> 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<sub>50</sub> 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<sub>50</sub> 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<sub>50</sub> value was observed in population



from Kovilpatti (0.308) followed by Madurai (0.452  $\mu\text{g}/\mu\text{L}$ ), Coimbatore (0.777).

#### *Endosulfan*

Nalgonda population recorded a maximum LD<sub>50</sub> value to endosulfan (13.240  $\mu\text{g}/\mu\text{L}$ ) followed by population from Guntur (13.155  $\mu\text{g}/\mu\text{L}$ ). Lowest LD<sub>50</sub> value was observed in population from Madurai (0.740  $\mu\text{g}/\mu\text{L}$ ) followed by Kovilpatti (0.906), Coimbatore (1.025).

#### *Quinalphos*

Maximum resistance to quinalphos was observed in Nalgonda population (20.937  $\mu\text{g}/\mu\text{L}$ ) followed by Guntur population (12.564  $\mu\text{g}/\mu\text{L}$ ). Least resistance was noticed in Madurai population (0.5  $\mu\text{g}/\mu\text{L}$ ).

#### *Chlorpyrifos*

Maximum resistance to chlorpyrifos was recorded in Guntur (11.038  $\mu\text{g}/\mu\text{L}$ ) followed by Nalgonda (9.480  $\mu\text{g}/\mu\text{L}$ ). Minimum resistance was observed in Madurai population (0.36  $\mu\text{g}/\mu\text{L}$ ) followed by Kovilpatti population (0.463) and Coimbatore (1.128).

#### *Cypermethrin*

Raichur population recorded a maximum LD<sub>50</sub> value to cypermethrin (22.40  $\mu\text{g}/\mu\text{L}$ ) followed by population from Guntur (10.92), Nalgonda (9.984). Lowest LD<sub>50</sub> value was observed in population from Madurai (0.143  $\mu\text{g}/\mu\text{L}$ ) 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<sub>50</sub> values for Cry 1Ac ranged from 0.147 to 1.095  $\mu\text{g}/\text{ml}$ .

Table 2. Correlation matrix showing the relation between xenobiotics and between xenobiotics and Cry 1Ac delta endotoxin

	Carbaryl	Monocrotophos	Endosulfan	Quinalphos	Chlorpyrifos	Cypermethrin	Cry1Ac
Carbaryl	1						
Monocrotophos	0.19	1					
Endosulfan	0.63	0.48	1				
Quinalphos	0.62	0.37	0.93*	1			
Chlorpyrifos	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<sub>50</sub>/LC<sub>50</sub> 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 xenobiotics 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 chlorpyrifos ( $r = 0.50$ ). 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.

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### 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<sub>50</sub> 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<sub>50</sub> value to cypermethrin (11.309 µg/µl) followed by population from Nalgonda (8.281), Guntur (7.920). Lowest LD<sub>50</sub> value was observed in population from Madurai (1.648 µg/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 <sub>50</sub> µg/µl	Fiducial limits		Slope	Chi-square Value	RR*	RR**
		Lower	Upper				
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

\*\*Susceptible strain

### 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

Location	Larval weight (g)		Larval length (cm)	
	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

Location	Pupal length (cm)		Pupal weight (g)	
	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

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

\* Significant at 5% level

**CONCLUSION** It was clear that cypermethrin resistance levels varied greatly from location to location and it is evident that populations from northern part of south Indian cotton ecosystem recorded higher resistance folds for cypermethrin as well as higher phenotypic attributes when compared 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.

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### 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<sub>50</sub> of plant extracts such as garlic, onion and Neem and also, entomopathogenic agents such as mushrooms (*Metarhizium anisopliae*) and nematodes (*Steinernema* and *Heterorhabditis*); 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. Topical 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 Analysis 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 *Metarhizium anisopliae*, which LD<sub>50</sub> was 2.1X10<sup>11</sup> 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 pyrethroids. 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 glutathione-S-

transferases, besides piretroid compounds showed an additional mechanism called demolished resistance, and finally organophosphorate insecticides became insensible to the acetylcholinesterase.

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 entomopathogens 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<sub>50</sub> 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  $5 \times 10^9$  conidias/g, *Steinernema carpocapseae*  $10 \times 10^6$  nematodes/vial and *Heterorhabditis bacteriophora*  $10 \times 10^6$  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  $2.1 \times 10^{11}$  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 Martín, Hidalgo, Jalisco, México.

Treatment	Regression Equation	LD50*	Fiducials Limits 95%	LD95*	X <sup>2</sup>	r <sup>2</sup>
Garlic extract		No effect				
Onion extract		No effect				
Neem extract		No effect				
<i>Metarhizium anisopliae</i>	Y=0.0008X+4.885	2.1X10 <sup>11</sup> *	(17.5x10 <sup>10</sup> -2.5x10 <sup>11</sup> )	19.3x10 <sup>11</sup>	0.0534	0.91
<i>Steinernema carpocapseae</i>	Y=0.0312X+38.11	209.45**	(138.97-329.46)	136509.49	0.0048	0.80
<i>Heterorhabditis bacteriophora</i>	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. carpocapseae* 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. carpocapseae*, 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. carpocapseae* 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.

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### 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<sub>99</sub> of F<sub>50</sub> 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<sub>1</sub>) 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.

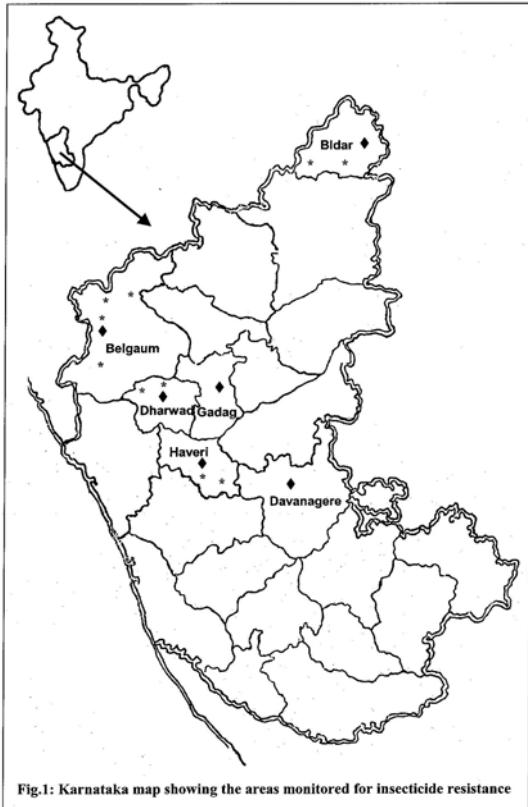


Fig.1: Karnataka map showing the areas monitored for insecticide resistance

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).

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**FIGURES AND TABLES**

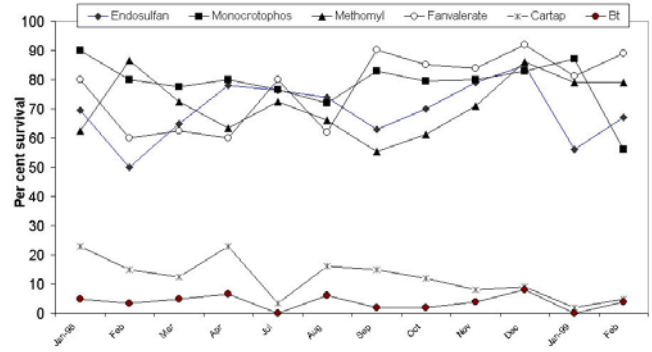


Fig.2: Dynamics of insecticide resistance in DBM at Dharwad

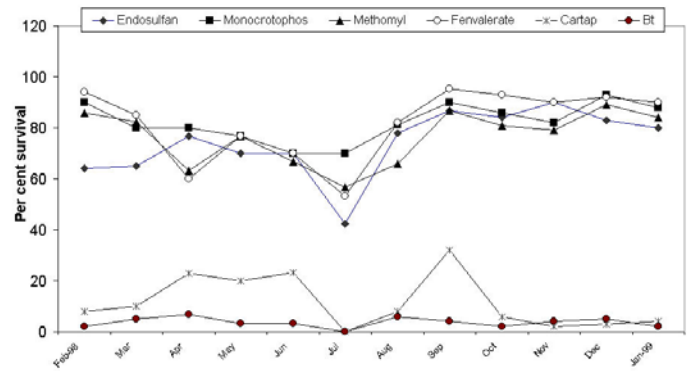


Fig.3: Dynamics of insecticide resistance in DBM at Belgaum

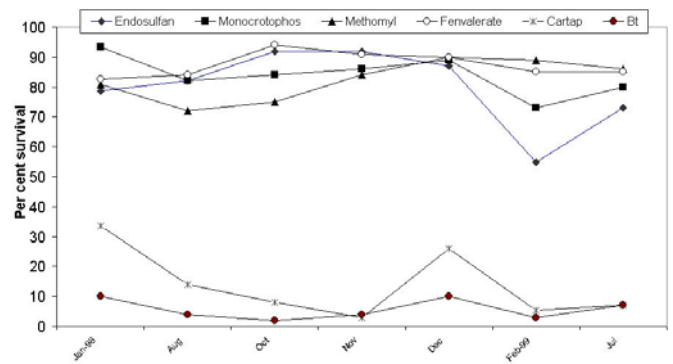


Fig.4: Dynamics of insecticide resistance in DBM at Haveri

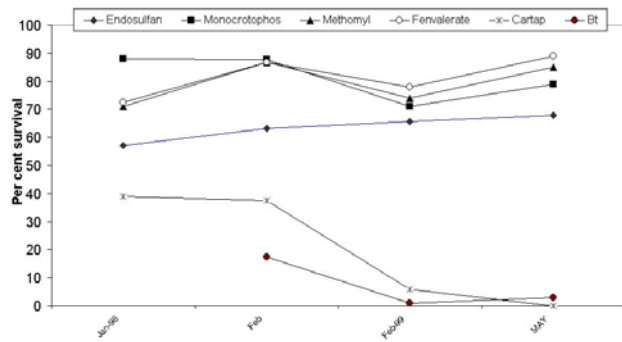


Fig.5: Dynamics of insecticide resistance in DBM at Bidar

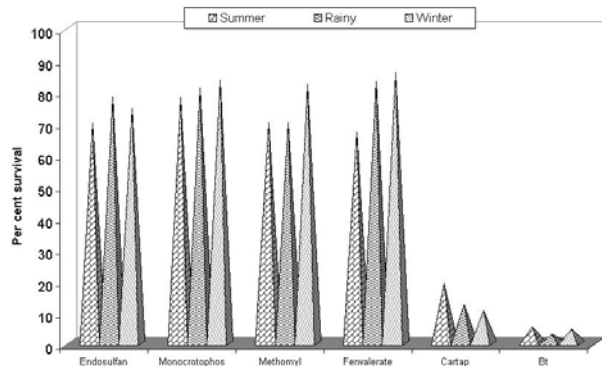


Fig.6: Seasonal variations in resistance levels in DBM

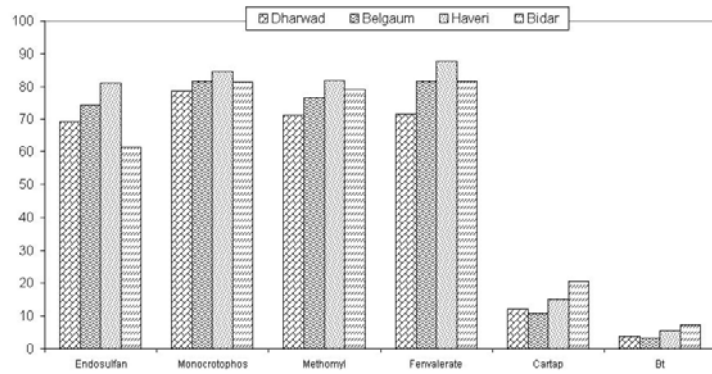


Fig.7: Resistance levels in DBM at different locations

District/Village	Month	Endosulfan	Monocrotophos	Methomyl	Fenvalerate	Cartap	Bt
<b>1. DHARWAD</b>							
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
<b>2. GADAG</b>							
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
<b>3. BIDAR</b>							
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
<b>4. BELGAUM</b>							
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
<b>5. DAVANGERE</b>							
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

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**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<sub>4</sub> 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<sub>50</sub> values for the F<sub>1</sub> hybrid was calculated. The results indicated the incompletely dominance of resistance in *P. xylostella* to these Bt toxins.

**RESULTS AND DISCUSSION** The results in Table 1 indicate that Cry 1Aa was most potent among the Bt toxins screened (1.25 ng/ cm<sup>2</sup>), where as the Cry 1B



and Cry 2A recorded 30.62 and 31.34 ng/cm<sup>2</sup> 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 <sub>50</sub>	LC <sub>90</sub>	LC <sub>95</sub>	Slope (± SEd)	Fiducial limits		Chi-square values
	ng/cm <sup>2</sup>				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 F<sub>1</sub> (RXS) to different ICP' s

Toxin /ICP	Population	Generation	LC <sub>50</sub>	Fiducial limit at LC <sub>95</sub>	Slope	RR <sup>a</sup>	D <sup>b</sup>	h <sup>c</sup>	n <sup>d</sup>
Cry1A	Cry1A -SEL	F <sub>9</sub>	2.06	3.71-6.04	4.54	2.11	--	--	240
	UNSEL	F <sub>9</sub>	0.97	2.07 - 3.69	2.82	--	--	--	240
	F <sub>1</sub> (Cry1A R.X.S)	F <sub>1</sub>	1.92	4.09 - 8.15	3.45	--	0.817	0.908	240
Cry1B	Cry1B -SEL	F <sub>9</sub>	114.54	272.88 - 496.45	3.24	4.71	--	--	240
	UNSEL	F <sub>9</sub>	24.27	81.63 - 299.77	2.03	--	--	--	240
	F <sub>1</sub> (Cry1B R. X.S)	F <sub>1</sub>	56.64	89.83 - 153.10	5.2	--	0.1	0.55	240
Cry2A	Cry2A -SEL	F <sub>9</sub>	38.86	94.50 - 119.83	6.39	2.85	--	--	280
	UNSEL	F <sub>9</sub>	20.62	44.49 - 80.67	3.55	--	--	--	240
	F <sub>1</sub> (Cry 2A R.X.S)	F <sub>1</sub>	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<sub>1</sub> progeny from mass crosses between the selected sub population and unselected subpopulation expressed that LC<sub>50</sub>s of F<sub>1</sub> 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.

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## FIGURES AND TABLES

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

Toxin	Generation	Dose ng/cm <sup>2</sup>	Survival percentage	'n'
Cry1A	F <sub>4</sub>	2.61	22	200
	F <sub>5</sub>	3.058	29.5	200
	F <sub>6</sub>	3.494	36.5	200
	F <sub>7</sub>	3.93	41	200
	F <sub>8</sub>	4.36	43	200
Cry1B	F <sub>4</sub>	46.69	38	200
	F <sub>5</sub>	54.48	45.5	200
	F <sub>6</sub>	62.22	47	200
	F <sub>7</sub>	70.04	52.2	200
	F <sub>8</sub>	93.39	59.5	200
Cry2A	F <sub>4</sub>	38.26	35	200
	F <sub>5</sub>	42.09	42	200
	F <sub>6</sub>	45.91	54	200
	F <sub>7</sub>	53.57	51.5	200
	F <sub>8</sub>	57.39	57.5	200

n – Number of larvae treated.

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## 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<sub>50</sub>) 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<sub>50</sub> of organophosphates with LD<sub>50</sub> of synergist with organophosphates.

#### FIGURES AND TABLES

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

Months	Per cent Resistance to			
	Quinalphos	Monocrotophos 1.0	Chlorpyrifos	Profenofos
	0.75 µg/larva	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)	Prof - (Prof + 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	

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## 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 limeticola* 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 (chlorothalonils) 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<sub>50</sub> 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).

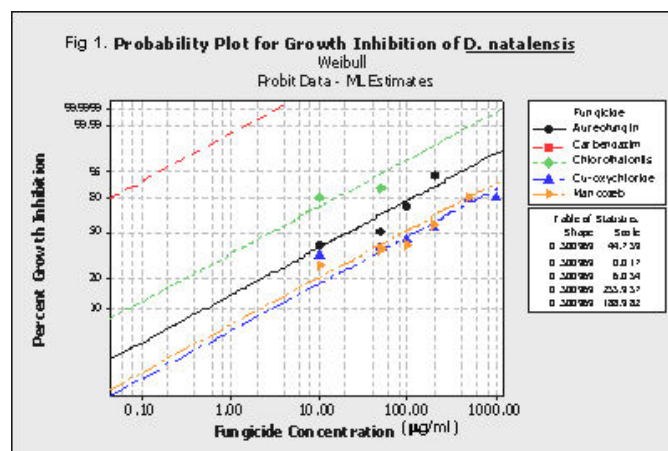


Table 1. ED<sub>50</sub> value for different fungicides against stem-end rot causing pathogens in citrus

Fungicides	ED <sub>50</sub> values (µg/ml)			
	<i>D. natalensis</i>	Cg-1 <sup>a</sup>	Cg-2 <sup>b</sup>	<i>G. limeticola</i>
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			
	<i>D. natalensis</i>	Cg-1 <sup>a</sup>	Cg-2 <sup>b</sup>	<i>G. limeticicola</i>
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			
	<i>D. natalensis</i>	Cg-1 <sup>a</sup>	Cg-2 <sup>b</sup>	<i>G. limeticicola</i>
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

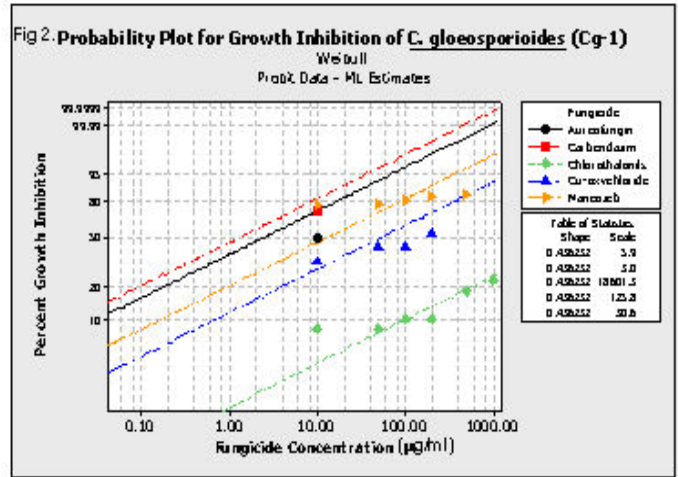
\* Complete growth inhibition at all the concentrations

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

*C. gloeosporioides* Isolate Cg-1

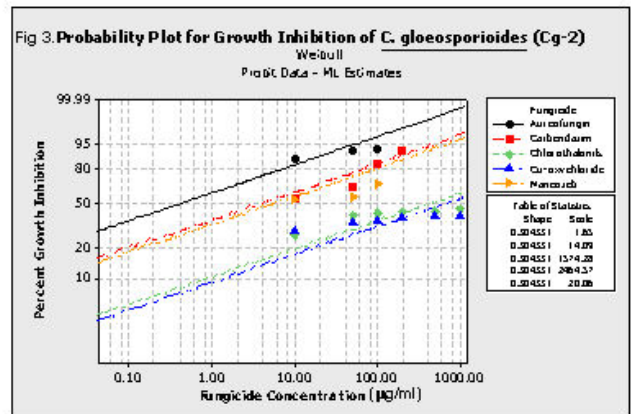
Aureofungin and carbendazim at concentration of 50 µg/ml were most effective in inhibiting mycelial growth of *C. gloeosporioides* Isolate Cg-1 and inhibited 100 per cent mycelial growth at this concentration (Figure 2). Mancozeb was also effective having ED<sub>50</sub> value 0.34 µg/ml (Table 1). Relative 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).



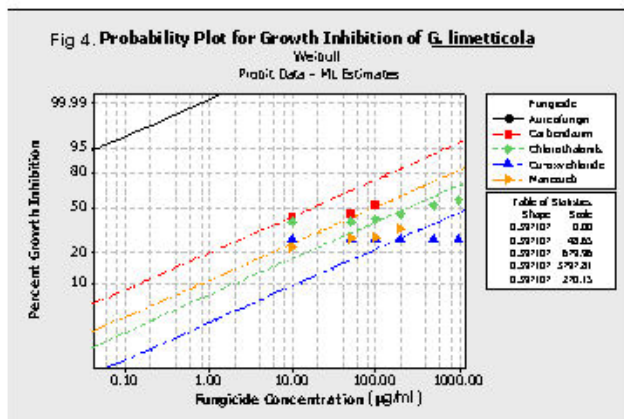
*C. gloeosporioides* Isolate Cg-2

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).



### *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 ED<sub>50</sub> value 0.34 and 17.40 µg/ml. Chlorothalonil was effective against only *D. natalensis* having ED<sub>50</sub> 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 (CaCl<sub>2</sub>.2H<sub>2</sub>O) concentrations in post-harvest treatments of guavas

(*Psidium guajava* L.) 'Branca de Kumagai' by the temperature differential method (fruits at 26°C and solution at 5°C 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.

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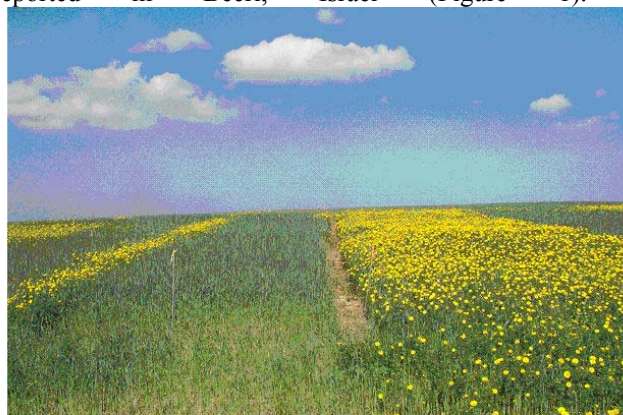
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## Herbicide Resistance

### 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 ai/ha tribenuron and 480 g ai/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'ATGAAAYGKCAAGAGYTRGC'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, pyriithiobac-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 <sub>50</sub> (g a.i./ha) <sup>a</sup>		R/S
		S	R	
Tribenuron	SU	2.5	>1822	>729
Imazethapyr	IMI	21	192	9
Flumetsulam	TP	36	>160	>4
Pyriithiobac-Na	PTB	271	3381	12
Propoxycarbazone-Na	SCT	56	>2688	>48

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

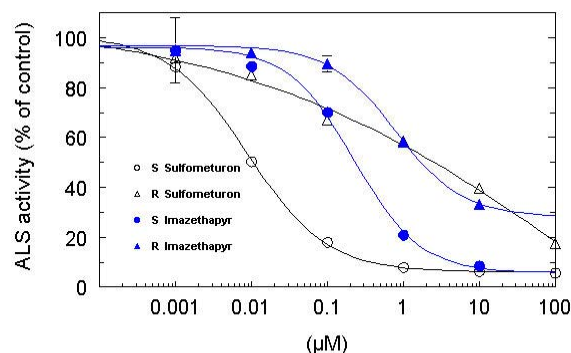


FIGURE 2. Inhibition of ALS extracted from S and R biotypes of *C. coronarium* from Gilat by sulfometuron and imazethapyr.

**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 where resistance has established and should be either replaced or combined with herbicides having different mode of action.

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### 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.E.m ±		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
<i>Azadirachta indica</i> leaf extract	4.0 (0.0)	100.0
<i>Ocimum sanctum</i> leaf extract	18.7 (25.6)	62.7
<i>Datura stramonium</i> leaf extract	30.0 (33.2)	40.0
<i>Allium sativum</i> 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

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## 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 sporulation 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 sensitivity 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.

Site	DMI moderately insensitive isolates (%)			Strobilurin (QoI) resistant isolates (%)		
	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* and rate per A (application date or interval)
1	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)
pumpkin			
2	60 A	5.4	Bravo and Ridomil/Copper (late July), Quadris alternated with Nova + contact fungicides (7 to 10 day program)
pumpkin			
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	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)
pumpkin			
4	6A	14.7	Bravo + Kocide (late July), Quadris once, Bravo + Nova (10-day schedule)
5	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)
pumpkin			
7	30 A	17.5	Alternated among Bravo, copper, and Stylet-oil on a weekly schedule. 1-2 applications of Quadris and Nova.
pumpkin			
8	2A	18.3	None: organically-produced crop
pumpkin			
9 pumpkin	1A	24.5	None: organically-produced crop
10	20 A	31.3	Manex 1 qt every 10 days
pumpkin			

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 nontreated 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.

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## 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>

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## 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

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## 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 truly 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](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.

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