Resistant Pest Management Newsletter

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Resistance Management from Around the Globe

Baseline Resistance

Base-line Values for Insecticide Susceptibility of an Indian Laboratory Strain of Diamondback Moth, *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae)

INTRODUCTION Diamondback moth (DBM), *Plutella xylostella* (L.) is a cosmopolitan pest of crucifers and its ability to develop resistance to insecticides deployed in its management has been well documented in different parts of the world (Georghiou, 1981). The resistance in DBM covers all major classes of insecticides *viz*., organochlorines, organophosphates, carbamates, pyrethroids, microbial insecticides and various IGR compounds (Cheng *et al*., 1992). In India, it has been not possible to know the exact situation in respect of insecticide resistance in DBM due to non availability of truely susceptible strains and lack of base-line values (Saxena *et al*., 1989), though comparative studies of DBM populations in different parts of country have shown occurrence of multiple resistance (Chawla and Joia, 1992; Renuka and Reghupathy, 1996)

Nearly three decades ago, Chawla and Kalra (1976) had emphasized the need for establishing base-line data for insecticide susceptibility using standard bioassay techniques for the correct appraisal of the problems of resistance in DBM. Here we present the base-line values for 26 insecticides that were established using a susceptible laboratory strain of DBM.

MATERIAL AND METHODS *Insects*: A susceptible strain of DBM was developed by rearing the insects in the laboratory for 58 generations under insecticde free conditions. The laboratory culture was intiated by using DBM larvae collected from cabbage fields around Hebbal, Bangalore, India. The insect was reared on mustard seedlings by adopting the mass -rearing technique developed by Liu and Sun (1984) with suitable modifications.

Insecticides: Twenty-six insecticides (formulated / technical) belonging to different chemical groups *viz.*, organochlorines, organophosphates, carbamates, pyrethroids, tertiary amines, acylurea compounds, *Bacillus thuringiensis* products, thiourea compounds and one insecticide mixture were used in the study (Table 1 & 2).

Table 1. Probit analysis of dosage-mortality response of the susceptible diamondback moth, *Plutella xylostella* to organochlorine, organophosporous and pyrethroid insecticides.

Insecticides	Trade name and Formulation	LD_{50} (µg a.i. g-1)	Fiducial limit (95%) (µg a.i. g-1)
Endosulfan	Thiodon 35 EC	111.94	80.61-154.27
Acephate	Asataf 75 SP	87.95	53.11-131.69
Chlorpyriphos	Corobon 20 EC	312.14	275.05-356.35
Diazinon	Basudin 20 EC	177.34	125.92-268.94
Dichlorvos	Nuvan 76 EC	97.69	71.24-127.41
Malathion	Cythion 50 EC	86.95	52.32-124.64
Methyl parathion	Metacid 50 EC	7.93	5.72-10.01
Monocrotophos	Nuvacron 36 EC	41.22	27.89-57.66
Phosalone	Zolone 35 EC	104.87	85.80-125.55
Profenofos	Curacron 50 EC	29.26	22.91-37.21
Quinalphos	Ekalux 25 EC	220.71	151.37-299.60
Triazophos	Hostathion 40 EC	111.14	67.48-165.94
Carbaryl	Sevinflo 42 F	158.72	113.90-214.24
Methomyl	Lannate 24 L	16.03	11.34-21.64
Alphamethrin	Technical grade (97 % purity)	2.09	1.41-3.11
Cypermethrin	Technical grade (94 % purity)	1.35	0.71-2.13
Deltamethrin	Decis 2.8 EC	0.41	0.20-0.66
Fenvalerate	Sumicidin 20 EC	0.29	0.16-0.55
Profenofos + Cypermethrin	Polytrin – C- 44	8	5.97-10.32

Bioassay: For bioassay of the conventional contact insecticides, the topical application method (FAO method 21) as outlined by Busvine (1980) was adopted. Appropriate dilutions of insecticdes were prepared in analytical grade acetone. At least five concentrations of the insecticides were used in each bioassay. Thirty-third instar larvae, each weighing 1.40mg, were treated in batches of ten for each concentration of the test insecticide. 0.3 ml of

Table 2. Probit analysis of dosage-mortality response of the susceptible daimondback moth, *Plutella xylostella* to tertiary amines, acylurea compounds and B.t. products

Insecticides	Trade name and Formulatiion	LC 50 (µg a.i. ml-1)	Fiducial limit-95% (µg a.i. ml-1)
Tertiary amines			
Cartap hydrochloride	Padan 50 SP	133.37	100.39-176.02
Acylurea compounds			
Flufenoxuron	Cascade 10 DC	0.002	0.0015-0.0028
Teflubenzuron	Nomolt 50 SP	0.038	0.030-0.047
***B. t.* products**			
Biobit	Biobit 29 % (potency 32000 IU/mg)	0.1322	0.05-0.268
Dipel 8L	Dipel 8L 3.5 % (potency 17600 IU/mg)	0.0083	0.0052-0.0124
Centari	Centari 3 % (potency 15000 IU/mg)	0.125	0.0806-0.1769
Thiourea compound			
Diafenthiuron	Polo 50 SC	17.05	13.25-21.58

insecticide solution was applied on the thoracic dorsum of each larva using a Top R microsyringe. The control larvae were treated with acetone alone. The treated larvae were maintained in petri-dishes containing excised mustard leaves at 25 ± 1º C in a BOD incubator. In the case of cartap hydrochloride, acylurea compounds and B. t. products, 'leaf - dip' method was employed (Tabashnik *et al*, 1987). The larval mortality counts were recorded 24-72 h after treatment and the data was subjected to probit analysis.

RESULTS AND DISCUSSION The median lethal dose of endosulfan (111.94 µg ai g -1) to the susceptible strain was about 90-fold lower than the LD_{50} value for a field strain of DBM established in 1976 (Kalra and Chawla, 1983). The susceptibilty of the laboratory strain to 11 organophosphorous compounds varied to a greater extent. Methyl parathion was the most toxic compound followed by profenofos, malathion, acephate, dichlorvos, phosalone, triazophos, diazinon, quinalphos and chlorpyriphos (Table 1). Similarly, in Japan, Hama (1983) observed profenofos to be more toxic to a suceptible strain of DBM than dichlorvos, diazinon, acephate and chlorpyriphos. Among the two carbamate insecticides, methomyl was about 10 times more toxic than carbaryl to the susceptible strain. These results in conformity with those of Hama (1983), and Chow and Cheng (1983).

In the case of synthetic pyrethroids, fenvalerate was highly toxic to the susceptible strain followed by deltamethrin, cypermethrin and alphamethrin (Table 1). However, studies by Liu *et al*. (1984) and Sun *et al*. (1986) indicated that deltamethrin was more toxic to the susceptible strains of DBM than fenvalerate and cypermethrin. The LC_{50} of cartap hydrochloride to the susceptible strain was lower (133.37 µg ai ml -1) in the present study compared to 290 µg a.i. ml -1 reported by Chow and Cheng (1983). Among the acylurea compounds, flufenoxuron was more toxic to the susceptible strain than teflubenzuron with LC_{50}'s of 0.002 and 0.038 µg ai ml -1, respectively (Table 2). Fauziah *et al*., (1992) also observed flufenoxuron to be more toxic than teflubenzuron with LC_{50}'s of 0.007 and 0.018 µg ai ml -1, respectively. Of the two B. t. *kurstaki* products, Dipel 8L was more toxic than Biobit. The LC_{50} of Dipel 8L is lower than that (0.12 and 1.77 µg ml -1) reported by Shelton and Wyman (1992) and Kobayashi *et al*., (1992), respectively .The LC_{50} of the B. t. *aizawai* product, Centari was lower than 0.21 µg ai ml -1 reported by Syed (1992). The LC_{50} of diafenthiuron observed in the present study was lower than that (245.90 µg ai ml -1) observed by Cheng *et al*., (1992).

Among the neurotoxins, pyrethroids were the most toxic compounds to the susceptible strain followed by organophosphates and carbamates. Further, the susceptibility of the laboratory strain to various organophosphates varied greately as reported by Chow and Cheng (1983) and Hama (1983). Though it is not possible to compare the actual base-line values of various insecticides with those obtained in similar studies by other workers because of different sources of susceptible strains used and different bioassay techniques adopted, the comparision of relative toxicities of various comopounds to the susceptible DBM strains indicate that findings of this study are more or less in agreement with the results of other such studies. The base-line values of different insecticides established in the present study can be used to quantify resistance in field populations of DBM in India.

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Generating baseline data for insecticide resistance monitoring in Sugarcane scale insect, *Melanaspis glomerata G.*

ABSTRACT Thiamethoxam was found to be toxic to *M. glomerata* followed by imidacloprid, dimethoate and methyldemeton, the LC_{50} of these insecticides to first generation of *M. glomerata* being 0.1584, 0.1705, 73.2891and 71.2700 ppm, respectively.The LC_{50} of these insecticides to third generation of *M. glomerata* was comparable to that of first generation indicating the susceptibility of the insect to these chemistries.

KEYWORDS: *M. glomerata*, baseline data, thiamethoxam

INTRODUCTION Sugarcane is one of the important commercial crops in the tropics and serves as the main source of sugar in the world. Sugarcane is known to be attacked by about 228 insects and non-insect pests in India (David and Nandagopal, 1986). Among them, scale insect, *Melanaspis glomerata G*. is causing severe outbreak in Tamil Nadu. In India, it remained as a minor pest till mid fifties. However, it was first observed in epidemic form at Pugalur in Trichy district of Tamil Nadu during 1956 (Narayanaswamy *et al*., 1957) which resulted in heavy loss of yield and quality. In subsequent years, the scale insect attained a major pest status and at present it is regular assuming devastating proportions in large cane areas of several states. Damage caused by the pest is varied. Reduction in germination of buds, inhibition of cane growth, reduction in cane yield even up to 63.40 per cent (Tembhekar, 1965), in juice up to 41.10 per cent (Prabhakara Rao *et al*., 1976), in commercial cane sugar by 35 per cent (Tembhekar, 1965) and also losses in jaggery production have been recorded. Hence acute toxicity studies were carried out for thiamethoxam, imidacloprid, dimethoate and methyldemeton against the scale, *M.glomerata* for three successive generations.

MATERIALS AND METHODS

Mass multiplication of scale insect

The scale insect was obtained from the sugarcane research station, Melalathur, Vellore district Tamil Nadu and was mass cultured in the Insectary, Department of Agricultural Entomology, Tamil Nadu Agricultural University. Two budded healthy sugarcane pieces, sliced from six months old canes of COC 671 were waxed at cut ends by dipping in melted paraffin wax to prevent desiccation and entry of pathogenic organisms. These cane bits were spread in trays (45 x 45 cm) that were painted black on the inner sides. Scraped bits of the stem, containing female in crawler extrusion stage from infested canes were collected from field and distributed uniformly over the healthy cane bits spread in the tray. The trays were then covered with black cloth to provide darkness. After 24 hours, canes bits were uniformly rotated for even

distribution of the emerging crawlers. After removing the wax at the lower cut end, the cane bits with established crawlers were transferred to cages and suspended vertically in plastic trays (45 x 22 cm) containing water. The entire setup was covered with mylar film cages (50 x 30 cm) with muslin cloth at the top. The scale insects were allowed to multiply on stem pieces at 28±5º C.

Baseline toxicity: Acute toxicity study was carried out for thiamethoxam, imidacloprid, dimethoate and methyldemeton against the scale insect for three successive generations. Infested sugar cane setts were sprayed using atomizer by appropriate dilutions of the test insecticide and shade dried. Setts sprayed by water alone, served as control. The treated setts were planted in a plastic bucket containing moist sand to maintain the turgidity. Each treatment was replicated thrice. Observation on the mortality of scales was recorded after 24 hours. LC_{50} and LC_{90} values, susceptibility index and rate of resistance declined were worked out (Regupathy and Dhamu, 2001).

RESULTS AND DISCUSSION *M. glomerata* was found to be highly susceptible as indicated from low LC_{50} value of 0.1584 for thiamethoxam (Table 1), 0.1705 for imidacloprid (Table 2), 73.2891 for dimethoate (Table 3) and 71.2700 for methyl demeton (Table 4). The LC_{95} values being 2.1773, 1.4608, 1156.9496 and 784.5087 ppm respectively for thiamethoxam, imidacloprid, dimethoate and methyl demeton respectively. The susceptibility of *M. glomerata* was tested without selection pressure to insecticides by rearing the population in glass house condition for three generations. The results indicated that the susceptibility increased as indicated from low LC_{50} and LC_{95} values.

The LC_{50} value for other pests is in close range. The LC_{95} values of *C. lanigera* was 1.2193 for thiamethoxam; 1.0462 for imidacloprid; 1094.5928 for dimethoate and 836.0015 ppm for methyl demeton (Vijayaraghavan and Regupathy, 2005). The LC95 values of 3745.0 ppm of methyl demeton (Hollingsworth *et al*., 1994) and 49.1667 and 418.4538 ppm for dimethoate and methyl demeton for *A. gossipii* (Praveen and Regupathy, 2004) and 2722.7 and 4466.8 ppm for dimethoate, methyl demeton respectively for *A. devastans* (Jeyapradeepa and Regupathy, 2002) were comparable.The LC_{50} and LC_{95} values declined when the generation proceeds. This results were in line with that obtained for *A. gossypii* by Praveen and Regupathy (2004), for *A. devastans* by Jeyapradeepa and Regupathy (2002) and for *N. lugens* by Sujatha and Regupathy (2003).

The LC_{50} values for third generation declined to 0.1253 ppm for thiamethoxam, 0.1490 for imidacloprid,72.0896 ppm for dimethoate and 66.4577 for methyl demeton; the LC_{95} values being 1.8113, 1.1296, 1033.2325 and 717.3333 ppm for thiamethoxam, imidacloprid, dimethoate and methyl demeton respectively. The results obtained were in line with the findings of Senthil kumar and Regupathy (2004); the LC_{50} values being 1.4289 - 45.3130 for thiamethoxam, 1.7144 - 75.2177 for imidacloprid and 11.4153 - 656.7360 for dimethoate to *C. viridis*. The susceptibility index varied between 1.01 and 1.26 based on LC_{50} and it was in the range of 1.09 - 1.29 based on LC_{95} values for all the chemistries tested. The rate of resistance decline was negative for all the insecticides tested, indicating the susceptibility of the test insect. In terms of number of generations required for 10 fold decrease in LC_{50}, a numerically high value was obtained for methyl demeton (99.00), imidacloprid (51.28), dimethoate (41.66) and thiamethoxam (29.49) (Table 5). The LC_{95} values are suggested as tentative diagnostic doses to monitor the development of insecticides resistant in case of field control failure.

Table 1. Acute toxicity of thiamethoxam to *M. glomerata*

Generation	Regression equation	χ^2	LC_{50} (ppm)	Fiducial limits		LC_{95} (ppm)	Fiducial limit	
				LL	UL		LL	UL
1	Y=1.8205 +1.4453x	2.2277	0.1584	0.1145	0.2192	2.1773	1.0141	4.6745
3	Y=2.0249+1.4181x	1.5814	0.1253	0.0872	0.1801	1.8113	0.8495	3.8621

Table 2. Acute toxicity of imidacloprid to *M. glomerata*

Generation	Regression equation	χ^2	LC_{50} (ppm)	Fiducial limits		LC_{95} (ppm)	Fiducial limit	
				LL	UL		LL	UL
1	Y=1.0654+1.7631x	0.5377	0.1705	0.1313	0.2213	1.4608	0.7985	2.6723
3	Y=0.9357+1.8701 x	1.0039	0.149	0.1132	0.1963	1.1296	0.6328	2.0163

Table 3. Acute toxicity of dimethoate to *M. glomerata*

Generation	Regression equation	χ^2	LC_{50} (ppm)	Fiducial limits		LC_{95} (ppm)	Fiducial limit	
				LL	UL		LL	UL
1	Y=-1.9638 +1.43139x	0.3659	73.2891	53.8927	99.666	1156.9496	482.1041	2776.4387
3	Y=-1.6288 +1.36456x	0.3232	72.0896	50.2819	103.3559	1033.2325	506.2545	2108.7603

Table 4. Acute toxicity of methyl demeton to *M. glomerata*

Generation	Regression equation	χ^2	LC_{50} (ppm)	Fiducial limits		LC_{95} (ppm)	Fiducial limit	
				LL	UL		LL	UL
1	Y=-2.6631+1.5791x	0.4493	71.27	50.4669	100.6496	784.5087	371.0201	1658.8153
3	Y=-2.6779+1.5921 x	1.1731	66.4577	49.4149	89.3786	717.3333	364.6289	1411.2077

Table 5. Susceptibility index and rate of resistance decline in *M. glomerata*

Insecticides	Susceptibility index		Rate of resistance decline	
	LC_{50}	LC_{95}	R	G
Thiamethoxam	1.26	1.2	-0.0339	29.49
Imidacloprid	1.14	1.29	-0.0195	51.28
Dimethoate	1.01	1.12	-0.0024	41.66
Methyl demeton	1.07	1.09	-0.0101	99

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Arthropod Resistance

Evaluation of Various Insecticides on the Cotton Whitefly, *Bemisia tabaci* (Genn); Population Control and Development of Resistance in Sudan Gezira

ABSTRACT The effects of endosulfan (chlorinated hydrocarbon), deltamethrin (pyrethroid) and chlorpyrifos (organophosphate) on the control of the cotton whitefly, *Bemisia tabaci* (Genn), were investigated during the 1989\1990 season with five applications in the field. The results indicate that endosulfan was a more effective insecticide than chlorpyrifos. However, the three pesticides did not perform well, as they did not decrease the pest population below the Economic Threshold Level (ETL) (i.e. 200 adults/100 leaves). The decreasing efficacy of the three insecticides, with increasing application, suggest the presence of multiple resistance in the Sudanese cotton whitefly *B. tabaci* in the Sudan Gezira. The result showed that the rate of resistance development increased with the increased intensity of selection i.e. the magnitude of the population exposed to insecticide and the population killed. The results show that pesticide application did not significantly enhance the grade quality of the cotton.

KEY WORDS: Whitefly, *Bemisia tabaci*, Chlorpyrifos, Deltamethrin, Endosulfan, Multiple resistance, Resistance.

INTRODUCTION The cotton whitefly, *Bemisia tabaci* (Genn), is an economically important pest of cotton in Sudan Gezira. Since cotton brings in close to half the country's foreign exchange, plant protection in cotton is highly important.

Chemical control is the primary method used to manage the whitefly population in the world and cotton receives more pesticides than any other crop. Insecticides used include: DDT, endosulfan, malathion, parathion, methyl parathion, dimethoate, dicrotophos, monocrotophos, profenofos, quinalphos, chlorfenvinphos, sulprofos, dichlorovos, fenthion, methomyl, aldicarb, carbofuran, cypermethrin, permethrin, deltamethrin, resmethrin and amitraz (Wardlaw *et al*., 1976; Watve *et al*., 1977; Abdeldaffie, 1984; Ahmed, 1984; Prabhaker *et al.*, 1989).

Although survey for natural enemies involve parasites, predators and pathogens of *B. tabaci* has

been done in worldwide they are hymenopterous parasites *Encarsia lutea* (Masi), *Encarsia nigricephala, Encarsia quaintancei, Encarsia formosa, Encarsia transvena* (Timberlake), *Eretmocerus mundus* (Mercets), *Eretmocerus diversiciliatus* (sliv.) *Prospaltella sp., Amitus bennetti* and *Amitus aleuroglanduli* (Gameel, 1966b; Schmutterer, 1969 and Gameel and Abdelrahman, 1978; Viggiani and Evans 1994; Brash *et al*., 1994; Rodriguez *et al*., 1994; Castineiras, 1995). The whitefly eggs and first larval stages are preyed on by the phytoseiid mites' *Abmlyscius aleyrodis* (El-Badry) and *Typhlodormus sudanicus* (Aef.) (Gameel and Abdelrahman, 1978). *Scymanus marginalis, Chrysopa flava* (scopoli) *Chrysopa carnea* (Stephens) *Chrysopa exterior, Crytopeltis varlans, Delphastus pallidus, Theridula gonygaster, and Theridula spp.* has also been reported (Sharaf Eldin, Sallem and Omer, 1978; Castineiras, 1995). The fungus *Paccilomyces forinosus* (Bain) has been found infesting the cotton whitefly (Nene, 1973; Castineiras, 1995). However the chemical control is the primary method to mange the whitefly population in Gezira and the whole world.

The major problem with frequent pesticide applications is insect resistance. When this occurs the usual practice is to increase the dosage and frequency of pesticide application, which only exaggerates the problem (Bettini *et al*., 1970; Brown and Pal, 1971; Busvine, 1980; Georghiou, 1986). The emergence of whitefly as a serious pest of cotton in the Sudan followed the application of DDT that was used to control Jassid, *Empoasca lybica* (de Berg), and the American bollworm (ABW) *Helicoverpa armigera* (Hub) (Dittrich *et al*., 1987). Since cultural or biological control of whitefly is limited protection aside from plant up rooting has primarily depend upon pesticide application, and biological control methods have never been used for controlling cotton pests in Gezira (Hassan, 1969). The whitefly exists from the beginning to end of cotton season and high densities of whitefly has a detrimental effect on yield and grade of cotton (Gameel, 1969; El-Tayeb *et al*., 1978; Bull, 1982).

In Gezira scheme, pesticide use for cotton was initiated in 1945/1946 (Osman and Balla, 1985). Prior to 1963, an average of one spray or less per season was employed to control Jassids, and to some extent whiteflies and flea beetles, *Podogrica puncticollis* (Weise). The whitefly became a serious pest of cotton in the early 1960's. At that time the pest was usually controlled with one to two seasonal sprays of dimethoate or endrin (Gameel, 1969; El-Tayeb *et al.*, 1978). From 1964/1965 to 1967/1968, the mean number of sprays applied per season ranged from 3 to 5. The additional sprays were necessary because the ABW became an important pest of cotton, and the whitefly was increasing in importance. By 1968/1969 whitefly infestation became serious and its honeydew secretions that caused lint stickiness adversely affected the quality of the cotton. To overcome this problem, the frequency of pesticide application was increased to control the late-season whitefly population (El-Tayeb *et al.,* 1978). And by 1968 6 to 7 applications per season were routine. About twenty-eight insecticides and various compatible combinations are registered for use on cotton, and seventeen insecticides are used to control the whitefly. Of these the most widely used are torbidan, endosulfan, dimethoate, and monocrotophos.

Previous studies have demonstrated that, the whitefly has developed resistance to the pesticides: such as dimethoate, monocrotophos, malathion, methyl parathion, carbofuran, DDT, resmethrin, methomyl, dichlorvos, chlorpyrifos, sulprovos, fenthion, parathion, permethrin, and amitraz (Wardlaw *et al*., 1976; Watve *et al.*, 1977; El-Hag, 1981; Dittrich and Ernst, 1983; Prabhaker *et al.*, 1985; 1989; Dittrich *et al.*, 1985; Ahmed *et al.*, 1987; Abdeldaffie *et al.*, 1987; Yassin *et al.*, 1989; El-Zubier, 1990).

In the Sudan, as a consequence of wide spread pesticides application resistance of *B. tabaci* was demonstrated for the first time in the 1981/1982 season, and has been monitored since then. The over-reliance on broad-spectrum insecticides may account for the serious resistance problem in *B. tabaci* (Dittrich and Ernst, 1983). The highest resistance was reported for dimethoate and monocrotophos (OPs), which were used in large quantities (Dittrich and Ernst, 1983; Dittrich *et al.*, 1985; Ahmed *et al.*, 1987; Abdeldaffie *et al*., 1987; Yassin *et al*., 1989).

The present work examines the effectiveness of endosulfan (chlorinated hydrocarbon), deltamethrin (pyrethroid) and chlorpyrifos (organophosphate) in controlling the cotton whitefly.

MATERIALS AND METHODS The field trials were conducted in Sudan at the University of Gezira farm at Neshishiba, Wad Medani (14º 24'N 33º 29'E, 407m above sea level) during the 1989/1990 season. The experimental area reserved for the trials was 2.5 feddans (Fed=4200m) for each season. From this only one fed. was sown with cotton and the neighbouring plots (i.e. the rest of the area) were left bare to minimize the migration of pest from one plot to another. The area was sown in mid-August with cotton, *Gossypium barbadense*. Spacing was 80cm between ridges and 50cm within ridges. Five seeds were planted per hole, but seedlings were eventually thinned to three per hole. In both seasons the entire experimental area was treated with the pre-emergence herbicide, stomp (pendimethalin) (50% EC) applied by tractor-mounted sprayers at the rate of 800g a.i. per fed. All plots received nitrogen fertilizer at the rate of 2N (80Kgs) per fed. in the form of urea immediately after thinning.

Supportive hand weeding was done twice and irrigation was conducted fortnightly.

Three insecticide treatments and an untreated control were compared in a randomized complete block design (RCBD) with three replications [plot size was 16x21m (i.e. 0.08 fed)].

Insecticides Tested and Method of Application The insecticides tested were (a) endosulfan (Rhonc Poulenc, France) 50% effective concentration (EC) at 405g a.i. per fed. (i.e. 65ml/plot), (b) chlorpyrifos (Dow chemical Ltd., England) 4.8% EC at 480g a.i. per fed. (i.e. 67.5ml/plot) and, (c) deltamethrin (Dow chemical Ltd., England) 2.5% EC at 6.25g a.i. per fed. (i.e. 20.8ml/plot). All the chemicals were commercial formulations applied at the dosage recommended by the Agricultural Research Corporation (ARC) of the Sudan.

Insecticides were dissolved in water and administered by pressurized knapsack sprayers at a spray volume of 24 L/fed. (2L per plot). The pressure was calibrated at 2-3 bar. The first insecticide application was delayed to allow the build-up of the whitefly population.

The field experiments were conducted in season between October 1989 and January 1990. The field were sprayed with insecticides in 1989 on November 2, 19, and December 6, 23, and in 1990 on January 9.

Field Insect Counts: Routine counts at regular intervals were made to assess population trends of the adult whiteflies. Adults were counted using a random sample of twenty plants per plot, selected on a diagonal course within the plot. For each plant five fully-grown leaves (two apical, one middle and two basal) were examined. Each leaf was carefully turned over and the number of adults on the lower surface was recorded using a counter. Counting was usually done early in the morning when adults were inactive.

Data Collection: The pre-spraying counts were conducted 24hrs before spraying and post-spraying counts were taken 24hrs after application. The data collected were used to determine: (a) Mean numbers of the initial whitefly population before insecticide application. (b) Mean numbers of whitefly population immediately prior to insecticide application throughout the season. This parameter reflects the relative residual effect (RE) of the insecticide. (c) Mean population numbers following each pesticide application. This shows the effects of the insecticide. (d) The relative general performance (GP) of the tested chemicals was determined by averaging the population throughout the 1989/1990 season with the exception of the first count.

Quality Tests: A seed cotton sample was taken from each plot, ginned and taken to the laboratory for stickiness testing and grade.

Statistical Analysis: The data collected were subjected to analysis of variance (ANOVA) Percent decrease in Whitefly population subsequent to spraying was obtained by the formula:

$$\% \text{ Drop} = \frac{P1 - P2}{P1} \times 100$$

Where:
P1 = mean of the pre-spray population.
P2 = mean of the post-spray population.

RESULTS The whitefly population was assessed in the control plots during the period from mid-October to early-January. A minimum of 93 adults per 100 leaves were counted during mid-October, 1989 and a maximum population of 6680 adults per 100 leaves was counted at the end of December 1989. The average throughout the season was 2627 adults per 100 leaves (Table 1; Fig. 1).

Fig 1. Population trends of whitefly adults in cotton under three insecticides treatments compared with the untreated control.

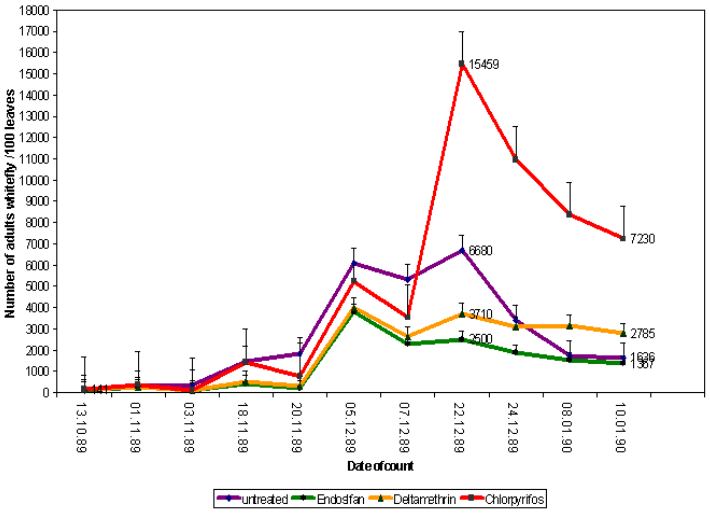


Table 1. Average number of adults Whiteflies in the control plots between October 1989 and January 1990

Date	No of adults per 100 leaves										
	Oct.13th	Nov.1st	Nov.3rd	Nov.18th	Nov.20th	Dec.5th	Dec.7th	Dec.22nd	Dec.24th	Jan.8th	Jan.10th
Mean	93	293	342	1483	1847	6064	5325	6680	3410	1727	1637
C.V.%	27.1	24.9	20.6	13.5	11.1	8.4	17	1.7	17.2	13.5	9.2
SE±	26.15	42.86	18.58	74.4	50.81	233.75	241.04	70.54	290.26	289.49	111.29
Average	2627.4										

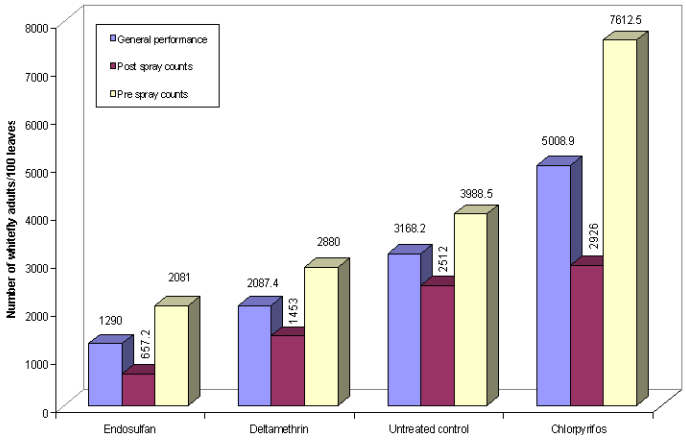
Table 2. Effects of various insecticides on the number of adult whiteflies on cotton

Treatment	1st counts * Adults/100 leaves	post-spray counts (average of 4 counts) Adults/100 leaves	Pre-spray counts (average of 5 counts) Adults/100 leaves	General performance (average of 9 counts) Adults/100 leaves
deltamethrin	139 a	2880.5 ab	1453 ab	2087.4 ab
endosulfan	274 a	2081 a	657.2 a	1290 a
control	293 a	3988.5 abc	2512 abc	3168.2 abc
chlorpyrifos	385 a	7612.5 bc	2926 bc	5008.9 c
SE±	42.86	1345.01	649.96	707.16
CV%	6.2	16.2	19.3	18.4

Means followed by the same letter in the same column are not significantly different (p=0.05).

* prior to insecticide spray.

The results of the pre-spray counts showed that the number of whitefly adults after treated with endosulfan, deltamethrinand chlorpyrifos were 2081, 2880.5, and 7612.5 per 100 leaves respectively. The mean number of adults in the control plots was 3988.5 per 100 leaves (Table 2, Fig. 2). The number of whitefly adults in the endosulfan treated field were significantly lower (p=0.05) than the number of adults in the chlorpyrifos treated plot.



After spraying with pesticide, the mean adult whitefly population in the endosulfan, deltamethrin, and chlorpyrifos plots were 657.2, 1453, and 2926 per 100 leaves respectively (Table 2; Fig. 2). The mean population in the control plots was 2512 per 100 leaves. Plots treated with endosulfan demonstrated significantly fewer (p=0.05) whitefly adults than the chlorpyrifos treated fields.

The general performance (G.P), of endosulfan, deltamethrin and chlorpyrifos was 1290, 2087.4, and 5008.9 adults per 100 leaves respectively. The mean number of adults in the unsprayed plots was 3168.2 per 100 leaves. The number of whitefly adults in the endosulfan treated plots was significantly lower (p=0.0 5) than the number of flies counted in the plots treated with chloropyrifos.

The evaluation of the insecticides in terms of percent decrease in adult whitefly population showed that chemically mediated mortality fluctuated within the growing season (Table 3). Twenty-four hours after the initial spraying with three insecticides there was a marked percentage decrease in whitefly population of 60.2-73.2%. The effectiveness of the insecticides decreased throughout the season and following the fifth application there was a decrease in whitefly population of 11.5-13.5%.

Table 3. Percent drop in the whitefly adults population 24 hours after the application of different insecticide on cotton during season 1989/90.

Treatment	Spry number					Mean
	1st	2nd	3rd	4th	5th	% drop
chlorpyrifos	73	46	33	29	14	28.8
endosulfan	67	55	39	27	10	39.8
deltamethrin	60	41	34	17	12	32.6

The yield and grade quality of the cotton following pesticide treatment is shown in Table 4. Chlorpyrifos and endosulfan treated cotton gave yields of 118.67 and 117.33 Lb./plot respectively, while the control plots gave a yield of 84.33 Lb./plot cotton, which was not significantly different from the yield of cotton obtained following deltamethrin treatment (114.67 Lb./plot). There was no significant difference in grade quality of the cotton.

Table 4. Affect of insecticide treatment on cotton yield and quality

Treatment	No. of Sprays	Yield lb/plot	Lint stickiness grade*	Grade
Control	0	84.33 a	3-Feb	4
Deltamethrin	5	114.67 ab	3-Feb	4
Endosulfan	5	117.33 bc	2	X4
Chlorpyrifos	5	118.67 bc	3	4
SE	-	8.95	-	-
CV %	-	3.6	-	-

* Honeydew grading at the minicard at 65% R.H. and 27 oC

0=free, 1=light, 2=moderate, 3=heavy and 4=very heavy

DISCUSSION The results from the pre-spray counts showed that endosulfan was significantly more effective than treatment with chlorpyrifos. In assessment of the post-spray counts, endosulfan was again, statistically more effective against the whitefly than chlorpyrifos. These results agree with the work conducted by Osman (1985) reported that endosulfan was a more effective insecticide than chlorpyrifos. However, all chemicals did not perform well, since none of them was able to drop the pest populations below the ETL (i.e. 200 flies/100 leaves).

In the post-spray counts, results also agreed with the findings of Osman (1985) which indicated that endosulfan was the best treatment; chlorpyrifos came late in the order of efficacy. However, the populations were also high when compared to the ETL (endosulfan 424.4 and chlorpyrifos 528.4 adults per 100 leaves). Again, Eisa (1989) demonstrated that endosulfan was the best chemical when compared to others.

With regard to the GP, endosulfan and deltamethrin were the best treatments. The infestation level in chlorpyrifos plots was not significantly different from those of the untreated control (Table 2, Fig. 1). Also, the populations were very high when compared to the recommended ETL. The results came in line with the fieldwork of Osman (1985) who found that endosulfan was the best chemical which was not significantly different from chlorpyrifos.

The percent mortality of the adults caused by the insecticide application decreased with the progression of the season; chlorpyrifos decreased from 73.5% to 13.5%, endosulfan from 67.2% to 10.1%, and deltamethrin from 60.2% to 11.5%. This may be due to an increase in whitefly resistance following intensive insecticide use (Wardlow *et al*., 1976). The detoxification mechanisms of insecticides take place in insects through metabolic degradation, which decreases the efficacy of the insecticide (Dittrich *et al*., 1985, 1986; Ishaaya *et al*., 1987).

The results obtained with the insecticide endosulfan agreed with the field work results reported by Osman (1985), and Eisa (1989), and the laboratory work results reported by Ahmed *et al.* (1987), and Yassin (1987). The pyrethroid deltamethrin was perhaps less toxic because the pyrethroids are esters that are metabolized through the action of detoxification esterases and oxidases, and are also directly excreted (Dittrich *et al*., 1985; Ishaaya *et al.*, 1987). The organophosphates (OP's) have been under extensive and intensive use since 1960 (e.g. dimethoate, monocrotophos and dicrotophos). Ahmed (1984) demonstrated that *B. tabaci* of the Sudan Gezira has developed resistance to dimethoate. Osman (1985) and Yassin (1987) proved that chlorpyrifos is less toxic to whitefly than endosulfan and endosulfan/chlorpyrifos. The high incidence of the whitefly adults in the chlorpyrifos plots may be a result of the negative impact of the insecticide to the natural enemies of the whitefly.

The decreasing efficacy of the three insecticides compare to the control were ineffective with increasing application suggests the presence of multiple-resistance in the Sudanese cotton whitefly, *B. tabaci* in the Sudan Gezira. Chemical control is currently the only management technique used on cotton against the whitefly in the Sudan. Until other forms of control can be developed and effectively implemented, heavy reliance upon insecticides, with its accompanying problem of resistance, will probably continue.

Furthermore, the effect of these chemicals should be studied physiologically and biologically so as to be able to determine the fate of these chemicals *in vivo* and *in vito.*

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Temporal and Toxicological Dynamics in the Cover Spot Patterns of the Colorado Potato Beetle in South Ural

INTRODUCTION The Colorado potato beetle (CPB) *Leptinotarsa decemlineata* Say is a dangerous pest of potatoes over a broad area in Europe and ex-USSR. This species diversified by high degree of polymorphism, ecological liability and the most adaptive potential among species of the genus *Leptinotarsa* [1]. The inductors of microevolution transformations are not only environmental hydrothermal factors, but also coevolution host plants immunity and anthropogenic factors, in the first place, insecticides intensively applying.

Resistance of CPB is registered for this moment to 40 insecticides of the different types [2]. Also noted polymorphism of CPB perceptivity degree to endotoxin of *Bacillus thuringiensis* and as a consequence - presence of probably resistance potential to Bt-transgenic (*Bacillus thuringiensis*) potatoes [3].

Estimation of CPB adults' susceptibility conducted in 1984-85 years allowed characterization of the population formed at that time as homogenous concerning pyrethroids susceptibility level [10]. In 1996 year the pest formed the resistance to pyrethroids; the appearance of small portion of high-resistant individuals was noted. In the next years there was demonstrated that in Bashkortostan prolonged unrelieved pyrethroids treatments led to appearance in the some regions of the local populations with high resistance levels [11].

Checking of the genetic processes in CPB populations is necessary for improvement of the potatoes protection systems that requires the reliable markers. There was noted earlier that certain phenes of cover spots of different body parts of the CPB mark its resistance to insecticides [4 - 9].

There was shown earlier that as a result of insecticides influence the change of phenotypic structure in pest populations observed [4 - 9]. Perhaps it is a consequence of selective survival of individuals resistant to insecticide effects and marked with certain phenes of cover spots.

The purpose given work was determination of factors, influencing upon population structure dynamics of CPB in territory of Bashkortostan by analysis the cover spot patterns.

MATERIALS AND METHODS The samples of the CPB (adult males and females of overwintered and second generations) were collected in 1989-1990 and 2000-2005 years in Bashkortostan. In toxicological experiments were defined the LC_{50} values of several insecticides: carbophos, 100 g/Kg, JV (malathion); decis, 2.5% KE (decamethrin); actara, 250 g/Kg, VDG, (the thiometaxam); mospilan, 200 g/Kg, RP, (acetamyprid). Using a microliter pipette "MK16" a 1 μl/ individual of an ethanol solution of insecticide was applied topically thorax. Insects in control groups were treated with ethanol. After account of mortality (on 7th day after treatment) was conducted individual description of phenomorphs for all individuals taken in experiment.

RESULTS AND DISCUSSION For significant reflection of microevolution processes and clear determination of the population borders within the framework of area of the species the phenogenetic analysis is applicable [12]. It Is considering that for elucidation of the concrete population borders the refusal of "purely genetic" methods, such as methods of the crossbreeding, entails the necessarily of the differences estimation for groups under investigation not by one or several phenes, but by groups of ten, or even hundred phenes. At the same time, as it seems, there is possible to dispense with the smaller amount of phenes if for analysis to select groups of phenes, closely related by functions, but referring to different levels of biosystems organization.

Spot cower patterns of CPB adults became the subject of attention and detail researching still since the beginning of the XX century [13]. In the rather much number of studies, devoted the variability of the sign, all authors divide phenes to groups coinciding with parts of body: 1) head, 2) pronotum, 3) elytra , 4) abdomen and 5) legs. In our researching were used the initial 3 groups.

Phenes of head tracery are dividing in turn into two groups. The first of them - vortex tracery, that is the middle spot, mostly heart-shaped [13]. In the Russian investigators articles were described different variations of this tracery [5, 8]. Generally, the vortex tracery variations reflect the differences in melanization degree, thus dividing into several groups by this sign is justifiable. Proposed by E.P. Klimets [5] dividing into 3 groups **m - o - III** by our observations is insufficiently, in particular as far as concerned the most intensity of pigmentation: we found the variations with still more intensity, and we propose to describe them as morphs **3** and **M** (fig.1 a).

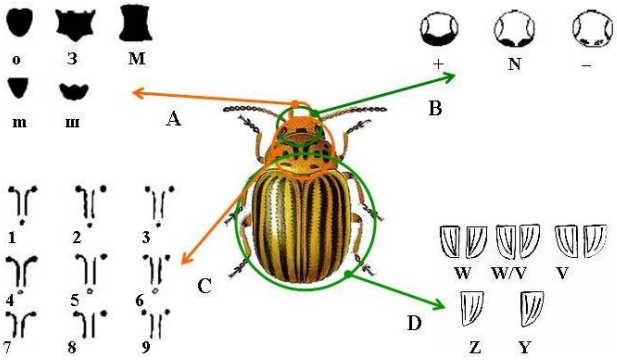

Fig. 1. Cover spot patterns of CPB. A – vortex, B – occiput, C – pronotum, D – elytra.

The second groups of phenes -the tracery of back of the head (occiput), which consists of two symmetric spots, early described [13] as oval spots. By our observing, the intensity of these spots pigmentation is describing in general by 3 morphs: Subnormal (**Sub**, or -) - weekly melanizated, clear separated spots ; Normal (**N**) - spots with intensive pigmentation, which are no merged and no reach the back border of eyes; and Extremal (**Ex**, or +) - intensively pigmented spots, which are merged in proximal edges and reach the back border of eyes (fig.1 b).

The cover spot patterns of pronotum were used repeatedly for the CPB polymorphism analysis; in the recent time the most acceptable is the classification by Sergey Fasulati [6], uniting the phenomorph variability to 9 groups (fig.1 c).

The tracery of elytra define the species name - decemlineata. In the some articles [5, 8] are described the variations of this group of phenes. We added to this classification the annual registering phenes **Y** and **Z** (fig. 1 d).

In fig.2 shown the ratio of meeting frequency for CPB phenes in general excerpts and in excerpts of individuals survived after the insecticide treatments. There is possible to pick out the set of phenes for excerpt of survived adults, with frequency predominated the frequency of the same phenes in total excerpt.

встречаемости которых преобладают над таковыми в общей выборке

Therefore, for the vortex they are "m" and "3", for back of the head - "+"; "3", "6" and "9" - for the pronotum. Among the elytra phenes are predominate "w" and "w/v". In the field population, frequencies of these patterns were 0.204, 0.102, 0.515, 0.217, 0.215, 0.120, 0.829 and 0.114 accordingly.

Since the tracery phenes genetically can be coded by big number of genes [12, 14], there is possible that some of them are located near the genes "responsible" for resistance in CPB to insecticides, marking thereby the resistant individuals and populations as a whole.

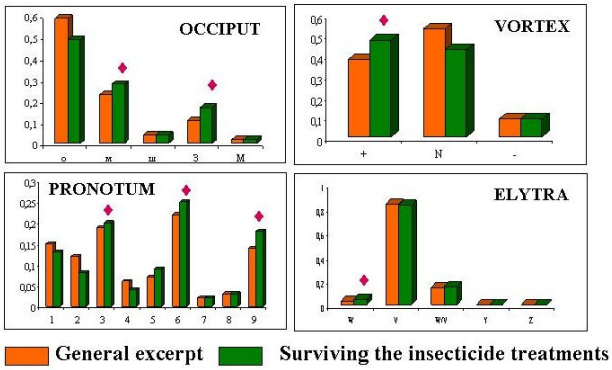

Fig. 2. Differences of cover spot patterns frequencies in CPB. Red spot – predominate of phenes in survived adults.

We shall consider the changes of the ratio chosen by us marking phenes during the row of the years, as well as level LC_{50} for decis (most broadly applicable preparation for CPB control) in Ufa region of Bashkortostan. In particular, value LC_{50} for given preparation for period from 1989 - 1993 to 2000 - 2003 years increased since 0.0015% before 0.014% accordingly that is to say nearly in 10 once. Fig. 3 illustrates the correlations between resistance level to insecticides and frequency of marking phenes in local populations of the CPB. It Is seen that increase of the LC_{50} value has a cognate directivity with increasing the frequencies of marking phenes.

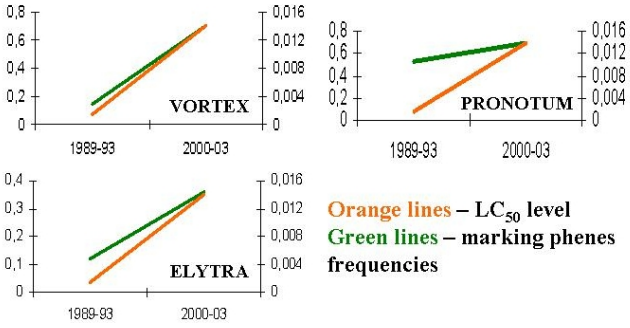

Fig. 3. Increasing of marking phenes frequencies and resistance level in total excerpts of CPB.

It was noted also the reduction of biodiversity level (Shannon-Weaver index [15]) during last 10 years that is, in our opinion, the result of insecticide pressure (Fig.4). Herewith in the course of selection the elimination of individuals with susceptible genotypes occurs that brings about reduction of the genetic diversity in populations.

CONCLUSION Thereby, under the total trend to accumulation of the marking phenes portion among the adults of CPB for specified years, turns attention on itself that for the first 5 years of the observations (1989 - 1993 years.), during which in Bashkortostan was winnowed still active goal-directed CPB control in all-republican scale with attraction of the agricultural aviation, mass treatments with pyrethroids modern

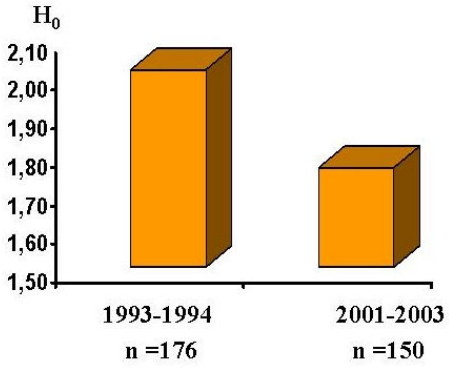

Fig. 4. The biodiversity level in karmaskalinskaya local population of CPB (values of Shannon-Weaver index, H_0).

phosphororganic preparations, the accumulation of the marking phenes portion under the action of insecticidal press developed quicker, than in total excerpt though as a whole picture possible to consider identical. Exactly this facts follows to consider the basis for enabling the account of phenetic data to the system of the CPB monitoring in territory of the Russia.

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Bioeffectivity of Some Botanicals and Conventional Insecticides Against *Plutella xylostella* (L.) from IPM Fields.

The diamondback moth (DBM), *Plutella xylostella* (L.) acquired the status of a major pest of vegetable crucifer crops in India. In recent years, with the rapid development and introduction of off-season

varieties, the cole crops are grown almost through out the year providing a year round food supply for the DBM. Farmers usually apply chemical insecticides quite frequently and excessively to counter this pest on vegetable cole crops, particularly the cabbage and cauliflower. Due to the indiscriminate application of various insecticides the DBM has developed resistance to the most of these commonly used insecticides and become highly problematic in several parts of the country (Raju, 1996; Raghupathy, 1996; Gujar & Mohan, 2002; Singh *et al*., 2005). Further the biological characteristics of the insect and agronomical practices of commercial crucifer production accentuated the development of resistance at place as the farmers are more relay on monocropping and insecticides.

Our earlier studies as well as related studies from other parts of the country on monitoring resistance in DBM to most commonly used insecticides have clearly indicated the development of resistance to many of the major groups of the insecticides *viz*., chlorinated hydrocarbons, organochlorines and pyrethroids (Raju, 1996; Kalra *et.al.*, 1997; Nirmal & Singh, 2001; Seenivasagan & Phokela., 2001). Quite often such control failures and out breaks of DBM on cabbage and cauliflower were also reported Usha Chauhan, 1995; Joia & Udeaan, 1998; Singh *et al*., 2005 claiming major threat for commercial vegetable cole crops production.

As a resistance management strategy a region focus insecticide resistance management program for DBM was developed and implemented during 2002 - 2004. This program emphasized using alternative methods and techniques in a coordinated manner. In this program 12 farming families were selected for implementing the management strategies of DBM. The program featured a cost effective scouting system involving presence / absence of DBM and its most common larval parasitoid, *Cotesia plutellae* Kurdjmov on plants and proven action threshold for cabbage and cauliflower cultivers. At first the nursery beds were covered by Agronets after germination from a height of about 2 ft to prevent the egg laying by DBM and *Spodoptera* in the nursery. Thus, 3 to 4 sprays of insecticides on an average given by the farmers to control insect pests in the nursery were totally avoided and the healthy, insect free nursery were transplanted in the main field. The average land holding of 12 farmers for commercial cultivation of these crucifer vegetables was 1.25 acres. The recommended package of practices for growing both theses crops were followed through out the cropping period and the program also included a resistance management strategy using selective insecticide in rotation to maximize the impact of natural enemies.

After 15 days of transplantation the crop was given two sprays of NSKE 5% at 10 days interval on the occurrence of the DBM in the main field. It was followed by application of two sprays of pongamia soap 4% (*Pongamia pinnata*) formulations at an interval of one week. Later with the initiation of curd formation the crops were treated with *Bt* var. *Kurstaki* formulation at 1kg/ha. With all these treatments and with prevailing larval parasitoid population in the field, the DBM population was maintained below ETL till the curd attained a respectable size. The 4th instar larvae as well as pupae were collected from different fields at the end of the 1st cropping season of the program and reared in the laboratory on natural diet. The 2nd instar larvae (0.56 ± 0.03 mg mean weight) of F1 generation have been subjected to susceptibility tests including bioefficacy and bioassay to various commonly used insecticides by the farmers at their recommended field concentrations.

The leaf residue bioassay (leaf-dip) method has been used for assaying *P. xylostella* susceptibility. Leaf discs of 8 cm dia. were cut and treated individually by dipping in desired concentrations of endosulfan, cypermethrin, cartap hydrochlorode quinalphos and pongamia soap solution for 5-7 sec. and dried under shade. These treated leaf discs were placed in petriplates after drying and a batch of 15 DBM larvae of above specific age were released. Each treatment was replicated thrice and the larvae were subjected to 2 hours starvation before being released on the treated leaf disc. The treated leaf discs were changed with fresh leaf discs next day and the exposed larvae were examined at 24 hours interval. The dead and moribund larvae were counted up to 120 hours of exposure. The per cent mortality observed was subjected to analysis to variance to find out the differential susceptibility of DBM to various test insecticides. The median lethal concentration (LC_{50}) and their corresponding 95% confidential limits of various insecticides were estimated through LDP analysis as described by Finney, 1974. The median lethal concentrations of commercial grade of insecticides used in the present study which killed 50 per cent of the test population of two day old second instar P. xylostella larvae were worked out. The LC_{50} values of endosulfan, cypermethrin, quinalphos and cartap hydrochloride were computed with mortality data obtained after 48 h whereas, LC_{50} values of NSKE and pongamia treatments were computed with 72 h mortality data. The changed susceptibility status of DBM test populations was also assayed by using the following formula:

$$\text{Susceptibility status} = \frac{\text{LC 95 of the treated chemicals}}{\text{Recommended field concentration of the chemical}}$$

RESULTS AND DISCUSSION The impact of insecticidal treatments showed significant variation in per cent mortality at different hours of treatment exposures.

After 24 hours of exposure a maximum of 34.31 mean per cent mortality was observed with cypermethrin 0.01 % followed by cartap hydrochloride 0.06 % (26.91), endosulfan 0.08 % (22.84), quinalphos 0.06 % (22.22), NSKE 5 % (12.98) and pongamia 4 % (15.20) treatments (Table 1). The mean per cent mortality in control treatment was observed to be zero and the per cent mortality in various insecticidal treatments was statistically significant when compare to the mortality in control.

Table 1. Bioefficacy of various insecticidal treatments including botanicals on two days old 2nd instar P. xylostella larvae

Treatments	Dose	Cumulative mean* per cent mortality after				
		24 h	48 h	72 h	96 h	120 h
Endosulfan	0.08%	22.84^{c}	80.74^{d}	84.99^{bc}	84.99^{c}	84.99^{c}
Cypermethrin	0.01%	34.31^{d}	75.38^{cd}	79.98^{b}	79.98^{b}	79.98^{b}
Quinalphos	0.06%	22.22^{c}	75.55^{cd}	86.00^{c}	86.00^{c}	86.00^{c}
Cartap hydrochloride	0.06%	26.91^{c}	72.00^{c}	80.12^{b}	85.05^{c}	85.05^{c}
NSKE	5.00%	12.98^{b}	38.12^{b}	82.22^{bc}	90.63^{d}	95.49^{d}
Pongamia	4.00%	15.20^{b}	37.22^{b}	92.59^{d}	99.09^{e}	99.09^{d}
Control	-	0.00^{a}	0.00^{a}	4.00^{a}	6.56^{a}	8.30^{a}
C.D. (0.05)	-	6.85	5.76	5.3	4.55	4.47
C.D. (0.01)	-	10.3	8.66	7.97	6.84	6.73

* Mean of three replications

The mean per cent mortality after 48 hours of exposure ranged from 37.22 to 80.74 per cent in all the insecticidal treatments having maximum mortality recorded with endosulfan. The result obtained after 48 hours of exposure with endosulfan, cypermethrin and quinalphos treatments were statistically at par but differ significantly with NSKE and pongamia treatments. Cypermethrin and quinalphos treatments recorded 75.38 and 75.55 mean per cent mortality of DBM larvae, respectively and were at par with mortality data recorded in cartap hydrochloride treatment (72.00 %). The mean per cent mortality in NSKE 5% treatment was 38.12 and with pongamia was as low as 37.22 per cent after 48 hours of treatment. No mortality of DBM larvae was observed in control treatment after 48 hours of treatments. However, after 72 hours of exposure the maximum mean per cent mortality of 92.59 per cent was recorded with pongamia 4% spray followed by 86.00 per cent with quinolphos, 84.99 per cent with endosulfan, 80.12 per cent with cartap hydrochloride, 82.22 per cent with NSKE 5% and 79.98 per cent with cypermethrin treatments. Thus, the mean per cent mortality recorded after 72 hours of exposure to pongamia 4% spray was significantly differed from the mortality of DBM larvae in other insecticidal treatments. Lowest mortality of 79.98 per cent was recorded with cypermethrin among insecticidal treatments and this value was significantly differed from the mean per cent mortality recorded with quinalphos treatment. A maximum of 4 per cent mortality was recorded in control treatment at this hour of exposure (Table 1).

The cumulative mean per cent mortality after 96 hours of exposure to various chemical treatments was observed to be 99.09, 90.63, 86.00, 85.05, 84.99 and 79.98 per cent with pongamia, NSKE, quinalphos, cartap hydrochloride, endosulfan and cypermethrin, respectively. Again, the mean per cent mortality being highest in pongamia and NSKE, differed significantly between each other and also differed with mean per cent mortality recorded with the four conventional insecticides. Even after 120 hours of exposure no change in per cent mortality with endosulfan, cypermethrin, quinalphos, cartap hydrochloride and pongamia treatments was observed but the cumulative mean per cent mortality with NSKE 5 % was observed to be as high as 95.49 differing significantly from the larval mortality recorded with other conventional insecticidal treatments (Table 1).

The susceptibility status of two days old 2nd instar larvae to all the test insecticides was low. The Maximum susceptibility of DBM larvae with a susceptibility status of 0.0139 was observed with pongamia 4 % followed by NSKE 5 % (0.0170), cartap hydrochloride (0.0533), cypermethrin (1.3800), endosulfan (1.4187) and quinalphos (2.3550) (Table 2). The relative susceptibility status of the DBM larvae to various insecticides revealed that the DBM larvae were 1.69 times more susceptibility to pongamia 4 % when compare to quinalphos. The NSKE 5 % treatment when compared to quinalphos treatment was found 1.38 times more effective. The endosulfan and cypermethrin treatments were 1.46 and 1.68 times more effective than quinalphos treatment to 2nd instar DBM larvae, respectively.

The impact of all four conventional test insecticides along with two botanicals on sensitivity of DBM larvae clearly showed that the initial mortality was more in conventional insecticidal treatments. But

Table 2. Susceptibility status of *P. xylostella* second instar larvae to selected insecticides

Insecticides	Heterogenety (x^2)	Regression equation	LD_{50}	Fiducial Limit		Susceptibility status
				Lower	Upper	
Endosulfan	1.68	Y= 4.941+4.263x - 7.055	0.046	0.042	0.05	1.4187
Cypermethrin	1.4	Y= 4.770+7.265x - 6.408	0.008	0.007	0.01	1.38
Quinalphos	1.87	Y= 4.858+2.822x –4.281	0.037	0.032	0.042	2.355
Cartap hydrochloride	2.03	Y= 0.700+1.85x – 4.199	0.028	0.019	0.037	0.0533
NSKE	3.38	Y= 4.897+4.534x –7.005	3.67	3.42	3.944	0.017
Pongamia	5.01	Y= 5.133+4.262x –5.934	2.28	2.089	2.5	0.0139

at later stage the mortality in two botanical insecticidal treatments clearly showed as much as 95.49 and 99.09 per cent, significantly well above the rest of the three insecticidal treatments. The maximum mortality in these conventional insecticidal treatments was only 86.00 per cent with quinalphos and as low as 79.89 per cent was observed with cypermethrin treatment. The variation in susceptibility to these insecticides could be due to frequent exposure of DBM to these chemicals in the field and the possible development of resistance and stability in the field populations. The present studies revealed that less than 15 to 20 per cent surviving individuals in the conventional insecticidal treatments could be a real threat in developing true resistant population. However, with the removal of selection pressure to insecticides in field, the prevailing resistance factor was brought down to many a fold in the season. Otherwise, our earlier studies clearly showed more than 40 fold resistance in DBM field population to many of the commonly used insecticides, including pyrethroids (Raju, 1996). The same scenario was also reported from other parts of the country where the insecticides are the main stay in combating DBM population in the field (Raghupathy, 1996; Kalra *et.al*., 1997; Gujar & Mohan, 2002) Therefore, induction of NSKE and pongamia extracts as field sprays in IPM schedules of DBM could be promisive in reducing the pest intensity and the ultimate damage to crucifer vegetables.

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First New World Report of Q Biotype of *Bemisia tabaci* (Gennadius) Reveals High Levels of Resistance to Insecticides.

Whiteflies possessing unusually high levels of resistance to a wide range of insecticides were discovered in 2004 while conducting annual resistance monitoring in Arizona cotton (Table 1). The multiply-resistant whiteflies were obtained from poinsettia plants purchased at a retail store in Tucson. They were subjected to biotype identification in three of our laboratories. PAGE electrophoresis of non-specific esterases (Byrne *et al*. 2000), and sequencing of the mitochondrial cytochrome oxidase I gene (780 bp) (Brown 2001) confirmed the first detection of the Q biotype of *Bemisia tabaci* in the New World.

The Q strain, named Poinsettia'04, was resistant to two insect growth regulators, pyriproxyfen and buprofezin. These selective insecticides have provided the foundation for a resistance management

program that has been highly successful in Arizona cotton for ten years (Dennehy *et al*. 1996, Ellsworth and Martinez-Carrillo 2001). Poinsettia'04 was virtually unaffected by pyriproxyfen in egg bioassays (Figure 1) and was strikingly reduced in susceptibility to buprofezin in nymphal bioassays (Figure 2). The Poinsetttia'04 strain also possessed unusually low susceptibility to the neonicotinoids, acetamiprid, imidacloprid, and thiamethoxam, and to mixtures of fenpropathrin and acephate, (Dennehy *et al*., 2005).

Table 1. Locations from which whiteflies were collected in 2004 and brought to the EARML facilities in Tucson for testing. All 26 collections from cotton and melons were the B biotype of *Bemisia tabaci* . Only one of six collections from ornamentals was the Q biotype. Similar biotype evaluation of statewide samples collected in 2001 and 2003 yielded only the B biotype.

Location	Biotype	Host	Collection Date
1. Casa Blanca, AZ	B	cotton	9/10/2004
2. Casa Grande, AZ	B	cotton	9/20/2004
3. Coolidge, AZ	B	cotton	9/10/2004
4. Cotton Center, AZ	B	cotton	10/4/2004
5. Holtville, CA	B	cotton	8/1/2004
6. Laveen, AZ	B	cotton	10/18/2004
7. Marana, AZ	B	cotton	10/19/2004
8. Maricopa Agric. Center, AZ	B	cotton	8/18/2004
9. Paloma, AZ	B	cotton	10/22/2004
10. Parker Valley, AZ	B	cotton	9/26/2004
11. Picacho, AZ	B	cotton	9/20/2004
12. Queen Creek, AZ	B	cotton	8/30/2004
13. Stanfield, AZ	B	cotton	10/4/2004
14. Yuma, AZ	B	cotton	8/3/2004
15. Yuma Agric. Center, AZ	B	cotton	8/3/2004
16. Avondale, AZ	B	melons	6/22/2004
17. Citrus Park, AZ	B	melons	6/14/2004
18. Coolidge, AZ	B	melons	7/21/2004
19. Harquahala Valley, AZ #1	B	melons	6/14/2004
20. Harquahala Valley, AZ #2	B	melons	9/24/2004
21. Marana Agric. Center, AZ	B	melons	8/24/2004
22. Palo Verde Valley, CA	B	melons	6/22/2004
23. Somerton, AZ	B	melons	6/3/2004
24. Stanfield, AZ	B	melons	6/12/2004
25. Wellton, AZ	B	melons	6/3/2004
26. Yuma Agric. Center, AZ	B	melons	6/3/2004
27. Maricopa Agric. Center, AZ	B	lantana	6/2/2004
28. Phoenix, AZ, Wholesale GH	B	ruellia	2/10/2004
29. Tucson, AZ, Retail GH #1	B	hibiscus	7/6/2004
30. Tucson, AZ, Retail GH #2	B	lantana	4/12/2004
31. Tucson, AZ, Retail GH #3	--Q--	poinsettia	12/10/2004
32. Tucson, AZ, Retail GH #4	B	poinsettia	12/10/2004

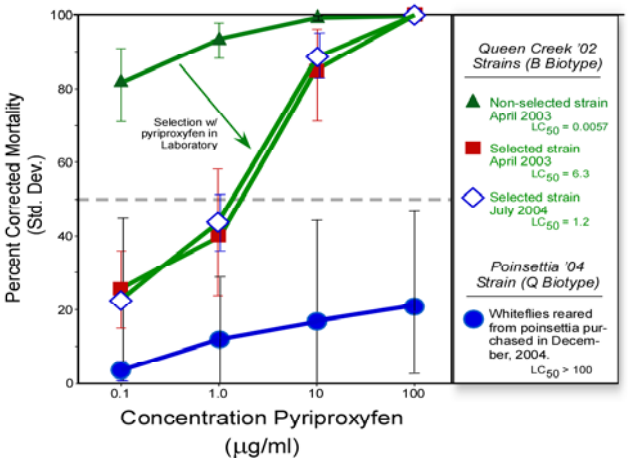

Figure 1. The Poinsettia'04 strain of *Bemisia tabaci* was essentially unaffected in 5-day leaf-dip bioassays of eggs. It possessed strikingly higher resistance to pyriproxyfen than any field or laboratory-selected strains evaluated from Arizona. The Queen Creek'02-R strain was the B-biotype of *B. tabaci.* This strain was intensively selected with pyriproxyfen for over two years in the laboratory.

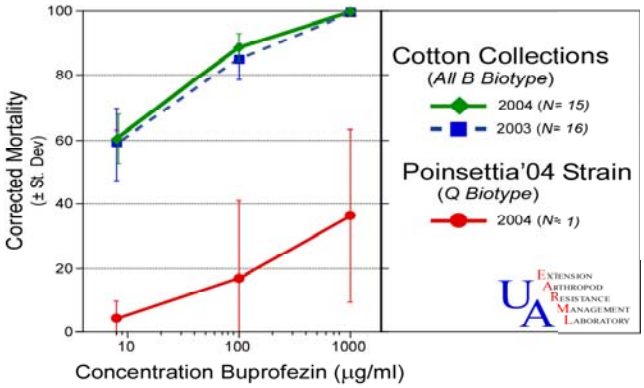

Figure 2. The Poinsettia'04, Q biotype, strain of *Bemisia tabaci* was strikingly less susceptible to buprofezin than were any whiteflies collected from Arizona. Shown are grand mean corrected mortality (± standard deviation) values of whiteflies collected from throughout Arizona cotton in 2004 (15 locations sampled) and 2003 (16 locations sampled). First instar nymphs on seedlings were dipped in buprofezin solutions and evaluated 9 days later for mortality.

In the Mediterranean Region, the Q biotype has been associated with severe resistance to neonicotinoid and insect growth regulating insecticides (e.g., Horowitz *et al.* 2005, Nauen and Denholm 2005). Comparative analysis with reference sequences available in the Brown laboratory indicated that CO1 sequences from Poinsettia'04 were most closely related to haplotypes from southern Spain greenhouses (1992-2003), sharing 98.0-99.7% nucleotide identity. The next closest relatives for which sequences were available were populations from Morocco (collected 1999-2000), and Israel (collected 2001-04), with which they shared 97.0-99.0% and ~94.0% nucleotide identity, respectively.

It appears that the Q biotype has been detected at a relatively early stage in its spread in the US. No Q biotypes have been detected in field or row crops in Arizona. However, track-back inspections by regulatory agencies yielded Q biotypes at the wholesale nursery that produced the plants on which Q biotypes were found in Tucson, as well as at the location from which the wholesaler obtained poinsettia propagation material. These findings indicated the likelihood that Q biotypes were disseminated widely throughout the US on propagation material in 2004. This deduction has been supported by surveys conducted by cooperating states. At the end of 2005, the Q biotype had been detected on ornamental or nursery plants in 18 U.S. states, and in Guatemala (Osborne 2005).

Severe economic losses to agriculture, resulting from introduction of the B biotype of *B. tabaci*, have been chronicled by pest managers in many areas of the US. With this hindsight, the potential threat posed by the Q biotype is indisputable. Although, it is not possible to predict with accuracy the future spread of the Q biotype in the US, or the severity of associated control and virus problems, there is a critical need to formulate contingency plans for its management. Information regarding the geographical distribution, insecticide resistance, pest status, and virus/vector relationships of this invasive biotype will be essential for formulating such plans. Additionally, regulatory efforts to limit the further spread of the Q biotype within the US and to thwart further importation on plant materials produced offshore will be critical for management of this new problem.

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Control of Melanagromyzae sojae Zehnt. and YMV Disease Transmitted by *Bemisia tabaci* (Genn.) of Soybean by Seed Treatment with Systemic Insecticides

ABSTRACT Efficacy of thiamethoxam at different doses were evaluated against stemfly and YMV transmitted by whitefly during cropping seasons of 2003 and 2004 on soybean as seed treatments. In 2003 all the treatments were found effective in reducing the infestation by both the pests and increasing the seed yield. The tunneling varied between 24.18 and 39.3 per cent during 2003 and 1.28 to 29.68 per cent during 2004. The seed yield in treated plots varied from 15.92 Q/ha to 22.58 Q/ha against 13.87 Q/ha in control plot in 2003 while it was 17.55 to 24.44 Q/ha against 16.78 Q/ha in the control plot in 2004. The B : C ratio in 2003 due to different treatments was low (between 0.74 and 4.4) while it was high (9.57 to 61.67) during 2004.

KEY WORDS Soybean, stem fly, YMV, systemic insecticides, seed treatment

INTRODUCTION Amongst the insect pest complex of soybean, stem fly, *Melanagromyzae sojae* Zehnt. And white fly, *Bemisia tabaci* Genn. pose serious problems in achieving higher productivity of soybean. Seed coating with systemic insecticides holds promise, as they not only protect the developing seedlings but also later stages of the crop against various insect pests. These treatments are not only economical but also fit better into the usual farming practices with an added advantage of the least disruptive effect on the environment and to the useful fauna. Siddiqui and Trimohan (2000) reported seed treatment with Thiamethoxam 70 WS @ 3 g /kg seed very effective in controlling stem fly infestation and yellow mosaic incidence transmitted by white fly. The present experiments were conducted to find out the efficacy of Thiamethoxam at lower doses to reduce the cost of control. Another systemic insecticide, Oxy-demeton methyl was also tested at different doses.

MATERIALS AND METHODS The trials were conducted at the farm of Indian Agricultural Research Institute, New Delhi during kharif season of 2003 and 2004. Soybean variety Pusa 16 was sown in July end in plots of 2.25 x 3 m size. The trials were laid in a Randomized block design and replicated thrice. Row length was 3 m while spacing between the rows and between the plants were 37.5cm and 10 cm respectively. All the recommended agronomic practices were followed to raise the crop. In the first year i.e. kharif of 2003 two different formulations of Thiamethoxam , *viz.*, 25 Wg and 70 WS were evaluated in the field. From the data of 2003 the best treatment was selected and an experiment was laid in 2004 to find out the efficacy of Thiomethoxam at lower doses. The details of all the treatments in both the years have been listed in the Table 1and 2. The insecticides were applied as seed treatment.

The efficacy of the treatments were evaluated on the basis of tunneling per cent in case of stem fly at flowering and at harvest and YMV disease incidence at flowering in all the rows of all the treatments separately on a rating scale of 1-9, where 1 was given to healthy plants and 9 to those with maximum disease incidence (Siddiqui et al., 1996; Siddiqui and Trimohan, 1999). Grain yield was also recorded. For recording the stem fly infestation, ten plants per replication from each treatment were uprooted at random at flowering stage, the stems were split open and the length of stem and that of tunnel due to stem fly damage were measured. The data on the incidence of the aforesaid pests were subjected to statistical analysis (analysis of variance). The tunneling percentages were transformed to angular values before subjecting the data for analysis (LeClerg, Leonard and Clark, 1962). The seed yields were also recorded after harvest from each plot. Thereafter, the yield was worked out on per hectare basis. The benefit : cost (B:C) ratio was also worked out.

RESULTS AND DISCUSSION Results showed that seed treatment with Thiamethoxam at all the doses neither produced adverse effect on the germination of soybean nor phytotoxicity to the emerging seedlings. YMV disease incidence was significantly less in Thiamethoxam 70 WS @ 3g /kg seed treatment (rating 2.0) and Thiamethoxam 25 W G @ 6g /kg seed treatment (rating 2.5) (Table 1). In other treatments including untreated control ratings ranged from 3.0 - 7.50 on a scale of 1 - 9. Stemfly infestation, both at flowering and at harvesting in treatments with Thiamethoxam 25 W G @ 5g /kg seed, was significantly less than the remaining treatments. Significantly higher grain yields were recorded in T-5 (Thiamethoxam 25 W G @ 6g /kg seed treatment), T-3 (Thiamethoxam 25 W G @ 4g /kg seed) and T-4 (Thiamethoxam 25 W G @ 5g /kg seed)

During 2004, results obtained on the efficacy of lower doses of Thiamethoxam 70 WS revealed that all the treatments were significantly better than control (Table 2). However only Thiamethoxam 70 WS @ 2g/kg seed could effectively control both stem fly infestation and yellow mosaic incidence transmitted by white fly.

Table 1 . Efficacy of different Thiamethoxam formulations against major insect pests of soybean

S.No.	Treatment	Dose	Mean per cent tunnelling due to stem fly At flowering	At harvest	Mean YMV disease incidence due to white fly (rating scale 1-9)	Seed Yield Q./ha. Plot	Benefit : Cost Ratio
T1	Thiamethoxam 25 WG	2g/kg seed	27.49(21.45)	33.00(29.78)	3.25	16.29	0.74
T2	Thiamethoxam 25 WG	3g/kg seed	28.27(22.55)	30.93(26.48)	3	16.66	0.99
T3	Thiamethoxam 25 WG	4g/kg seed	26.71(21.30)	31.19(25.72)	3.25	20.17	4.25
T4	Thiamethoxam 25 WG	5g/kg seed	24.18(16.88)	26.30(19.76)	2.75	19.99	2.62
T5	Thiamethoxam 70 WS	6g/kg seed	25.61(19.25)	27.21(21.68)	2.25	22.58	4.44
T6	Thiamethoxam 70 WS	3g/kg seed	25.03(18.31)	29.72(25.19)	2	18.8	2.4
T7	Thiamethoxam 25 WG Spray	0.2g/l 2 DAG	34.35(31.85)	34.97(32.89)	5.75	15.92	4.1
T8	Control	-----	39.32(39.67)	38.84(39.40)	7.5	13.87	
	S Em +		1.54	2.21	0.24	0.11	
	C D at 5%		4.52	6.49	0.7	0.32	

Figures in parenthesis are original values whereas, outside are transformed

Table 2. Efficacy of different insecticides against major pests of soybean

Treatment	Dose	Mean per cent tunnelling due to stem fly At flowering At harvest	Mean YMV disease incidence due to white fly (rating scale 1-9)	Seed Yield (Q./ha.)	Benefit: Cost Ratio
Thiamethoxam 70 WS	1g/ kg seed	7.14 (15.40) 11.23(19.41)	2	22.22	13.6
Thiamethoxam 70 WS	1.5g/ kg seed	4.38(11.7 9) 9.96(18.19)	2	24.44	12.77
Thiamethoxam 70 WS	2g/ kg seed	1.28(6.29) 5.98(13.84)	2	24.44	9.57
Control	-----	29.30(32.73) 31.55(33.97)	4.66	16.78	
S Em +		2.57 2.3	0.163	14.1	
C D at 5%		7.92 6.91	0.56	N.S.	
C D at 1%			0.85		

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Cellular Immune Reactions Participating in Resistance Formation of Colorado Beetle (*Leptinotarsa decemlineata* Say) Larvae and Imago to a Biopreparation for Potato

INTRODUCTION Fast development of Colorado beetle resistance to chemical and biological insecticides stipulates complication and actuality of the problem of this dangerous potato pest quantity regulation. Study of *L. decemlineata* defensive reaction mechanisms will allow to more purposefully approach a problem of its quantity control. Recently we have reported about immunization of *L. decemlineata* larvae and adults by unlethal bitoxybacillin (BTB) concentration increasing insect's survival and activity of the humoral defensive reactions during repeated infection (Gayfullina *et al.*, 2005). The aim of this work was a study of the cellular immune responses during initial stage of infectious process in *L. decemlineata* larvae and imago infected per os with BTB consistently increasing concentrations. This investigation was made through determination of ratio of the different hemocyte types which takes an active part in defense reactions such as phagocytosis and encapsulation of foreign objects as well as synthesis and secretion of the humoral immunity factors. Primary treatment of *L. decemlineata* was accomplished with most unletal BTB concentration which didn't cause mortality significantly distinguished from control variant, and secondary treatment - with BTB LC_{50} preliminary determined for larvae and imago.

METHODS

Insects: L. decemlineata larvae and imago were collected from potato field and reared on potato foliage in glasses with volume 0,5 dm3. Artificial infection of insects was conducted through feeding of potato foliage moistened with BTB solution (*Bacillus thuringiensis var. thuringiensis* product). Larvae was primary treated at third instar with 0.01% BTB solution and secondary - at fourth instar with 0.1% BTB. Imago was primary treated with 0.1% BTB and repeatedly - a ten days later with 0.5% BTB. Change dynamics of the enumerated indices was determined during first twenty-four hours after single and two-fold treatment of *L. decemlineata.* Insect mortality was valued by quantity calculation of died larvae and imago seven days later treatment with BTB LC_{50}.

Haematological analysis: Haemolymph was collected by pricking of a dorsal vascular with a pin and sucking the emerging haemolymph into a capillary. Haemolymph smears were fixed with ethanol and stained with azure-eosin. Haematological analysis was carried with microscope Carl Zeiss Jena at ´800.

Statistical analysis: Received data was carried out with using arithmetical mean, arithmetical mean error and Student t-criterion.

RESULTS AND DISCUSSION Fourth instar larvae preliminary treated in third instar with 0.01% BTB differed from control individuals by almost two-fold increase of the spherulocytes and oenocytoids portion (Fig. 1). Preliminary treatment of the larvae with BTB unletal concentration also changed the different haemocyte ratio during repeated infection in comparison with single infected individuals namely provoked increase of the spherulocytes and oenocytoids portion during the first hours after treatment with BTB LC_{50}. In *L. decemlineata* imago preliminary treatment with unletal BTB concentration provoked increase of the portion of spindle-formed

plasmatocytes forming from rounded plasmatocytes and directly from prohaemocytes and decrease of spherulocytes and oenocytoids portion at the repeated infection moment (Fig. 2). After repeated infection significant increase of the spindle-formed plasmatocytes, spherulocytes and oenocytoids portion was registered in imago haemolymph.

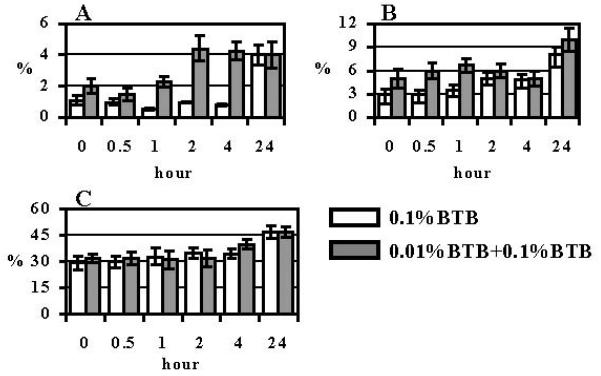

Fig. 1. Portion of *L. decemlineata* larvae haemolymph defensive cells under the single and two-fold treatment with BTB (P<0.05).
A - spherulocytes, B - oenocytoids, C - spindle-formed plasmatocytes.

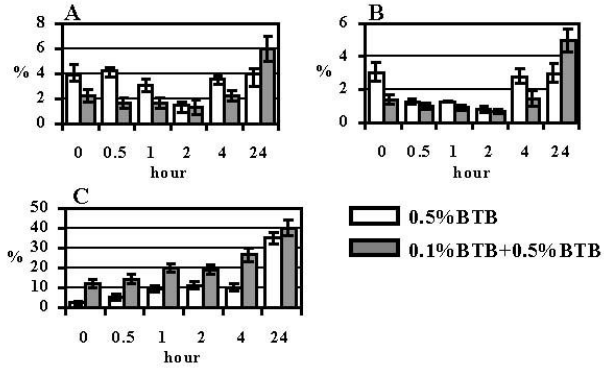

Fig. 2. Portion of *L. decemlineata* imago haemolymph defensive cells under the single and two-fold treatment with BTB (P<0.05).
A - spherulocytes, B - oenocytoids, C- spindle-formed plasmatocytes.

Oenocytoids and spherulocytes are determined as "metabolic" haemocytes synthesizing and secreting in plasma some proteins and haemolymph adipose matter and taking part in detoxication processes (Landim, 1985; Glupov, Bachvalov, 1998). In addition these cells reveal in the cytoplasm and inclusions phenoloxidase activity that indicates on their role in the processes of melanin formation during parasite encapsulation and melanization under metamorphosis and healing injury (Ribeiro, 1995). Spindle-formed plasmatocytes are defined as " immunocompetenciv" haemolymph cells realizing phagocytosis and encapsulation of the pathogens (Glupov, Bachvalov, 1998; Ling *et al.*, 2005). Infection development in different species insects is accompanied by quantity increase of the spindle-formed plasmatocytes which are generated from rounded plasmatocytes and directly from prohemocytes (Bathurin, Bathurina, 1984; Ling *et al.*, 2005; Saltykova *et al.*, 2005). So present data demonstrates that the consequence of preliminary action of BTB unletal concentration on *L. decemlineata* larvae is increase of the "metabolic" haemocytes portion raising the larvae humoral defense degree during first twenty four hours after treatment BTB LC_{50}. In imago action of BTB unletal concentration stimulates done early augmentation of the spindle-form plasmatocytes providing insects with raise level of the "immunocompetenciv" haemocytes during infection initial stage and preparing the haemolymph cellular system to more effective pathogen isolation and elimination. It is necessary to note that preliminary treatment of *L. decemlineata* imago with BTB unletal concentration causes also increase of the "metabolic" haemocytes portion under repeated infection indicating the humoral factors participation in imago defensive reaction. Early it had been shown that the result of immunization of *A. mellifera* worker bees is more rapid increase of active phagocytes and spherulocytes portion after repeated infection in comparison with nonimmunized individuals (Saltykova *et al.*, 2005). It is suggests that increase of the portion of active phagocytes and haemocytes taking part in the humoral defensive processes may be common reaction on the repeated infection development for imago stage of Insect different orders.

Research attention to insects ability for increase of resistance to infection through the immunization - immunity acquisition - had been began to spare as long ago as first decades of 20-th century. Possibility of immunity acquisition through active and passive immunization with use of different virulence pathogens had been shown in different species insects by V. Nedrigaylov, S. Metalnikov, A. Hollande, A. Paillot and D. Tateiwa in the 1908-1930-ies (Kusnecov, 1948). The main cause of the immunization had been notice to be increase of cell sensibility, phagocytosis activation, acceleration of all cellular reactions which stay "more energetic and sensible" to the microbe used for immunization. Suppositions and conclusions of these works had been corroborated by later studies. So, defensive strengths induction in immunized insects displaying in increase of the phagocytic activity of haemolymph cells had been shown (Bathurin, Bathurina, 1984), ability of insect's haemocytes formed capsules around foreign objects to form the "memory relative to encapsulation reaction" had been described (Matz, 1987), specific immune memory had been discovered in cockroaches (Dunn, 1990). At last recently stimulate action of unletal BTB concentration on development of *L. decemlineata* humoral defensive reactions displaying in larvae and imago in stabilization of the antioxidant enzymes activity and increase of the specifical hemagglutinins titer as so as in increase of the phenoloxidase activity

in imago had been shown by us (Gayfullina *et al*., 2005). However, in spite of these facts, immunity acquisition by insects have been called in question and actively disputed up to now.

Present work results demonstrate the stimulate action of unletal BTB concentration on *L. decemlineata* cellular immune system displaying in increase of the defensive haemolymph cells ratio during first hours of repeat infection development. Observed changes of the cellular immunity components at the physical isolation of gut pathogen during first hours after the beetles treatment with BTB suggest the existence of mediators signalizing about the penetration of infectious agent into the gut and in short time activating defensive mechanisms. Biogenous amines seem to be most likely candidates on the role of such mediators. Mammals biogenous amines representatives - adrenergic compounds - are known to play significant role in the phagocytic activity regulation through the influence of cAMP and phosphatidylinositiol signal pathway metabolites on the processes controlling phagocyte cytoskeleton reorganization (Orlova, Shirinov, 2004). Some of biogenous amines are reported to regulate phagocytes functional activity in insects too. So octopamine modulates plasmatocytes locomotory activity through the actin-based cytoskeleton namely stimulates increases in intracellular Ca^{2+} in haemocytes, elevates inositoltriphosphate levels and causes formation of long filopodia with F-actin core (Diehl-Jones *et al.,* 1996). In addition in insects of some species majority of haemocytes have been detected to not be in plasma, but borders to tissues and dorsal vascular presenting some analogue of vertebrate reticuloendothelial apparatus and in case of need passes to free-circulating state (Kusnecov, 1948). Perhaps during first hours of infection development some haemocytes pass from settled state to circulation causing sharp changes of haemolymph cells ratio.

CONCLUSIONS Thus stimulation of *L. decemlineata* initial cellular immune reaction in response to repeated infection have been shown under the action of bacterial preparation unletal concentration don't causing serious pathological changes in insect organism and mortality distinguished from control. This stimulation should be noted to remains during change of larvae ages. In common with early published data indicative of increase of the humoral defense systems activity and insects survival under repeated treatment with BTB (Gayfullina, 2005) present results are evidence of *L. decemlineata* ability for increase of resistance to infection through the immunization. As a whole stimulation of the cellular and humoral immune systems of *L. decemlineata* and other species insects (Kusnecov, 1948; Bathurin, Bathurina, 1984; Matz, 1987; Dunn, 1990; Saltykova *et al.,* 2005) by immunized doses of pathogen can be one of the mechanisms of insect-pests resistance development to biopreparations.

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Insecticide resistance level in *Leptinotarsa decemlineata* Say population in the South Ural

INTRODUCTION The Colorado potato beetles (CPB) *Leptinotarsa decemlineata* Say (Coleoptera, Chrysomelidae) is a serious pest (fig.1, A) of potatoes around the world [1] including European part of ex-USSR [2]. This beetle was introduced to Bashkortostan (South Ural, Russia) since 1976 year in two regions - Kumertausky and Arkhangelsky (see fig.1, B) and since 1977 - in Alsheevsky region [3]. These hotbeds were reported to be liquidated, but since 1979 year CPB was settled down firmly in all Bashkortostan territory. The main means of the CPB control on initial stage was an applying of organophosphorus insecticides, but since 80-years a different pyrethroids replaced them. For 20 years in laboratory of biochemistry of insect adaptability of Institute of Biochemistry and Genetics of Ufa scientific center, CPB insecticide resistance monitoring was conducted.

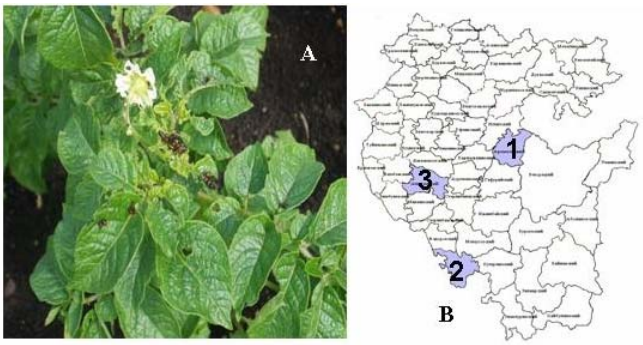

Fig.1. A – The CPB larvae on potato. B – appearance of CPB in Baskortostan: 1 - Arkhangelsky, 2 - Kumertausky, 3 - Alsheevsky regions.

MATERIALS AND METHODS The samples of the CPB (adult males and females of overwintered and second generations) were collected in 1989-1990 and 2000-2005 years in Bashkortostan (table 1). In toxicological experiments defined the susceptibility of CPB to several insecticides: carbophos,. 1.0%; decis, 0.01%; aktara and mospilan, 0.001%, using a topical treatments. 10 individuals were treated and transferred into Petri dishes. Insects in control groups were treated with ethanole. Experiments were conducted in 2 replications.

Table 1. List of collected CPB local populations in Bashkortostan (2004 – 2005).

No in map	Populations name	No in map	Populations name
1	Abzelilovskaya	12	Karmaskalinskaya
2	Arkhangelskaya	13	Meleuzovskaya
3	Askinskaya	14	Mechetlinskaya
4	Aurgazinskaya	15	Miyakinskaya
5	Blagovarskaya	16	Salavatskaya
6	Buzdyakskaya	17	Sterlitamakskaya
7	Davlekanovskaya	18	Tuimazinskaya
8	Duvanskaya	19	Ufimskaya
9	Dyurtyulinskaya	20	Khaibulinskaya
10	Iglinskaya	21	Scharanskaya
11	Ilischevskaya	22	Burzyanskaya

After account of mortality (on 3rd day) with Abbot's formula [4] was calculated the susceptible\resistance ratio or S\R individuals, accordingly.

RESULTS AND DISCUSSION Data of 1984-85 years [5] allowed to evaluate CPB populations at that period as susceptible ones. So, applying of 0.0092% decametrin (decis) led to 100 per cent mortality (see fig. 2A). However, in 1996 year the resistant individuals were observed (fig.2B), even after applying of preparation in 0.075% concentration [6].

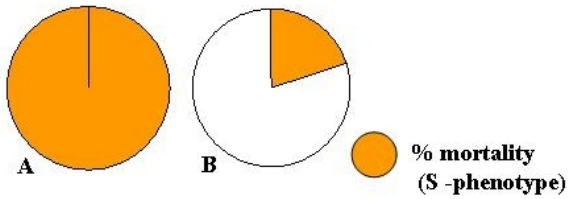

Fig 2. Increasing the pyrethroid resistance in CPB. A – 1985, B-1996.

S\R ratio of CPB adults from different populations after topical application of decis or carbophos in 2005-2006 years shown in fig.3. The resistance to decis is evidently wide-spread, but some local populations contain more than 90% R-phenotype, whereas the others contain less than 10% (e.g., Burzyanskaya population). On the average, in all Bashkortostan territory nearly 62% of adults staying still susceptible to decis. Resistance to organophosphorus insecticides spread much widely - number of susceptible individuals is less than 25%. It is compelling attention the mosaic spreading of resistant populations in the territory of Bashkortostan. Similar local appearance of resistant CPB micropopulations was noted also in Moscow and Nizhegorodsk regions in Russia [7, 8]. Such a phenomenon can be explained by wide spreading of potato monoculture, reducing the migration activity of CPB.

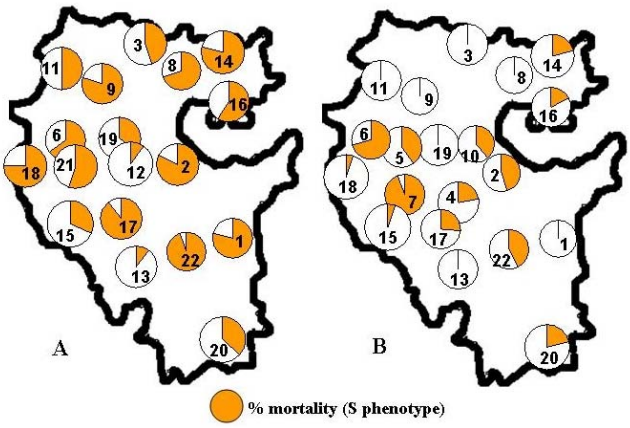

Fig. 3. S\R phenotype ratio (2004-2005). A-decis, B-carbophos. See table 1 for population names.

The special anxiety causes the discovering of CPB populations, containing individuals resistant to the new insecticide mospilan, which is in use only since 2003 year. An average mortality caused by diagnostic dose of mospilan composed still 72.6%. Resistance to actara, which is applying since the same time, is developing more slowly: the diagnostic dose of actara caused 92.8% mortality, but in 205 year in Ufimskaya population was registered mortality 20% from actara.

To provide the potato protection in such a situation, producers must to 0 elucidate, what an insecticides are effective still, and have to rotate preparations regularly trying to retard the resistance forming to each insecticide. If the pyrethroids and organophosphorus compounds are still middle-effective in the region , they can be used rarely. Such a tactics in Stavropol region of Russia led to some reversion the pyrethroid resistance, that is a return of susceptibility in CPB [9].

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Geographical Variation in the Susceptibility of Diamondback Moth, *Plutella xylostella* L. (Lepidoptera: Yponomeutidae) to *Bacillus thuingiensis* Products and Acylurea Compounds.

INTRODUCTION The diamondback moth (DBM), Plutella xylostella (Linnaeus) is one of the most destructive insect pests of cruciferous vegetables in the world. This insect attacks cruciferous vegetables, particularly cabbage, broccoli and cauliflower. The world production of these crops is over 42.2 million tons and the global estimate of the cost of its control is about one billion US $ per annum (Talekar and Shelton, 1993). Indiscriminate use of insecticides and the year-round availability of host crops have contributed to the development of resistance in this pest to almost all kinds of insecticides including *Bacillus thuringiensis* based products and acylurea compounds (Sivapragasam *et al*., 1996, Ferr'e and Van Rie, 2002).

Resistance in *P. xylostella* to different insecticides has been reported from several states like Punjab, Haryana, Uttar Pradesh, Karnataka, Tamil Naidu, and Andhra Pradesh (Sannaveerappanavar, 1995, Mehrotra and Phokela 2000, Mohan and Gujar, 2000). Since early 1990's, *B.t.* products have been extensively used against DBM in vegetable growing areas around Bangalore (Malur), but their use has been very limited in other areas of Karnataka state. Similarly, the acylurea compounds are presently being used by farmers only in the areas around Bangalore. Here we report the susceptibility of DBM populations to three *B.t. kurstaki* products in these major cabbage growing areas of Karnataka state, India.

MATERIAL AND METHODS Field populations of *P. xylostella* were collected from three major vegetable growing areas *viz*., Malur, Hassan, and Belgaum to study the variation in their susceptibility to *B. thuingiensis* products and acylurea compounds. The insects were reared to F_1 generation in the laboratory on mustard seedlings as described by Liu and Sun (1984) with suitable modifications. A susceptible strain of DBM which had been maintained in the laboratory since 1992 (144 generations) without exposure to any insecticides/xenobiotics was used for comparison..

The susceptibility of the field populations to three *B.t. kurstaki* based products *viz.*, Biobit, Delfin and Dipel, and two acylurea compounds viz., flufenoxuron (Cascade 10DC) and lufenuron (Match5EC) was determined by employing "leaf dip" method. At least five concentrations of each insecticide were used in each bioassay. For every dose, three batches of fifteen larvae each were maintained. Uniform sized fresh mustard leaves were dipped in aqueous insecticide fluids containing 0.1% soap for ten seconds and then dried under shade for one hour. The treated leaves were placed in petridishes (10x1.5 cm) and fifteen third instar larvae were released on treated leaf in each petridish. The treated larvae were maintained at 25±1º C and the mortality was recorded at 72 hours after the treatment. The data was subjected to probit analysis (Finney, 1971) to determine the LC_{50} values. In all the bioassys F_1 progeny were utilized. The resistance level was computed by comparing with LC_{50} values of susceptible strain and field populations.

RESULTS AND DISCUSSION The variation in susceptibility of three field populations of DBM to *B.t.* products and acylurea compounds is presented in Table 1 and 2, respectively.

Table-1: Resistance to *B.t.* products in the field strains of *Plutella xylostella* L. collected from major cabbage growing areas of Karnataka (India)

Population and Insecticide	Susceptible strain LC_{50} (µg a.i.ml^{-1})	Field strain LC_{50} (µg a.i.ml^{-1})	Resistance ratio (RR)*
MALUR			
Biobit	2.07	90.71	43.95
Dipel 8L	0.21	9.88	47.03
Delfin	0.14	3.54	24.91
HASSAN			
Biobit	2.07	10.29	4.97
Dipel 8L	0.21	1.01	4.79
Delfin	0.14	0.13	0.93
BELGAUM			
Biobit	2.07	16.22	7.84
Dipel 8L	0.21	1.51	7.19
Delfin	0.14	1.33	9.5

* Resistance ratio (RR) = Ratio of LC_{50} for field strain to that of susceptible strain.

Table-2: Resistance to acylurea compounds in the field strains of *Plutella xylostella* L. collected from major cabbage growing areas of Karnataka (India)

Population and Insecticide	Susceptible strain LC_{50} (µg a.i.ml^{-1})	Field strain LC_{50} (µg a.i.ml^{-1})	Resistance ratio (RR)*
MALUR			
Flufenoxuron	0.27	3.5	12.96
Lufenuron	0.03	0.19	6.63
HASSAN			
Flufenoxuron	0.27	0.18	0.66
Lufenuron	0.03	0.002	0.06
BELGAUM			
Flufenoxuron	0.27	0.19	0.7
Lufenuron	0.03	0.01	0.33

* Resistance ratio (RR) = Ratio of LC_{50} for field strain to that of susceptible strain.

Resistance to B.t. products: Among the three field populations studied, the Malur population showed the highest level of resistance (16.8 - to 47.03-fold) to the three *B.t*. products followed by Belgaum (7.19 - 9.5-fold) and Hassan populations (0.93- to 4.97- fold). No resistance was observed against Delfin in the Hassan population, the RR being less than one. This shows the geographical differences in susceptibility of DBM populations to B. thuringiensis kurstaki endotoxins. The variations in the resistance ratios for the three field populations could be attributed to insecticide usage pattern in those locations. In Malur area, crucifers are cultivated all-round the year and the farmers have used *B.t.* products extensively since early 1990's which has resulted in higher levels of resistance to these products. In Belgaum area, farmers still use the conventional insecticides to manage the pest and the B.t. products have been introduced in this area only recently. In Hassan area, these products have not been used extensively and most of the farmers not aware of the products. The geographical differences in susceptibility of P. xylostella to B.t. kurstaki products in India has also been reported by Chandrasekaran and Reghupathy, 1996; Perez and Shelton, 1997; Mohan and Gujar, 2000; Singh, 2002.

Resistance to acylurea compounds: As with *B.t.* products, the highest level of resistance to flufenoxuron and lufenuron was observed in Malur area. The DBM populations of Belgaum and Hassan did not show resistance to these compounds. Acylurea compounds have been used by the farmers in Malur area for over two years. But, in Hassan and Belgaum their use in the management of DBM just started. In India, studies by Mohan and Gujar (2003) indicated that five field populations of DBM were more tolerant to flufenxuron compared with the IARI-17-65 strain.

The present study has clearly shown the geographical differences in susceptibility of *P. xylostella* to *B.t.* products and acylurea compounds. It is hoped that the findings of this investigation would help in formulating appropriate insecticide resistance management (IRM) strategies for effective management of DBM in different locations.

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Laboratory Selection of Diamondback Moth, *Plutella xylostella* L. (Lepidoptera: Yponomeutidae) for Resistance to a *Bacillus thuingiensis* Product and an Acylurea Compound.

INTRODUCTION Diamondback moth (DBM), *Plutella xylostella* (Linn.) is a key pest of crucifers, particularly on cabbage and cauliflower in many parts of the world. The pest is known for its propensity towards quick development of resistance (Georghiou, 1990) and has developed resistance to almost all the recommended insecticides belonging to major chemical groups (Talekar and Shelton, 1993). In India, resistance in *P. xylostella* to different insecticides has been reported by several researchers (Sannaveerappanavar, 1995, Chandrasekaran and Regupathy, 1996, Raju, 1996 and Mohan and Gujar, 2000). In view of the ineffectiveness of conventional insecticides, farmers are currently using *Bacillus thuringiensis* based products and acylurea compounds in the management of DBM in areas around Bangalore, Karnataka (India). A study was taken up to investigate the rate of development of resistance to these compounds through laboratory selection, which would help in optimizing their utilization in the management of this pest.

MATERIAL AND METHODS To determine the response of DBM to selection with a B.t. *kurstaki* based product, (Biobit R) and an acylurea compound, flufenoxuron (Cascade 10DC) in the laboratory, a field population of DBM showing 3.01-fold resistance to Biobit was used. The insect culture was maintained on mustard seedlings by employing the mass-rearing technique of Liu and Sun (1984) with suitable modifications. Before the selection, a preliminary bioassay was carried out to determine the LC_{50} of two-insecticides. Concentrations giving approximately 90 per cent larval mortality were used for selection. Selection was achieved by exposing third instar larvae to mustard leaves, which were dipped in the aqueous suspensions of insecticides. Larvae were allowed to feed on treated leaf discs for 48 hrs. In each selection, more than 1000 larvae were

maintained. The surviving larvae were reared to the adult stage for further selection. The selection was continued for 9 generations. Bioassays were carried out after every 3 generations of selection. The doses of the insecticides were increased in each selection, depending upon the survival rate of larvae in the previous selection.

Table-1: Development of resistance to a *B.t.* product, Biobit in the field strain of *Plutella xylostella* L. after laboratory selection.

Generation Number	LC_{50} (µg a.i.ml^{-1})	Resistance ratio (RR)*	Increase in resistance (fold)
I	6.24	3.01	-
III	7.55	3.65	1.21
VI	10.18	4.92	1.63
IX	10.3	4.98	1.65

* Resistance ratio (RR) = Ratio of LC_{50} for field strain after selection to that of susceptible strain.

RESULTS AND DISCUSSION The laboratory selection of diamondback moth with Biobit resulted in higher median lethal concentrations (Table 1). After nine generations of selection, the LC_{50} of Biobit increased by only 1.65-fold when compared to the unselected strain. Similarly, studies by Wright *et al*., (1997) indicated very low rate of development of resistance (1.82-fold) after selection of DBM with Dipel 8L for three generations. Several other workers also have reported (Tabanshnik *et al*., 1991 and Mohan and Gujar 2000) increased resistance in DBM on selection with Dipel in the laboratory. Selection of DBM with fluenxuron resulted in increase in resistance in successive generations (Table 2). After 9 generations of selection, the LC_{50} of flufenxuron increased by 5.96-fold when compared to the unselected strain. Though no reports are available on the selection studies with this particular acylurea compound, many studies have been carried out with other acylurea compounds. Higher resistance ratios were obtained by Fauziah *et al*., (1992) after six generations of selection with chlorfluazuron and teflubenzuron (17.3- and 46.8-fold, respectively). Studies carried out by Iqbal *et al*., (1996) and Perng *et al*., (1988) also reported increase in resistance levels after the field population was selected with teflubenzuron in the laboratory. It is clear from the present study that, the pest is capable of developing resistance to B.t. and acylurea compounds under intensive selection pressure. Secondly, DBM acquires resistance to acylurea compounds at a much faster rate than to B.t products. Therefore, these products should be used sparingly in the field to prolong their effective life against DBM.

Table-2: Development of resistance to an acylurea compound, flufenoxuron in the field strain of *Plutella xylostella* L. after laboratory selection.

Generation Number	LC_{50} (µg a.i.ml^{-1})	Resistance ratio (RR)*	Increase in resistance (fold)
I	1.11	4.11	--
III	4.13	15.3	3.72
VI	5.09	18.85	4.59
IX	6.61	24.48	5.96

* Resistance ratio (RR) = Ratio of LC_{50} for field strain after selection to that of susceptible strain.

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Determination of the Susceptibility of White Grubs (*Phyllophaga* spp.) to Different Insecticides in Populations of San Martin Hidalgo, Jalisco, Mexico.

INTRODUCTION In the state of Jalisco the soil pest infest a surface of approximately 200,000 hectares, concentrated mainly in the center area, which are zones of deficient weather in their majority; although, there are also extensions where residual humidity is conserved from the rain cycles that allows to advance the plantation before the rain season, which favors the development of the white grub. The storm areas are the more affected by the soil plagues, due to the climate conditions (Felix, 1978, 1988).

The group of plagues that attack the root is constituted by the following genus: *Colaspis chapalensis, Diabrotica virguifera* and the complex of white grub constituted mainly by the genus: *Cyclocephala comata* Bats and *Phyllophaga spp.* (Morón, 1986, 1988, 1990, 2001).

The larvae of *Phyllophaga* represent one of the main problems of phytosanitarium character in the different crops localized in the entity as; corn, grasses, vegetables, etc., since it can attack any crop in its different phenological stages, being their critical period from 0 to 60 days. It is considered that this gender is distributed thoroughly in the center area of the state of Jalisco, one of the most productive areas agriculturally speaking. It is considered that if this plague is not controlled efficiently it can end up causing losses of approximately 70% of the normal production (Pérez, 1987).

The farmers use as main forms of handling of plagues the cultural and chemical control, generally used to protect the root area of the crop from this plague; due to the above-mentioned, there are in the market new and diverse insecticides, which have been used indiscriminately without taking into account the minimum permissible doses creating resistance in the plagues (PLM, 2003).

In the beginning the new insecticides exhibited an acceptable efficiency and through the years the levels of effectiveness have fallen notably, consequently, the recommended doses have been increased consecutively without obtaining favorable results on the control of these insects. In the last years it was demonstrated that the soil plagues like *Phyllophaga* and *Cyclocephala* present high resistance levels to the different insecticides used for their handling (Sediments, 1993). The previous situation gave us the margin to realize these works to know the levels of susceptibility of this plague to different insecticides used for its control. From where the necessity was seen of determining the susceptibility of white grub to different insecticides used for its control, in the area of San Martin Hidalgo, Jalisco.

MATERIALS AND METHODS The biological material of the population's of white grubs larvae was obtained from commercial parcels of crops sowed in the region mentioned above during the 2005 spring-summer cycle. Once collected, the field larvae were transported to CUCBA's laboratories (Centro Universitario de Ciencias Biológicas y Agropecuarias). At once they were weighed and separated in groups of 20 larvae that were placed in boxes of disposable polypropylene with a mixture of soil and organic matter. The technique of topical application proposed by the FAO was used, evaluating the following active ingredients: Benfuracarb, Chlorpyrifos, Diazinon and Carbofuran. For each insecticide treatment the active principles of commercial products were extracted with the dilution with acetone 99% of purity grade reagent. Once extracted the active ingredient according to the proportion weighs volume, using 7 dose ranges (those that are shown in Table 1.), preparing 10 ml of each dose for each treatment. The products were diluted in acetone with 99 technical grade in grade reagent putting them in an agitator during 24 hrs. For the case of Oncol (Benfuracab) the Commercial Material was used from the concentrated formulation. (Table 1)

Table 1. Dose Ranges of the insecticides evaluated to determine the susceptibility of white grubs larvae.

COMMON NAME	DOSE RANGES
1. Benfuracarb	62.5, 125, 250, 500, 1000, 2000 y 4000
2. Chlorpyrifos	62.5, 125, 250, 500, 1000, 2000 y 4000
3. Diazinon	62.5, 125, 250, 500, 1000, 2000 y 4000
4. Carbofuran	62.5, 125, 250, 500, 1000, 2000 y 4000

To determine the LD_{50} of each of the involved insecticides and pointed out in the previous table, 7 dose ranges were run and 20 larvae were used for each dose with 2 observation units and when mortality was presented in the witness it was corrected with acetone by means of the Abbott formula (1925) that is the following:

$$\%\,\text{mortality} = \frac{\text{Alive Larvae in the witness - Alive Larvae in the treatment}}{\text{Alive larvae in the witness}} \times 100$$

Once the bioassays were carried out, and the data of mortality calculated, these were transformed from lethal concentrations average LC_{50} expressed in ppm to lethal dose average LD_{50} expressed in µg/g of live weight of the larva. Once the obtained data of the mortality in logarithmic scale was graphed it can be observed if the dispersion cloud corresponds to a certain lineal model, and later they are analyzed by means of the statistical method of Probit Analysis of Maximum Verisimilitude proposed by Finney (1971) mentioned for (Lagunes and Vazquez, 1994). These Probit analyses were carried out using the computer program (SPSS, 2001), with which the following parameters were obtained:

RESULTS AND DISCUSSION In order to establish a logical sequence that allows understanding clearly the findings of the present investigation, the information is presented in the following order:

Regarding the reliability of the results, in table 2 that corresponds to the location of San Martin Hidalgo, it was observed that the regression equations are positive as well as the coefficients of determination and the grade of probability. All the treatments had acceptable coefficients of determination since they were higher then 0.80 except Carbofuran that were in 0.70. It is necessary to point out that the trust level was high, with probability higher than 95% of dependability which means that if the works were repeated, of every hundred times we would obtain similar results 95% of the occasions. (Table 2)

Table 2. Statistical Estimators of the regression lines of dose-mortality of different insecticides used for the control of white grub (*Phyllophaga* sp) in San Martin Hidalgo, Jalisco.

Treatment	Regression Equation	R2	GL	P
1. Benfuracarb	Y= 45.3482 +	0.8	5	99
2. Chlorpyrifos	Y= 23.9 + 5.31x	0.8	4	97
3. Diazinon	Y= 24.0 +10.0x	0.9	5	99
4. Carbofuran	Y= 45.83 + 3.48x	0.8	4	95

R^2 = Coefficient of determination; GL = Grades of freedom; P = Probability (%)

In Table 3 the lethal doses average are indicated (LD_{50}) of the insecticide products that were evaluated in the location of San Martin Hidalgo, to estimate the susceptibility of larvae of Phyllophaga, where it is observed that the population was shown to be very sensitive to Benfuracarb presenting an LD_{50} of 0.1541 µg/g of live weight. Following are the treatments with Diazinon and Carbofuran with LD_{50} of 1.9 and 1.5 µg/g of live weight. The treatments to those that the white grub larvae shown few sensitive were the treatments based on Chlorpyrifos with an LD_{50} of 3.0 µg/g of live weight, being these less sensitive. In the case of the insecticide Benfuracarb, the LD_{95} was the lowest with 2.77 µg/g of live weight, followed by the treatment Diazinon with 24.45 µg/g of live weight. On the other hand with a very similar LD_{95} were Chlorpyrifos with 24.45 and Carbofuran with 4 µg/g of live weight. (Table 3)

Table 3. Answer of larvae of third instars of *Phyllophaga* sp to different insecticides of exposed populations to chemical control in San Martin Hidalgo, Jalisco.

Treatment LD_{50}	LD_{50}*	Fiducial limits at 95% confidence	LD_{95}*	Slope
1. Benfuracarb	0.1514	(0.11318-	2.776	11.80+1.39
2. Chlorpyrifos	3.1861	(2.7001-3.7583)	24.45	5.314+1.38
3. Diazinon	1.959	(1.703-2.215)	10.03	9.770+1.88
4. Carbofuran	1.4937	(1.059-1.9312)	42.7	3.489+1.09

* Dose expressed in µg/g of live weight

CONCLUSIONS

1. In benfuracarb concentrations in dose of 1,000 ppm control was observed for up of 85%.

2. The favorable answer of benfuracarb and the rest of the treatments to be able to carry out a Probit analysis were obtained starting from 62.5 ppm, with mortalities of 33%; with 1000 ppm there were mortalities of 88% and with 2000 ppm to 100%.

3. Statistically the answer of white grub Phyllophaga sp. to benfuracarb is different to the other treatments (Carbofuran, Diazinon and Chlorpyrifos).

4. In general we can say that from highest to lowest sensibility that the population of white grub (Phyllophaga sp) presented to the four molecules was BENFURACARB, CARBOFURAN, DIAZINON and CHLORPYRIFOS.

5. It is considered that the answer of the population of the white grub Phyllophaga coming from San Martin Hidalgo is normal, without having resistance features from the plague to the evaluated insecticides.

6. The laboratory dose of Benfuracarb equivalent to that of the field is the concentration from 500 to 1000 ppm.

7. The products based on Benfuracarb in the location of San Martin Hidalgo represents one of the best options in control of soil plagues since in this town the population of white grub showed to be very sensitive.

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Resistance to Insecticides in an Indian Strain of Diamondback Moth, *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae).

INTRODUCTION The resistance in diamondback moth (DBM), *Plutella xylostella* (L.) to all major classes of insecticides including microbial products and insect growth regulators has been reported from many parts of the world (Cheng *et al*., 1992). The resistance problem is especially serious in tropical countries because of continuous cultivation of crucifers throughout the year and rapid generation turn over of the pest in favourable warm climate (Liu *et al* ., 1981). As a result, DBM has become one of the most difficult pests to manage and its importance is reflected in the estimate that its control could cost approximately US$ 1 billion annually (Talekar, 1992). The management of DBM in India has depended primarily on the use of insecticides. A cabbage crop receives about 25 sprays in areas around Bangalore (Khan *et al.*, 1991). The reliance on this single approach has led to eventual breakdown of control over the years. Development of resistance in DBM to organophosphorous and pyrethroid insecticides has been observed in different parts of India (Saxena *et al*., 1989; Chawla and Joia, 1982; Renuka and Reghupathy, 1996). Here we report resistance in a field strain of DBM to insecticides commonly deployed in its management.

MATERIAL AND METHODS *Insects:* A strain of DBM resistant to insecticides was collected from infested cabbage fields near Bangalore, Karnataka. Larvae were reared in the laboratory on mustard seedlings and third instar F1 offspring were used in the bioassay. For comparison, a susceptible laboratory strain was used. This strain was developed by rearing the insect under insecticide-free conditions for over 58 generations. The insect culture was maintained on mustard seedlings by employing the mass-rearing technique of Liu and Sun (1984) with suitable modifications.

Insecticides: Formulated insecticides used in our study were: chlorpyriphos (Corobon 20 EC), dichlorvos (Nuvan 76 EC), monocrotophos (Nuvacron 36 EC), profenofos (Curacron 50 EC), triazophos (Hostathion 40 EC) methomyl (Lannate 24 L), deltamethrin (Decis 2.8 EC), fenvalerate (Sumicidin 20 EC), a combination formulation, profenofos + cypermethrin (Polytrin-C-44), cartap hydrochloride (Padan 50 SP), flufenoxuron (Cascade 10 DC), teflubezuron (Nomolt 15 SC), two

Bacillus thuringiensis kurstaki based products namely, Biobit (potency : 3200 IU/ mg), Dipel 8L (potency : 17600 IU/mg) and one B. t. aizawai based product, Centari (potency 15000 IU/mg).

Bioassay: For bioassay of the conventional contact insecticides, the topical application method (FAO method 21) as outlined by Busvine (1980) was adopted. Appropriate dilutions of insecticdes were prepared in analytical grade acetone (99.5 % purity). At least five concentrations of the insecticides were used in each bioassay. Thirty-third instar larvae, each weighing 1.40mg, were treated in batches of ten for each concentration of the test insecticide. 0.3 µl of insecticide solution was applied on the thoracic dorsum of each larva using a Top microsyringe. The control larvae were treated with acetone alone. The treated larvae were transferred to glass petri-dishes containing excised mustard leaves and were maintained in a incubator at 25 + 1ºC. The mortality counts were taken 24 h after treatment. In the case of cartap hydrochloride and stomach poisons viz., acylurea compounds and B. t. products, 'leaf - dip' method was employed for the determination of LC_{50} values. Fresh, uniform sized mustard leaves were dipped in aqueous insecticide solutions/suspensions containing 0.05 per cent soap (Labolene) for 10 seconds and then dried under shade for one hour. The treated leaves were placed in petri dishes (10X1.5 cm) and ten third instar larvae were released into each petri dish. The treated larvae were maintained at 25 + 1 º C and the mortality counts were recorded 72 h after treatment. The data obtained in both the bioassays were subjected to probit analysis for developing regression equations for dosage-mortality response and to establish the LD_{50} /LC_{50} values.

RESULTS AND DISCUSSION The results of the probit analysis are shown in the Tables 1 and 2. The dosage-mortality response of the field strain of DBM varied greatly from that of the susceptible strain thus indicating development of resistance to the insecticides tested. At LD_{50}'s, the degree of resistance to five organophosphorous insecticides in the field strain ranged from 5- to 227- fold (Table 1). While the highest level of resistance was observed against monocrotophos, the resistance ratios for triazophos, profenofos, dichlorvos and chlorphyriphos indicated

Table 1. Dosage-mortality response of susceptible (S) and resistant field (R) strains of *Plutella xylostella* to conventional Insecticides

Insecticides	Strain	n	LD_{50} (µg a.i. g^{-1})	Fiducial limit (95%) (µg a.i. g^{-1})	Slope ± SE	Resistance ratio *
Chlorpyriphos	S	180	312.14	275.05 - 356.35	4.50 ± 0.72	
	R	150	1674.91	1442.96 – 1911.21	4.28 ± 0.62	5.37
Dichlorvos	S	150	97.69	71.24 - 127.41	2.21 ± 0.37	
	R	150	1505.22	1313.74 – 1771.52	4.29 ± 0.71	15.41
Monocrotophos	S	180	41.22	27.89 - 57.66	1.46 ± 0.21	
	R	150	9367.12	7328.04 – 12441.55	2.48 ± 0.44	227.25
Profenofos	S	150	29.26	22.91 - 37.21	2.48 ± 0.33	
	R	150	1143.8	870.10 - 1437.89	2.38 ± 0.41	39.09
Triazophos	S	150	111.14	67.48 - 165.94	1.35 ± 0.27	
	R	150	5563.36	4968.09 – 8133.13	2.50 ± 0.47	59.06
Methomyl	S	180	16.03	11.34 - 21.64	1.70 ± 0.24	
	R	180	582.11	416.78 - 798.89	1.58 ± 0.23	36.31
Deltamethrin	S	180	0.41	0.20 - 0.66	0.98 ± 0.20	
	R	150	1153.77	905.17 - 1475.23	2.36 ± 0.47	2814.07
Fenvalerate	S	150	0.29	0.16 - 0.55	1.04 ± 0.26	
	R	150	8075.98	5578.59 – 10849.09	1.84 ± 0.39	27848.21
Profenofos + Cypermethrin	S	150	8	5.97 – 10.32	2.27 ± 0.33	
	R	150	704.43	499.92 - 933.53	2.09 ± 0.36	88.01

* Resistance ratio = LC_{50} resistant ÷ LC_{50} susceptible strains

Table 2. Dosage-mortality response of susceptible (S) and resistant field (R) strains of *Plutella xylostella* to newly introduced Insecticide.

Insecticides	Strain	n	LD_{50} (µg a.i. g^{-1})	Fiducial limit (95%) (µg a.i. g^{-1})	Slope ± SE	Resistance ratio *
Tertiary amines						
Cartap hydrochloride	S	180	133.37	100.39 - 176.02	1.98 ± 0.28	
	R	180	499.53	328.40 - 717.73	1.37 ± 0.22	3.75
***B. t.* products**						
Biobit	S	180	0.132	0.05 - 0.268	0.81 ± 0.61	
	R	180	1.06	0.81 - 1.40	1.00 ± 0.27	8
Dipel 8L	S	180	0.0083	0.005 - 0.012	1.42 ± 0.19	
	R	150	0.127	0.08 - 0.20	1.31 ± 0.21	15.3
Centari	S	150	0.125	0.081 - 0.177	1.55 ± 0.28	
	R	150	0.248	0.171 - 0.332	2.12 ± 0.41	1.9
Acylurea compounds						
Flufenoxuron	S	150	0.002	0.0015 - 0.0028	2.11 ± 0.36	
	R	150	0.0036	0.003 - 0.0043	3.97 ± 0.62	1.8
Teflubenzuron	S	150	0.038	0.03 - 0.047	2.95 ± 0.56	
	R	150	0.028	0.020 - 0.039	1.85 ± 0.32	0.72

* Resistance ratio = LD_{50} resistant ÷ LD_{50} susceptible strains

moderate to low levels. Organophosphoous insecticides are known to induce varying levels of resistance in DBM because of their highly diverse molecular structure (Liu *et al*.,1982 ; Cheng, 1986). Varying levels of resistance to organophosphate compounds in DBM has been documented by Saxena *et al*., (1989) and Chawla and Joia (1992). A moderate level of resistance (36-fold) was observed gainst methomyl. The resistance ratios for the synthetic pyrethroids tested were highest compared to organophosphorous and carbamate insecticides. The field strain showed very high degree of resistance to both fenvalerate (27,848 - fold) and deltamethrin (2,814- fold). This is in conformity with the very high levels of resistance to pyrethroids observed the world over compared to other groups of insecticides (Cheng *et al*., 1992). Resistance to a combination product, profenofos + cypermethrin, was more than the level observed against profenofos alone (Table 1).

After the failure of synthetic pyrethroids to control DBM, cartap hydrochloride, a tertiary amine, was widely used by the cultivators to suppress DBM populations since 1987. Our results showed that the pest has acquired only about 4-fold resistance to this insecticide in spite of its long usage in the field (Table 2). Though a maximum of 199-fold resistance to cartap hydrochloride has been documented in Taiwan (Liu *et al.*, 1982), in countries like Malaysia and Japan, the pest has shown only 7-fold and 16-fold resistance, respectively, even after its long usage over ten years (Noppun *et al.*, 1983; Kobayashi *et al.*, 1992) indicating that the rate of development of resistance to this compound is rather slow. During the past few years, Bt products have been extensively employed in the control of DBM around Bangalore. The pest has acquired 8- to 15- fold resistance to Bt kurstaki based products and only about 2- fold to a Bt aizawai product. The relatively low level of resistance observed against Bt aizawai product is in agreement with the findings of Syed (1992) and Shelton etal., (1993) who observed only 3- and 4- fold resistance to Bt aizawai, respectively, as against 113- to 461-fold to Bt kurstaki based products.

DBM has shown resistance to acylurea compounds within a short span of time wherever these were employed for the control of insecticide resistant populations (Cheng *et al*., 1990; Ismail and Wright, 1991). In our study, the resistance ratios for two acylurea compounds indicated that DBM has not acquired resistance to these insecticides in areas around Bangalore.

Our results show that over dependence on insecticides to control DBM over the years has resulted in development of multiple resistance to all classes of conventional insecticides, cartap hydrochloride and Bt products. As the pest is capable of developing resistance even to acylurea compounds, which are highly effective at present, the susceptibility to these insecticides should be conserved by optimizing their deployment. Also, there is need for evolving sound resistance management strategies to reduce the overall risks imposed by exaggerated use of insecticides.

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Selective Toxicity and Discriminating Dose of Lambda Cyhalothrin 5 CS Against *Earias vitella* Fab.

ABSTRACT The acute toxicity of lambda-cyhalothrin 5.0 CS was assessed to target pests *viz., Earias vitella* (Fab.), and non-target insects *viz., Trichogramma chilonis* (Ishii) and *Apis indica* (Fab.) in terms of LD_{50}/LC_{50} and LD_{95}/LC_{95}. The median lethal dose (LD_{50}) of lambda-cyhalothrin for F_1 population of *E. vitella* was 0.00394 µg larva^{-1} and LD_{95} value was 0.0174 µg larva^{-1}. The LD_{50} value of F_7 generation was 0.00267 µg larva^{-1}and LD_{95} was 0.0142 µg larva^{-1}. The susceptibility increased upto seven generations of insecticide exposure free culturing as evident from decline in LC_{50} and LC_{95} values. The susceptibility index (SI) of F_7 generation over F_1 was 1.4757 based on LD_{50} and 1.2185 based on LD_{95}. The rate of resistance decline (R) was -0.0241. The number of generations required for a 10-fold decrease in LC_{50} was calculated as 41.49. Based on LD_{95} of F_1 generation the tentative discriminating dose (DD) arrived was 0.017 µg larva^{-1}.Endosulfan was found to be less toxic than lambda-cyhalothrin to *T.chilonis* with the LC_{50} of 3.5012 and 1.0326 ppm and LC_{95} was 16.0166 and 19.2251 ppm respectively. Malathion was found to be less toxic than lambda-cyhalothrin to *A.indica* with LC_{50} of 12.8878 and 4.3569 ppm and LC_{95} was 61.7549 and 32.5778 ppm respectively. The selectivity ratio worked out for non-target organisms vs. target organisms revealed that lambda-cyhalothrin is selective to *A. indica* but non-selective to *T.chilonis* comparatively.

INTRODUCTION Of the new generation insecticide molecules, lambda-cyhalothrin is an eco-friendly and photostable synthetic pyrethroid. It has been used against a wide range of Lepidopteran (Rinkleff *et al*., 1995), Hemipteran (Greene *et al.*, 2001), Homopteran (Liu *et al*., 2001), Dipteran (Zhu *et al*., 2002) and Coleopteran (Yadav *et al*., 2001) pests and it also has some acaricidal activity (Yang *et al*., 2002).

However, it has also been reported that many insect pests have become resistant to lambda-cyhalothrin (Cho and Lee, 1994; Cameron and Walker, 1998). Since similar studies on spotted bollworm *E. vittella* are limited, the present study was undertaken to determine selective toxicity (LD_{50}) of lambda-cyhalothrin 5.0 CS to non-target insects like *Trichogramma chilonis* (Ishii) and honeybees, *Apis cerana indica* (Fab.) in relation to target pests, *E. vittella* and to fix discriminating dose for monitoring resistance to *E. vitella*

MATERIALS AND METHODS Culture of *E. vitella* was initiated at Insecticide Resistance Lab. Insectary, Department of Entomology, Tamil Nadu Agricultural University (TNAU), Coimbatore. Larvae of different instars were collected from cotton and okra from farmers field at Thondamuthur and reared on okra fruit. The choice of this food medium was based on earlier findings that development time was shortest on okra fruit (Vishwapremi and Krishna, 1974; Senapati *et al*., 1978; Ambegaonkar and Bilapate, 1984).

Fresh unripe okra fruits were collected from okra plants grown under insecticide free condition. The fruits were cut into pieces of 2.5 cm and placed in 12 well-cavity trays. Using camel brush, the larvae were transferred on fruits. Filter paper of same size as that of tray was placed over the fruits in order to absorb excess moisture thereby preventing rotting of fruits. The tray was then covered using plastic lid and fastened by a rubber band. Fresh fruits were provided on alternate days. The boat shaped cocoon were collected and placed in adult emergence cage (30x30x30 cm). Newly emerged adults were provided with ten per cent sugar solution fortified with ABDEC multivitamin solution in 5 ml glass vial with cotton wool wick placed in mouth to prevent the moths from drowning. Four to five tender okra fruits were kept in cages as oviposition substrates These fruits were removed the next day of egg laying and observed for hatching of eggs. The newly hatched first instar larvae were provided with cut pieces of okra fruit. This represented F_1 generation. The larva reached third instar on 6-7 days after hatching. Those with weight ranging between 0.0638 to

0.0857 g larva-1 and length of 0.8 to 1.0 cm alone were used for bioassay (Somasundaram, 1984). Thirty larvae were used to assess mortality per dose and remaining were sustained on okra fruit for development of subsequent generations.

The insecticide dilutions required for bioassay were prepared using technical grade insecticides of lambda cyhalothrin 5 CS formulation of 86.2 % purity diluted with analytical grade acetone and formulated product with water and technical grade insecticides of malathion (96.0% purity) and endosulfan (98.8 % purity).

Preliminary range finding tests were done to fix the test dose range causing 20 to 80 per cent mortality approximately. Based on this, 4 to 6 doses were fixed in geometric progression for which dilutions were prepared with analytical grade acetone. The experimental insects were treated starting from lower to higher concentrations of the test insecticide.

Bio assays - *E. vittella Fab:* Topical application method was followed for *E. vittella* because the chance of behavioural resistance or avoidance of treated surface is not possible and the amount of insecticide used for exposing each insect is exact. Third instar larvae were assigned to topical application on the dorsal thorax with 1.0 µl of required concentrations of technical insecticides in acetone with a Hamilton repeating dispenser. A minimum of 30 larvae were used per concentration. After treatment, larvae were held individually in their respective diets *viz*., okra fruit for spotted bollworm at 25 (± 2ºC) for 24 h when mortality was recorded. Larvae were considered dead if they were unable to move in a co-ordinated manner when prodded.

Apis cerana indica Fab: The Indian bees, *Apis cerana indica* were obtained from insectary. Dry film contact toxicity method was followed. Malathion was used as standard check. Filter papers of 7 cm diameter were impregnated with 0.5 ml of insecticide test solution and dried. The filter papers were then placed in plastic containers with sufficient aeration for bees. Worker bees collected from a single frame from a single colony, at about 2.00 p.m. were anaesthetized by keeping it in refrigerator for few minutes. Approximately 25 bees were quickly transferred with the help of paper used like a spoon into each of the containers. They were then covered with black cloth in order to reduce their flying or "balling" so as to bring about maximum contact with the treated surface of filter papers. The bees were kept in contact with the treated surface of filter paper for one hour, after which the bees were transferred into cages (40x40x40 cm) made of muslin cloth over iron frame and provided with cotton soaked in 40 per cent sucrose solution as source of food. The bees were observed for mortality 12 hrs later. Moribund honeybees were counted as dead.

Trichogramma chilonis Isida: T. chilonis wasps were collected from the Biocontrol laboratory, TNAU, Coimbatore. An aliquot of each concentration of the test insecticide was pipetted into test tube and rotated to have a uniform coating of the insecticide all over the inner surface. The tubes were then air dried to leave a thin dry film of the insecticide. Twenty adult wasps emerged from the parasitized egg cards were released into the tube and mortality was noted after 4 hrs. Endosulfan was used as standard check The median lethal dose (LD_{50}) and median lethal concentration (LC_{50}) of insecticide used were determined by Finney's probit analysis (Regupathy and Dhamu, 2001). Susceptibility index, the rate of resistance decline and resistance frequency were also calculated (Regupathy and Dhamu, 2001).

RESULTS AND DISCUSSION The LD_{50} and LD_{95} values for seven generations of *E. vittella* collected from Thondamuthur, Coimbatore are presented in Table 1. The median lethal dose (LD_{50}) assessed for F_1 population was 0.00394 µg larva-1 for lambda-cyhalothrin and LD_{95} value was 0.0174 µg larva-1 (Table 1). The susceptibility of F_7 generation was moderately increasing with an LD_{50} value of 0.00267 µg larva-1. The LD_{95} was also found to decrease from 0.0174 (F_1) to 0.01428 µg larva-1 (F_7) (Table 1). The baseline toxicity of lambda-cyhalothrin obtained in the present study for *E. vittella* was found to be less when compared to LD_{50} reported for other synthetic pyrethroids *viz*., fluvalinate (3.296 µg larva-1), deltamethrin (2.293 µg larva-1) (Somasundaram aand Regupathy, 1985), fenvalerate (0.79 mg litre-1) and cypermethrin (1.33 mg litre -1) (Saini *et al*., 1989).

Table 1. Acute toxicity of lambda-cyhalothrin to *E. vittella* by topical application

Generation	Regression Equation	Chi square χ^2	LD_{50} µg/µl	Fiducial limits		LD_{95} µg/µl	Fiducial limits	
				LL	UL		LL	UL
1	Y = -1.6103 + 2.5469x	1.9732	0.00394	0.003362	0.0046	0.0174	0.0122	0.0248
2	Y = -1.3275 + 2.4506x	2.525	0.00381	0.0032	0.0045	0.0179	0.0123	0.026
3	Y = -1.2945 + 2.4486x	1.4193	0.00372	0.003	0.0046	0.0175	0.0108	0.0284
4	Y = -1.6016 + 2.6138x	3.3995	0.00335	0.0027	0.00413	0.0143	0.0093	0.0219
5	Y = -0.9981 + 2.4250x	4.4295	0.00297	0.0023	0.00374	0.0142	0.00896	0.0239
6	Y = -0.7309 + 2.3479x	5.0766	0.00275	0.00216	0.00352	0.01385	0.00857	0.02239
7	Y = -0.4818 + 2.2589x	2.9291	0.00267	0.002066	0.00345	0.01428	0.00859	0.02374

Table 2. Acute toxicity of lambda-cyhalothrin to non-target insects

Insecticide	Regression Equation	Chi square χ^2	LC_{50} ppm	Fiducial limits		LC_{95} ppm	Fiducial limits	
				LL	UL		LL	UL
T.chilonis								
Lambda-cyhalothin 5.0 CS	Y = 1.0962 + 1.2952x	0.0631	1.0326	0.6477	1.6462	19.2251	4.7778	77.3595
Endosulfan	Y = -3.8284 + 2.4909x	0.42	3.5012	3.6915	4.5544	16.0166	9.047	28.3553
A. cerana indica								
Lambda-cyhalothrin 5.0 CS	Y = -1.8511 + 1.8826x	1.3489	4.3569	3.2691	5.8067	32.5578	13.7312	77.2922
Malathion	Y = -4.9352 + 2.4174x	1.4511	12.8878	10.2729	16.1683	61.7549	32.6375	116.8491

This clearly explicited that lambda-cyhalothrin has high insecticidal activity than other synthetic pyrethroids. The LD_{50} values of lambda-cyhalothrin to *E. vittella* was less when compared to LD_{50} against *H.armigera* 0.3 to 0.5 µg larva^{-1} (Tamilselvi and Regupathy, 2002) and 1.53 µg larva^{-1} (Ramasubramainan and Regupathy, 2004).This indicates that *E.vittella* is comparatively more susceptible than *H.armigera*. The susceptibility of *E. vittella* population was tested with relaxation of selection pressure to pesticides by continuously rearing the population in lab for seven generations. The susceptibility index at LC_{50} was 1.4757 and the number of generations required for 10- fold decrease at LC_{50} was 41.49. The rate of resistance decline was negative indicating that the susceptibility increased with succeeding generation (Table 3). The results indicated that susceptibility gradually increased with succeeding generation as evident from decline in LD_{50} value from 0.0394 (F_1) to 0.00267 µg larva^{-1} (F_7) and LD_{95} from 0.0170 (F_1) to 0.01428 µg larva^{-1} (F_7). Such reduction during relaxation of selection pressure had been demonstrated in *E.vittella* for fenvalerate and cypermethrin (Saini *et al*., 1989). The tentative discriminating dose (DD) arrived at based on LD_{95} of lambda-cyhalothrin for F_7 generation of laboratory cultured population of *E. vittella* was 0.017 µg larva^{-1}.The LC_{50} values of lambda-cyhalothrin and endosulfan to *T.chilonis* revealed that the latter is 3.39 fold less toxic than the former (Table 2). The toxic nature of synthetic pyrethroids to parasitoids was reported by House *et al*. (1985) and Somasundaram and Regupathy, (1985). Cleary and Scholz, (2002) reported the extreme toxic nature of lambda-cyhalothrin for the survival of *T. pretiosum* adults. Median lethal concentration of lambda-cyahlothrin by dry film method was 4.3567 ppm for *A. cerana indica* (Table 2) whereas it was 68 ng/ bee (Pilling and Jepson, 1993) by topical application in *Apis mellifera* (F.).Malathion was 2.96 fold less toxic than lambda-cyhalothrin. Moderate toxicity of malathion to honeybee was reported by Singh *et al.* (1974).Selectivity values are ratio of toxicity of insecticides to target pest divided by the toxicity to non-target species. For working out the selective toxicities, the application method should be the same to the target and non-target species. However in the present study, different methods of application were adopted *i.e*. topical application of exact dose for bollworms, leaf disc method for thrips and dry film method for non-targets. The selectivity ratio worked out for non-target organisms vs target organisms revealed that lambda-cyhalothrin is selective to *A. cerana indica* but non-selective to *T.chilonis* comparatively (Table 4). The non-selective nature to the parasitoid, *T.chilonis* might be due to the sensitivity of parasitoid to synthetic pyrethroids and its smaller size. White *et al*. (1990) reported the selectivity of lambda-cyhalothrin to three groups of natural enemies of *R. padi viz*., Episyrphus balteatus De Geer, Dacnusa sibirica Telenga and Trechus quadristriatus Shrank. The selectivity of pesticide used can be conferred in several ways. Environmental selectivity could achieved by exploiting the spatial or temporal asynchrony between a pest and its natural enemy so that the pest is exposed to the toxicant but the natural enemy is not. Physiological selectivity could be achieved when the compound is toxic to the pest but not to the natural enemy at the same concentration level. However the hazard posed by a formulated pesticide depends not only on the toxicity, but also on the dosage applied at field rate, the proportion of the dose that is available for transfer to non-targets. Thus hazard ratio (Smart and Stevenson, 1982), the ratio between application rate and toxicity gives an approximation of selectivity, how close the likely exposure of non-target to the toxicant.

Table 3. Susceptibility index and rate of resistance decline

Target pests	Susceptibility Index		Rate of resistance decline	
	LD_{50}/LC_{50}	LD_{95}/LC_{95}	R	G
Earias vitella	1.4757	1.2185	-0.0241	41.49

Table 4. Selective toxicity of lambda-cyhalothrin to non-target insects

LC_{50} of Non-target insects / LC_{50} (or) LD_{50} of Target pest	LD_{50}/LC_{50}	LD_{95}/LC_{95}
T. chilonis / E.vittella	262.08	1104.89
A. cerana indica / E.vittella	1105.81	1871.14

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Comparative Efficacy of Transgenic *Bt* and Non-transgenic Cotton against Insect pest of cotton in Tamil Nadu, India.

ABSTRACT The Bollgard gene that is expressed in transgenic cotton (Bt cotton) varieties has been adopted recently by cotton growing farmers in India to control cotton bollworm, *Helicoverpa armigera.* Replicated Field trials were carried out during 2002-2003 seasons to study and compare the impact of *Bt* and non *Bt* cotton on the insect pest with and without insecticides usage. The study results indicated that *Bt* crops were able to suppress the bollworm infestation even without the use of additional insecticides. The *Bt* crop plots required relatively lesser number of insecticidal applications as compared to the non *Bt* crop plots. The *Bt* crop plots showed better yield and cost benefit ratio than the non-*Bt* plots.

KEY WORDS: *Bt* cotton, cotton pest, *Helicoverpa armigera*

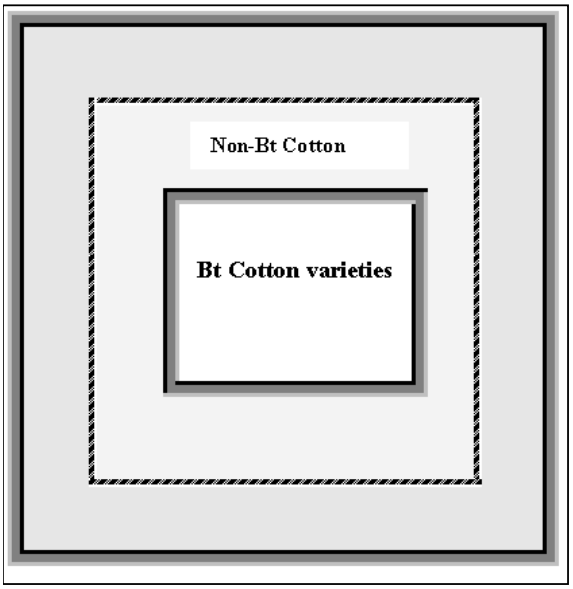
INTRODUCTION Genetic engineering technology has made it possible to develop new means of pest management. In particular, novel insecticidal proteins can be inserted into crop plants to enhance their host plant resistance, or into natural enemies to increase their speed of kill. Insect resistance to chemical insecticides and concern about the impact of residues on human health and the environment have driven agriculture to develop more sustainable approaches to managing pests. Pest, take a heavy toll in cotton and a third of the potential production is lost. Heavy reliance on chemical pesticides to control the pest and disease, has led to detoriation of health and environmental hazards. Biotechnology offers us the option in the form of B.t transgenic crops (genetically modified crops - GM crops) as a solution to the above-mentioned problem. Biotechnology offers a powerful tool for enhancing productivity by removing constraints to create higher genetic production potential and protecting crops from insects and thereby, reducing the use of chemical pesticides. It is not surprising therefore that, the best known success stories of biotechnology application for commercial agriculture is related to insect control In this paper an attempt has been made to evaluate and compare the effect of *Bt* and non *Bt* cotton on the insect pest complex in cotton ecosystem.

MATERIALS AND METHODS Filed experiments were laid at Kancheepuram district, Tamil Nadu, India, during 2002 - 2003 season. The experimental designs were randomized complete blocks with two treatments and four replications. Individual plots were approximately 0.5 acre (Figure 1). The two treatments consisted of Bollgard MECH -12 *Bt* cotton containing CrylAc and a conventional cotton variety (MCU-5). Separate plots were established to evaluate the comparative effects of additional insecticides treatments. Similarly other plots were laid to study the effect on non-target insects without any insecticidal application. The insecticidal application was done based on the economic threshold level (ETL), Ellsworth *et al.*, 1996; Ellsworth and Barkley, 2001). The entire cotton crops were maintained according to the crop production guide published by the Tamil Nadu Agricultural University, India (T.N.AU., 2003).

All the data will be subjected to suitable transformation and then statistically analysed for variance (Snedecor and Cocharan, 1967)

Insect Pest Assessment: Ten plants per replication will be selected at random. The sucking pests (Table 1) population will be assessed by counting the total number of insects from three leaves selected at random from top, middle and bottom parts of each plant. Healthy and infested squares and bolls will be recorded and the data will be subsequently computed into per cent infestation in each treatments including untreated control. Cotton yield (kapas) and local infestation will be recorded at every picking and it will be computed as q/ha. Observations will be recorded at fortnightly intervals (Dandale *et al*, 2001)

Fig.1 Planting Layout of the Field Experimental Plots for Bt crops



Bt cotton was planted in the center of the plot for one acre, 5 rows of non-Bt cotton was planted surrounding the Bollgard cotton plot.

{In accordance with the conditions laid down in the Genetic Engineering Approval committee (*GEAC, 2001*), Government of India under the environment protection act and the rules framed there under}

Table 1. Major Insect species attacking Cotton in Tamil Nadu

Common Name	Scientific Name	Type of Damage
Cotton Aphids	*Aphis gossypii*	Adults and nymphs suck sap from the leaves, producing honey dew and sooty mold.
Cotton Leaf hopper (Jassids)	*Amrasca biguttula Ishida*	Adults and nymphs suck sap from the leaves, producing honeydew and sooty mold.
Thrips	*Thrips tabaci*	Adults and immature cause distortion of leaves and terminals of seedling plants.
Cotton Whiteflies	*Bemisia tabaci*	Adults and nymphs suck sap from the leaves, producing honeydew & sooty mold.
Tobacco caterpillar	*Spodoptera litura*	Larvae feed on the leaves causing defoliation.
American Bollworms	*Helicoverpa armigera*	Larvae feed on squares, bolls and locules.
The Spotted Bollworms	*Earias insulana* and *E.vittella*	Larvae feed on squares, bolls and locules.

Table 2. Insecticides Recommended for use against Pest of Cotton in Tamil Nadu, India

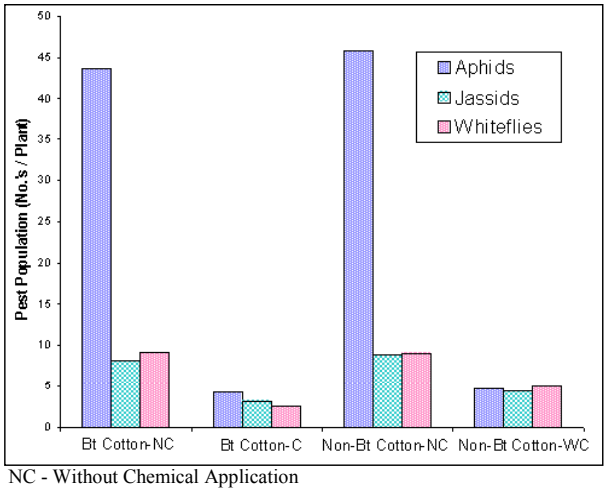
Insecticide Name	Dosage (in 500 Litre of water)	Type of Pest
Acetamiprid 20 % SP	10 gram	Cotton Aphids
Acephate 75% SP	292 gram	Cotton leaf hopper (Jassids)
Imidacloprid 17.8 % SL	0.10 - 0.125 Litre	Thrips
Imidacloprid 17.8 % SL	0.10- 0.125 Litre	Cotton White flies
Cypermethrin 10 % EC	50-70 gram	Tobacco caterpillar
Lambdacyhalothrin 5 % EC	15-25 gram	American Bollworms
Indoxacarb 14.5 % SC	75 gram	
Deltamethrin 2.8 EC	12.5 gram	
Cypermethrin 25 % EC	40-70 gram	
Cypermethrin 10 % EC	50-70 gram	The Spotted Bollworms
Indoxacarb 14.5 % SC	75 gram	The Pink Bollworms
Indoxacarb 14.5 % SC	75 gram	The Red Cotton Bug
Achook (Biopesticide)	0.00%	Cotton Aphids
Halt (Biopesticide)	1 Kg /ha	American Bollworms

RESULTS AND DISCUSSION *Effect of Bt and Non Bt crops on the insect pest of Cotton (Non Insecticides treated plots)*

Sucking Pest Population (Aphids, Jassids & Whiteflies) In the studies that did not involve the use of additional insecticides, the study results (Figure 2) indicated a gradual increase in the sucking pest population in the Bt and the non-Bt plots. The plots recorded pest population of 43.61 and 45.66 aphids per plant, 8.13 and 8.86 jassids per plant, 9.04 and 9.11 whiteflies per plant in the Bt and the non-Bt plots respectively. There was no significant difference among the pest population in the Bt and non-Bt crop plots. The second season studies showed similar trend.

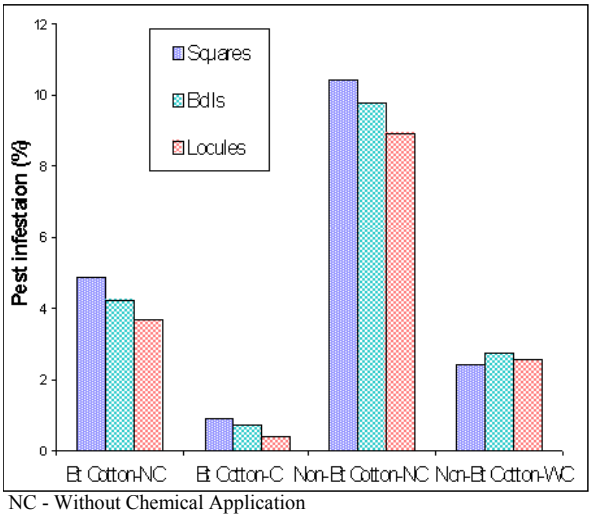
Bollworm complex (Squares, Bolls and Locules): The results of the present study revealed that the non-Bt plots attracted more Bollworm infestation than the Bt plots. The plots recorded 4.86 and 10.42 per cent (squares), 4.23 and 9.80 per cent (bolls), 3.67 and 8.93 per cent (locules) infestation in the Bt and the non-Bt plots respectively. The Bt plots were able to reduce the bollworm infestation without any additional insecticide applications. There was a significant difference in the bollworm infestation among the Bt and non-Bt crop plots (Figure 3). A similar trend was observed in the second season studies as well.

Figure 2. Effect of cotton type and insecticide use on the sucking pest of cotton.



NC - Without Chemical Application
C - With Application

Figure 3. Effect of cotton type and insecticide use on the Bollworm pest of cotton.



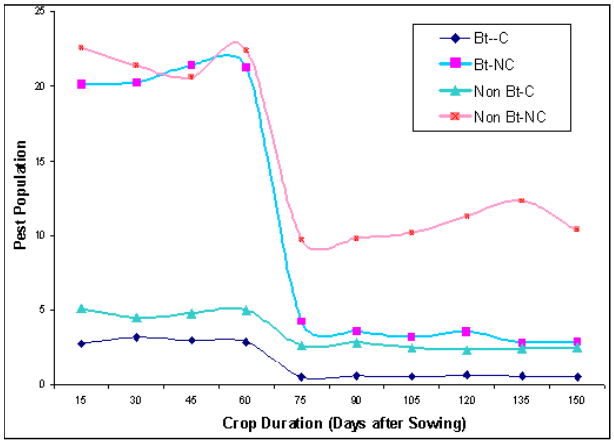
NC - Without Chemical Application
C - With Application

Insecticidal Treated Plots

Sucking Pest Population (Aphids, Jassids & Whiteflies) In the studies that involved the use of additional insecticides to control the sucking pest population, the study results indicated a gradual decrease in the sucking pest population in the Bt and the non-Bt plots. The plots recorded pest population of 4.20 and 4.75 aphids per plant, 3.11 and 4.47 jassids per plant, 2.43 and 4.91 whiteflies per plant in the Bt and the non-Bt plots respectively (Fig. 2). Similar observations were recorded in the second season studies as well.

Bollworm complex: The study results with respect to bollworm infestation in the insecticidal treated plots revealed that the bollworm infestation decreased in both the Bt and non-Bt plots. The plots recorded 0.91 and 2.40 per cent (squares), 0.71 and 2.74 per cent (bolls), 0.41 and 2.56 per cent (locules) infestation in the Bt and the non-Bt plots respectively. However the number of insecticidal application in the Bt plots was less as compared to the non-Bt plots (Figure 4). A similar trend was observed in the second season studies as well.

Figure 4. Efffect of Cotton Type and Insecticide Use on the Pest of Cotton at Different Crop Growth Stages



Effect of Bt and Non Bt crop on the cotton yield: The results data on yield data (Table 3) indicated that the Bt plots recorded better cotton yield (Kapas) of 17.21 q/ha and 8.58 q/ha in the insecticides treated and non-insecticides treated plots. The non-Bt crop plots recorded 9.82 q/ha and 3.33 q/ha in the insecticides treated and non-insecticides treated plots Effect of Bt and non-Bt cotton on the cost benefit analysis The cotton budget and analysis revealed a Benefit/ Cost (B/C) ratio of 1: 2.2 in the Bt crop plots, where as the non-BT showed a lower Benefit/ Cost (B/C) ratio of 1: 0.74, there by indicating that the value of investing in Bt crops can result in better profits.

DISSCUSSION A number of potential benefits and risks are associated with the use of transgenic crops in agricultural production systems. Among the benefits cited are significant reduction in conventional, broad spectrum insecticide use, improved suppression of target pests, better yield, reduction in production cost leading to increased profitability, and increased opportunity for biological control (Edge *et al*., 2001). There are also potential risks, including food safety, loss of susceptibility to Bt toxins in target pest, and direct or indirect detrimental effects on the non-target organisms (National Research Council, 2002; Shelton *et al.*, 2002)

Bt cotton provides a fairly high degree of resistance to the American bollworm (*Helicoverpa armigera*), the major insect pest in India. Field trials with these Bt crops have been carried out since 1997 and for the 2002/2003 growing season the technology was commercially approved. Its performance is hotly disputed among biotechnology advocates and opponents.

Relatively very few studies have been done to study the effect of transgenic crop on the insect pest, insecticide usage pattern and non-target organisms in large-scale agricultural production systems in India. Therefore, with this background in mind, the present study was planned and conducted.

The results of the field experiments conducted on the above aspect are discussed in the following pages

Effect of Bt and Non Bt crops on the insect pest of Cotton: Field experiments conducted to assess the insect pest incidence of cotton in the major cotton growing areas of Tamil Nadu revealed that the bollworm incidence is one of the major pests of cotton. The results of the present study (Figure 2 &3) indicated that the Bt crop provided effective control of bollworms, and Bt cotton varieties decreased overall levels of insecticide application for lepidopteran pest (Figure 4). The results of the present study are similar to the findings of many workers. Jenkins *et al*, 1997 had similar finding, he reported that Bt cotton provides effective control of the three major caterpillar pests in cotton. Moore *et al*, 1997 estimated that two Bt cotton varieties provided 90% control of bollworms. Mann and Mullins (1999) in their study showed that a 54 % higher insecticide efficacy was related to reduced bollworm feeding injury on Bt cotton vs. non-Bt cotton.

Table 3. Effect of BT and Non BT crop on cotton Yield

Crop	Yield Data (Q/ha)*	
	Bt – Cotton (Bollgard, MECH 12 Bt)	Non Bt- Cotton (MCU-5)
With Insecticidal Application	17.21a	9.82a
Without Insecticidal Application	8.58b	3.33b
CD (P= 0.05)	2.53	2.11
SE m (±)	0.79	0.66

* Cumulative of 12 pickings

CD: Critical difference at 0.05 level., SE (M): Standard Error of Mean

Effect of Bt and Non Bt crop on the cotton yield In the present study (Table 3) yields were compared between the Bt varieties and non-Bt varieties .The Bt plots recorded higher yield as compared to the Non-Bt

crop plots. Gianessi and Carpenter (1999) in their study found that the average percentage loss in yield before *Bt* cotton introduction was 3.7 %, whereas the average percentage loss in yield after *Bt* cotton introduction was 2.3 %. Mullins and Miller (1999) demonstrated yield advantage of 22.4 kg/ha that resulted from adoption of Bt cotton. Kerly, 1996 in a 75 field comparison of the 3 Bt cotton varieties and their non-Bt near isogenic lines, showed a lint increase of as much as 207.2 kg/ha. The results of the present study are similar to the above findings.

Effect of Bt and non-Bt cotton on the cost benefit analysis

Ismael *et al*, 2001 in their study reported that the non-transgenic cotton requires up to 8 or more sprays for bollworm control. Bt crops can eliminate this requirement almost completely and provide savings in time and labour, insecticide and equipment cost. These finding are similar to the results of the present study in which we found that the benefit cost ratio of Bt plots are better than the non Bt crop plots.

The broader impacts of genetically modified (GM) crops are still a matter of controversy, especially with respect to social ramifications as well as long-term environmental implications and sustainability. In the present study, we empirically analyze the effects of insect resistant *Bacillus thuringiensis* (Bt) cotton on the insect pest, non-target insects, soil microorganisms, pesticide usage pattern, and the benefit cost ration in comparison to the conventional cotton varieties. Based on the field and laboratory experiments in Tamil Nadu, the present project is summarized as follows:

The *Bt* crops provided a fairly high degree of resistance to the American bollworm (*Helicoverpa armigera*), the major pest in cotton ecosystem.

The study results presented here suggest that use of Bt crops did not have any consistent or detrimental effect on the Non target insect pest

The results data concerning to the effect of Bt crops on the soil microorganism showed no significant impact on the non target micro flora under field conditions

While there was no significant difference in the number of sprays against sucking pest, Bt crops were sprayed less number of times against bollworms than the conventional cotton varieties.

The present study results on the yield data indicated a significant increase in the yield, the filed trials results indicated that average yield of Bt cotton exceeded those of non-Bt cotton.

The present study results indicated that the Bt crop plots achieved a better benefit cost ratio of 1:2.2 as compared to the non-Bt crop plots.

Field studies integrate both direct and indirect effects; the preliminary results resented here suggest that use of Bt cotton may not have significant adverse effects on the non-target insects and micro flora. The Bt crops represents an extremely selective control method that may facilitate the broader use of biological control and Integrated Pest Management (IPM) in an agriculture system long dominated by the use of broad spectrum insecticides.

These present study results suggest the ecological and economic advantage of Bt cotton technology; however more research is needed into the technology's complex interaction with environmental systems, before conclusive statements about its sustainability can be made.

A major policy change is needed to invest more in public research, so that promising biotechnologies can reach the poor at affordable prices on a larger scale.

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The Antifeedant Activity of Allobetulin and its Derivates Towards the Colorado Potato Beetle Larvae

ABSTRACT Allobetulin and its four derivatives as antifeedants towards the larvae of 4th age of the Colorado potato beetle (*Leptinotarsa decemlineata*) was evaluated. It was revealed that allobetulin, ester of allobetulin and permetrin acid exhibit antifeedant activity and the latter possess insecticide activity.

INTRODUCTION Essential attention is spared to reduction of the human activity influence on surrounding environment at the last years. The growing rates of the resistance forming to organophosphorus, carbamats and organogalogenus insectoacaricides, including pyretroids, are noted now in populations of arthropods - agricultural pests, counting more than 500 species. Rotation of the insecticides is considered one of the most effective manner of overcoming resistance [1]. Antifeedants must occupy the worthy place in the system of the insectoacaricides rotation because of their specific influence on insects, and nontoxicity for mammals. Additionally, one of the main values of antifeedants is the lightness of their biodegradation. Because of this fact, there is continued searching for high active antifeedants from available sources for plants protection from pests, including potato beetle.

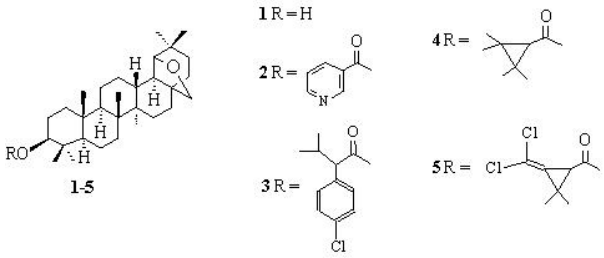
It is researching an antifeedant activity of triterpenoid in recently. So, betulinic acid, contents of which in bark of common birchs does not contain more than 0.025% [2], possesses by the antifeedant activity in respect to tobacco caterpillar *Spodoptera litura* F [3] moreover its derivates are more active, than acid itself. At the same time its bioprecursor betulin (the contents of which in bark of common birch reaches 30% [2]), is not active towards to larvae of the potato beetle [4], caterpillars of cotton armyworm [5], but its derivatives show significant activity.

The purpose of our work was an undertaking the antifeedant activity screening of triterpenoid of the oleanan group - allobetulin and its derivates towards larvae of the Colorado potato beetle (*Leptinotarsa decemlineata*). Allobetulin in small amount is kept in birch bark, however it is formed easy from betulin as a result of acid rearrangement [6]. About display of the antifeedant activity of the allobetulin and its derivatives did not communicate earlier.

MATERIALS AND METHODS Allobetulin 1 was obtained by the isomerization of betulin in CH2Cl2 in presence of p-TsOH [7]. 3β-O-nicotinat of allobetulin 2 was synthesized by interactions of allobetulin with chloranhydride of the nicotinic acid [8]. The esters 3-5 were received by the interaction of allobetulin 1 with chloranhydrides (2'-(4'-chlorfenyl)-3'-methyl)butanic, (2',2',3',3'-tetramethylcyclopropyl)carbonic and (2'-(b,b-dichlorvinyl)-3',3'-dimethylcyclopropancarbonic (the permetrinic) acids respectively in 75-89% yields [9] (Scheme 1).

Antifeedant activity of the compounds was

Scheme 1



assessed on larvae of the Colorado potato beetle L. decemlineata of begin IV age. Potato leaves were plunged on 5 sec in acetone solutions of the compounds 1-5. Then they were dried a little on air, weighted and placed in Petri dishes. The concentrations of the compounds 0.02%, 0.1%, 0.2% in three repeatings on each concentration were used. The control leaves were treated by the acetone only. Treated and control potatoes leaves were placed in Petri dishes and larvae of the Colorado potato beetle were placed between they. In a day the account was conducted: remained pieces of leaves were weighted and defined the weight of eaten food. The percentage of antifeedant activity was calculated as:

% of antifeedant activities = (O-C/100-C) 100 where: O- % remained treated food (in experience), C - % remained untreated food (in control) [10]. Significance of differences between the mean values was determined using Student's t-test.

RESULTS AND DISCUSSION Allobetulin 1 and 3β-O-permetrat of allobetulin 5 have shown antifeedant activity towards larvae of the Colorado potato beetle in all used concentrations (the table 1). 3β-O-Nicotinat of allobetulin 2 was active in two concentrations - 0.1% and 0.2%. The ester of allobetulin 3 has shown antifeedant activity only under the most high concentration (0.2%). Tetramethilciclopropylcarbonat of allobetulin 4 did not exhibit antifeedant activity. Allobetulin and 3β-*O*-permetrat of allobetulin had shown the most antifeedant activity from compounds investigated, (3β-O-(2'-(4'-chlorfenyl)-3'-methyl) butanoat of allobetulin had shown the least activity moreover only in the most high from used concentrations. The comparison of the compound structure with its antifeedant activity had shown that introduction to structure of the allobetulin molecule of chlorphenylbutyric and cyclopropancarbonic acids fragments negatively influences upon exhibiting of antifeedant activity. In the same time the derivative of allobetulin and nicotinic acid in small concentration did not exhibit any antifeedant action, but in the most concentration its activity was equal such as for allobetulin. Introduction to molecule of allobetulin of the permetrinyc fragment did not intensify its antifeedant activity, but this compound possessed of the insecticide activity (all larvae at moment of the account were in tremor condition). This is confirmed by literary data, according to which some pyrethroids including permetrine, possess of the antifeedant activity in respect of fall armyworm *Spodoptera frugiperda larvae* [11] and imago of the Mexican bean beetle E*pilachna variverstis* [12]. (Table 1)

Table 1. Antifeedant activity of allobetulin and its derivatives towards larvae of the Colorado potato beetle

Concentration of the compounds	Activity in % towards control for				
	1	**2**	**3**	**4**	**5**
0.02%	14.8 ± 1.6*	0	0	0	12.0 ± 1.3*
0.10%	16.7 ± 1.9*	10.3 ± 1.3*	0	0	13.7 ± 1.6*
0.20%	19.9 ± 2.2*	19.2 ± 2.3*	14.2 ± 1.5*	0	24.3 ± 2.4*

*significant difference as compared with control (P<0.05)

As it was already mentioned above, about antifeedant activities of allobetulin and its derivates earlier did not communicated: allobetulin and allobetulon did not shown the antifeedant activities in respect of bollworm *Heliothis zea* larvae [5]. In the same time, in our study allobetulin and some its derivatives show antifeedant activity towards larvae of the Colorado potato beetle *L. decemlineata* that confirms high specificity of antifeedants in its action.

CONCLUSION Thereby, triterpen derivatives of oleanan type 1, 2, 3 and 5 possess by the antifeedant action towards larvae of the Colorado potato beetle *L. decemlineata*, permetrat of allobetulin 5 combines antifeedant activity with insecticide action. Work is executed under financial support of RFBR (the project 05-03-32832).

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Insensitivity of Acetylcholinesterase in a Field Strain of the Fall Armyworm, *Spodoptera frugiperda* (J.E. Smith)

ABSTRACT Acetylcholinesterase (AChE) was purified from adult heads of the fall armyworm (*Spodoptera frugiperda*) by using a two-step procedure involving gel filtration and affinity chromatography. Both susceptible and field strains possessed two AChE isozymes, AChE-1 and AChE-2, with subunit molecular weights of 63.7 Kda and 66.1 kDa. The purified AChE had an apparent K_m value of 33.5 µM and a V_{max} of 7.07 µmol/min/mg protein in the susceptible strain. The apparent K_m and the V_{max} were 2.2 and 2.0-fold higher, respectively, in the field strain than in the susceptible strain. The purified AChE from the field strain was 17- to 345-fold less sensitive than that from the susceptible strain to inhibition by carbamates (carbaryl, eserine, methomyl, bendiocarb) and organophosphates (methyl paraoxon, paraoxon), insensitivity being highest toward carbaryl. The results support the notion that insensitive AChE played an important role in the insecticide resistance observed in the field strain.

INTRODUCTION The fall armyworm, *Spodoptera frugiperda*, is a serious lepidopterous pest of several important crops such as corn, cotton, peanuts, and soybeans. Recently, we reported that a strain of fall armyworm collected from Citra, Florida, showed high resistance to carbaryl (562-fold) and methyl parathion (35-fold) (1). The purpose of this research was to purify acetylcholinesterase (AChE) from adult heads of both field and susceptible strains and characterize the enzyme biochemically in order to verify if insensitive AChE was involved in the resistance.

MATERIALS AND METHODS Insects The field strain was collected from a corn field in the Plant Science Research Center, University of Florida in Citra, Florida, during the spring of 2002. The susceptible strain, which originated from the USDA in Tifton, Georgia, has been maintained in the laboratory without exposure to insecticides. Both strains were maintained on an artificial diet in environmental chambers at 25ºC with a 16:8 L:D photoperiod as described previously (2).

Enzyme preparation: Groups of 500 heads were removed from 7- to 10-day-old adults and homogenized in 25 ml of ice-cold 0.05 M sodium phosphate buffer, pH 7.5, containing 0.5% (v/v) Triton X-100, in a motor-driven tissue grinder for 30 sec, followed by differential centrifugation to obtain the soluble fraction (supernatant).

Purification of AchE: AChE was purified from the soluble fraction with a two-step procedure involving gel filtration and affinity chromatography. The affinity column used in this study was prepared according to the method of Pasteur et al. (3). To purify AChE, the soluble fraction prepared as mentioned above was first applied to a Sephadex G-200 column previously equilibrated with 0.05 M sodium phosphate buffer (pH 7.0) containing 0.1% (v/v) Triton X-100 and 0.05 M NaCl (designated as Buffer A). The column was eluted with the same buffer until no further protein was detected. Fractions of 1 ml were collected and analyzed for AChE activity.

The pooled AChE sample was applied to a procainamide affinity column previously equipped with Buffer A. The column was washed extensively with Buffer A until no further protein was detected. The bound AChE was then eluted with Buffer A containing 0.1 M procainamide. Fractions containing AChE activity was pooled and dialyzed against 0.05 M sodium phosphate buffer (pH 7.0) containing 0.1% (v/v) Triton X-100 overnight. Purified AChE was then concentrated by ultrafiltration on an Amicon Diaflo PM-10 membrane. Protein concentrations were determined by the method of Peterson (4) using bovine serum albumin as standard.

Enzyme Assays: AChE activity was measured with acetylthiocholine as substrate as described by Ellman *et al*. (5). In inhibition studies, inhibitors were dissolved in methyl cellosolve and then diluted with 0.1 M sodium phosphate buffer, pH 8.0. Biomolecular rate constant (K_i) for inhibition of AChE by insecticides was determined by the method of Aldridge (6).

Electrophoresis: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli (7). The procedure of nondenaturing PAGE was similar to the SDS-PAGE except that no SDS was used and it was performed at 4oC. Proteins were detected in gels with silver stain (Bio-Rad). The gel was also stained for AChE activity by the method of Karnovsky and Roots (8).

Kinetic Study: The Michaelis constant (K_m) and maximum velocity (V_{max}) for purified AChE were determined by Lineweaver-Burk plot using acetylthiocholine as substrate.

RESULTS AND DISCUSSION The results of the purification of AChE from both field and susceptible strains are summarized in Table 1. The gel filtration (Sephadex G-200) yielded 80% and 93% of the initial enzyme activity and purifications of 2.1- and 2.0-fold from the susceptible and the field strain, respectively. The affinity chromatography step yielded 23% and 31% of the initial enzyme activity and purifications of 57- and 56-fold from the susceptible and the field strain, respectively. The specific activity of AChE was 15.38 and 19.18 µmol/min/mg protein in the susceptible and the field strain, respectively.

Table 1. Purification of Acetylcholinesterase from Heads of Fall Armyworm Adults

Purification	Strain	Yield (%)	Specific activity (µmol/min/mg protein)	Purification factor
Soluble fraction	Susceptible	100	0.27	1
	Field	100	0.34	1.0
Sephadex G-200	Susceptible	80	0.56	2.1
	Field	93	0.69	2.0
Affinity Column	Susceptible	23	15.38	57.0
	Field	31	19.18	56.4

Analysis of the purified AChE by nondenaturing PAGE followed by AChE staining revealed two isozymes, AChE-1 (R_f = 0.037) and AChE-2 (R_f = 0.15), with AChE-1 being the major one (Figure 1). SDS-PAGE of the purified AChE preparation showed two protein bands with molecular weights of 63.7 kDa and 66.1 kDa, the former being a minor subunit (Figure 2, Table 1). In all instances, there was no difference in electrophoretic patterns between the susceptible and the field strain. Kinetic studies (Table 2) showed that the apparent K_m and the V_{max} were 2.2 and 2.0-fold higher, respectively, in the field strain than in the susceptible strain.

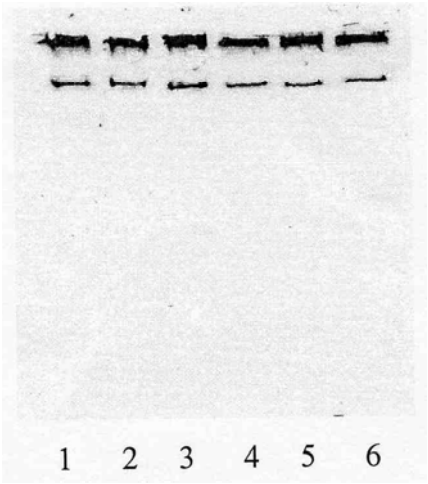

Fig. 1. Non-denaturing polyacrylamide gel electrophoresis of purified AChE from heads of fall armyworm adults. The gel was stained for AChE activity using acetylthiocholine as a substrate. Lanes 1-3, field strain; lanes 4-6, susceptible strain.

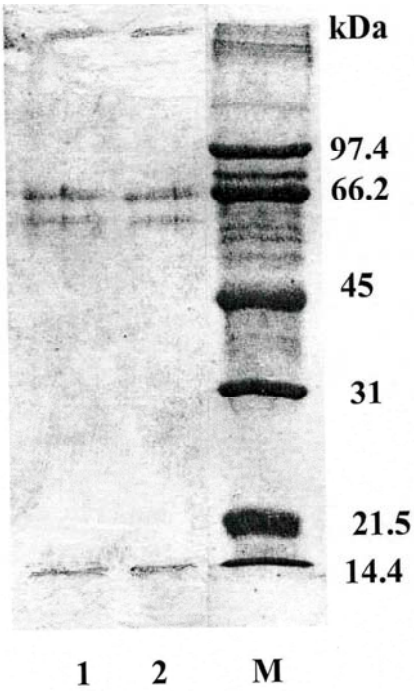

Fig. 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of purified AChE from heads of fall armyworm adults. Lane 1, field strain; lane 2, susceptible strain.

Table 2. Biochemical Characteristics of Purified Acetylcholinesterase from Heads of Fall Armyworm Adults

Strain	Km (µM)	Vmax	Subunit Mr (kDa)
Susceptible	33.5 ± 7.5	7.07 ± 1.5	63.7
			66.1
Field	74.5 ± 3.5	14.4 ± 2.5	63.7
			66.1

Data in Table 3 show that the purified AChE from the field strain was 17- to 345-fold less sensitive than that from the susceptible strain to inhibition by carbamates (carbaryl, eserine, methomyl, bendiocarb) and organophosphates (methyl paraoxon, paraoxon), insensitivity being highest toward carbaryl.

Table 3. Inhibition of Purified Acetylcholinesterase by Carbamate and Organophosphorus Insecticides in Fall Armyworms

Insecticide	Ki ($M^{-1}min^{-1}$)		
	Susceptible strain (S)	Field strain (R)	S/R
Carbamate			
Carbaryl	5.32×10^4	1.54×10^2	345
Eserine	1.28×10^6	6.30×10^4	20
Methomyl	2.31×10^5	1.39×10^4	17
Bendiocarb	$3.65\ 10^5$	2.48×10^3	147
Organophosphate			
Methyl paraoxon	6.30×10^5	2.24×10^3	281
Paraoxon	4.08×10^5	1.26×10^4	32

Our results support the notion that insensitive AChE played an important role in the insecticide resistance observed in the field strain. Insensitive AChE as a resistance mechanism to carbamates and organophosphates has been reported in numerous insect species including *S. littoralis*, *S. exigua* and *S. frugiperda* (1,9,10). Insensitive AChE has been shown to be due to point mutations (11).

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Fungicide Resistance

Baseline Sensitivity of Cucurbit Powdery Mildew (*Podosphaera xanthii*) to the Fungicide Quinoxyfen in New York State

INTRODUCTION Managing fungicide resistance is an essential component of managing powdery mildew in cucurbits. Powdery mildew is the most common foliar disease of cucurbits, occurring every year throughout the US. White powdery fungal growth develops on both surfaces of leaves and on stems. Resistant varieties are available, but application of fungicides continues to be the main management practice. It is critical to control powdery mildew on the lower surface, where this disease develops best. This is best accomplished with fungicides that are systemic, translaminar, or highly volatile. Unfortunately, products developed with these properties have had a high risk of developing resistance due to their single-site mode of action. The cucurbit powdery mildew fungus has demonstrated a high potential for developing resistance. It has developed resistance to the main fungicide classes at-risk for resistance currently registered in the US for its management: benzimidazoles, demethylation inhibitors (DMIs), and quinone outside inhibitors (QoI, aka strobilurins).

Quinoxyfen is a novel active ingredient in the fungicide Quintec (Dow Agro-Sciences, LLC) and is classified as the lone chemical in FRAC group 13. Quintec does not have a Section 3 US registration for powdery mildew on cucurbits; it was granted exemptions (crisis and emergency) to allow this use on non-edible peel cucurbits in New York for the 2004 and 2005 growing seasons and an exemption has been requested for 2006. Quintec has a unique mode of action, affecting G-proteins in early cell signaling. There is no cross resistance with other fungicides, and although it is not systemic, it moves into leaf surfaces and redistributes through a vapor phase.

The goal of this study was to provide the first step in fungicide resistance management by determining the baseline sensitivity of *Podosphaera xanthi*i to quinoxyfen, before its widespread use creates selection pressure for resistant strains. This information will be used in the future as a benchmark to determine whether fungicide use has resulted in a decline in

pathogen sensitivity. This would enable detecting shifts toward pathogen insensitivity before it results in control failure in the field.

MATERIALS AND METHODS: Isolates of *Podosphaera xanthii* were collected from pumpkin research fields at LIHREC in Riverhead, NY, during the 2004 and 2005 growing seasons. Fungicide efficacy experiments conducted in these fields included treatments with Quintec: it was tested alone in 2004 and in combination fungicide programs in both years. The 2004 isolates used in the study were selected from a larger group of isolates based on their sensitivity to quinone-outside-inhibiting (QoI, FRAC group 11) and demethylation inhibiting (DMI, FRAC group 3) fungicides.

A leaf disk bioassay was used to determine the baseline sensitivity of *Podosphaera xanthii* to quinoxyfen (McGrath et al 1996). Squash seedlings at the first true leaf stage were sprayed with six concentrations of quinoxyfen ranging from 0 to 10 ppm in 2004 and three concentrations ranging from 0 to 5 ppm in 2005 using a DeVilbiss bottle attached to a compressed air source (20 psi). Treated plants were allowed to dry overnight, then disks were cut from the cotyledons using a #9 cork borer (9 mm diameter). Three disks of each treatment were placed on water agar in divided petri plates (three treatments per plate plus a nontreated control). Disks were inoculated by transferring 10-20 conidia to each disk center. In each experiment, treated disks were inoculated with each mildew isolate, then plates were incubated for approximately 10 days at 24°C, at which time the control treatment showed good growth, with sporulating mildew covering an average of 30-50% of leaf disk area. For each fungicide concentration, growth of the mildew was considered to have occurred if sporulation was observed on 2 out of 3 disks. The percent leaf disk area colonized by sporulating mildew was recorded for each disk and averaged for each treatment.

RESULTS AND DISCUSSION Quinoxyfen baseline sensitivity varied among powdery mildew isolates. In 2004, all isolates tolerated 0.01 ppm, exhibiting some decrease in severity on these disks compared to 0 ppm (Table 1). Forty percent of the 15 isolates collected in 2004 were able to tolerate and grow at 1 ppm quinoxyfen and 27% tolerated 10 ppm. In 2005, 80% of the 20 isolates tested grew at 1 ppm and 50% tolerated 5 ppm quinoxyfen (Table 2). There appears to be a possible shift in sensitivity from 2004 to 2005; however, in 2004, isolates were collected at the start of powdery mildew development as well as after fungicides were applied, whereas in 2005 all isolates were collected late in the season after all fungicide applications for the efficacy experiment. Comparing

Table 1: Sensitivity to fungicides of *Podosphaera xanthii* isolates collected in 2004 from pumpkin in New York.

Isolate	Date Collected	Quintec Applied	Quintec Sensitivity	Nova Sensitivity	Flint Sensitivity
4f	8-Aug	No	VS	S	R
15j	22-Aug	No	VS	MR	R
29c	8-Aug	No	S	MS	S
2g	22-Aug	Yes	S	MS	S
16g	22-Aug	Yes	S	S	R
1f	22-Aug	Yes	S	MR	R
29w	22-Sep	No	S	MR	R
1x	22-Sep	Yes	S	MS	MR
29b	8-Aug	No	MS	S	S
6f	8-Aug	No	MS	S	S
4d	8-Aug	No	MS	MS	S
16f	22-Aug	Yes	MS	S	S
9z	22-Sep	No	MS	MR	S
6x	22-Sep	No	MS	MR	R
31z	22-Sep	Yes	MS	MS	MR

VS Very Sensitive: growth at only the lowest rate of Quintec tested (10 ppb).

S Sensitive: growth up to 1,000 ppb Quintec with no growth at 10,000 ppb, no growth at 20 ppm Nova, or no growth at 50 ppm Flint.

MS Moderately Sensitive: growth at all rates of Quintec tested (0, 10, 100, 1,000, 10,000 ppb) or limited growth at 20 ppm Nova.

MR Moderately Resistant: good growth at 50 ppm Nova or limited growth at 50 ppm Flint.

R Resistant: good growth at 50 ppm Flint.

Table 2: Sensitivity to fungicides of *Podosphaera xanthii* isolates collected in 2005 from pumpkin in New York.

Isolate	Date Collected	Quintec Applied	Quintec Sensitivity	Nova Sensitivity	Flint Sensitivity
R4 T6 A	20-Sep	No	VS	S	MR
R2 T1 B	3-Oct	No	VS	S	MR
R1 T1 B	3-Oct	No	VS	MS	R
R3 T2 A	20-Sep	Yes	S	MR	R
R2 T4 A	20-Sep	Yes	S	MR	R
R1 T5 A	20-Sep	No	S	MS	R
R2 T5 A	20-Sep	No	S	S	MR
R2 T6 A	20-Sep	No	S	MR	R
R3 T5 A	20-Sep	No	S	MS	R
R3 T5 B	3-Oct	No	S	S	R
R1 T5 B	3-Oct	No	S	MS	R
R3 T6 B	3-Oct	No	S	S	MR
R4 T1 A	20-Sep	No	MS	MS	R
R2 T2 A	20-Sep	Yes	MS	MS	MR
R1 T2 A	20-Sep	Yes	MS	S	R
R2 T2 A	20-Sep	Yes	MS	MS	MR
R4 T4 A	20-Sep	Yes	MS	MS	MR
R1 T2 B	3-Oct	Yes	MS	S	R
R1 T4 B	3-Oct	Yes	MS	S	R
R1 T6 B	3-Oct	No	MS	MS	R

VS Very Sensitive: growth at only the lowest rate of Quintec tested (10 ppb).

S Sensitive: growth at all rates of Quintec tested (0, 10, 100, 1,000, 10,000 ppb), no growth at 20 ppm Nova, or no growth at 50 ppm Flint.

MS Moderately Sensitive: growth up to 1,000 ppb Quintec with no growth at 10,000 ppb or limited growth at 20 ppm Nova.

MR Moderately Resistant: good growth at 50 ppm Nova or limited growth at 50 ppm Flint.

R Resistant: good growth at 50 ppm Flint.

isolates collected at the end of the season (20 Sep or later) reveals greater similarity between the 2 years. For the five isolates collected after fungicide treatments were completed in 2004, 100% tolerated 1 ppm and 60% tolerated 10 ppm. There appears to be an association between Quintec treatment and quinoxyfen sensitivity for the 2005 isolates but not the 2004 isolates. Isolates collected in 2005 from research plots where Quintec was applied tended to tolerate higher concentrations of quinoxyfen. No relationship was seen between quinoxyfen sensitivity and QoI sensitivity or DMI sensitivity in 2004 (Table 1) or in 2005 (Table 2).

Field experiments carried out in 2004 and 2005 have demonstrated that Quintec is providing excellent and consistent control of powdery mildew in multiple states and on multiple cucurbit crops. Quintec was the best product evaluated in fungicide efficacy trials in NY and North Carolina. In 2004 it provided 99% and 98% control on the upper and lower leaf surfaces, respectively, when applied at 4 fl oz/A to pumpkin in NY (McGrath, 2005) and 100% overall control in acorn-type winter squash at both 6 and 12 oz/A in NC (Holmes, 2005). In 2005, Quintec at 6 oz/A (82% control) also performed at the same statistical level as Pristine 18.5 oz/A (92%), which was the most effective treatment in the trial conducted in California on muskmelon (Turini, 2006). Field dose for Quintec applied at 4 fl oz/A and 50 gpa is 156 ppm quinoxyfen.

Variation in tolerance of the 35 *P. xanthii* isolates to quinoxyfen demonstrates that there may be a risk of resistance development in the pathogen population to this fungicide over time. The range in sensitivity illustrates the potential of the population to shift towards insensitivity. No relationship was found between quinoxyfen sensitivity and whether Quintec was applied to the research plot prior to isolate collection for the 2004 isolates, and there was no evident relationship between when an isolate was collected and its sensitivity to quinoxyfen; which suggests that risk of fungicide resistance development, at least on a short-term time scale, is minimal. However, association between Quintec treatment and quinoxyfen sensitivity found for the 2005 isolates suggests pathogen response to selection pressure from new chemistry can occur during a growing season.

Lack of relationship between quinoxyfen sensitivity and QoI sensitivity or DMI sensitivity is an important finding. In contrast, sensitivity to QoI and DMI fungicides appears to be related in *P. xanthii.* For example, in 2002, the first year that QoI resistance was detected in the US, 80% of QoI-resistant isolates tested were also moderately resistant to DMIs. Consequently, using either type of fungicide could select for strains resistant to both fungicide groups. A common strategy for managing resistance is alternating among fungicides in different chemical classes. Quinoxyfen is a valuable fungicide to use in rotation because it should help slow resistance development in *P. xanthii* populations to other high-risk fungicides. Additionally, Quintec has proven to be highly effective, an important characteristic for a fungicide used in an alternation program. The more effective a fungicide, the smaller the pathogen population that remains after treatment, and thus the lower the chance that strains will be present with resistance to another fungicide used in the rotation.

Resistance to fungicides is an ongoing problem in plant disease control. Continually monitoring the sensitivity of *P. xanthii* to quinoxyfen over time is expected to reveal shifts in the pathogen's sensitivity to this fungicide before resistance develops to the point of control failure. Ability to detect these changes will provide the opportunity to make changes in fungicide recommendations, which should extend the useful life of the fungicide. A more detailed report is posted at:

http://www.hort.cornell.edu/department/facilities/lihrec/vegpath/index.html#QuinBase

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Other Resistance

Induced Systemic Resistance of *Pseudomonas fluorescens* Against Root-Knot Nematode, *Meloidogyne incognita* in Tomato

INTRODUCTION Root-knot nematodes (*Meloidogyne* spp.) are major pathogens of tomato throughout the world, impacting both the quantity and quality of marketable yields. The yield loss due to the root-knot nematode in tomato is estimated to be up to 40 percent (Dasgupta, 1998). Currently biological control agents are being utilized to control plant parasitic nematodes Plant growth promoting rhizobacterium *Pseudomonas fluorescens* helps in boosting the plant growth and vigor by different mechanisms which may elicit a plant's normal defenses and improve its health and resistance to the plant parasitic nematodes (Rodriguez-Kabana *et al*, 1965). The bacterium achieves this mainly by bio-stimulation, bio-control, bio-fertilization and bio-remediation. Induced systemic resistance (ISR) of plants against pathogens is a widespread phenomenon that has been intensively investigated with respect to the underlying signaling pathways as well as to its potential use in plant protection. Elicited by a local infection, plants respond with a salicylic-dependent signaling cascade that leads to the systemic expression of a broad spectrum and long-lasting disease resistance that is efficient against nematodes. The bio-efficacy of *P. fluorescens* against root-knot nematode in tomato was tested under green house conditions.

MATERIALS AND METHODS *Evaluation of talc formulations of P. flourescens isolates on M. incognita under green house conditions*: 25 days old tomato seedlings of variety PKM-1 were planted in pots filled with 10 kg of steam sterilized soil. One week after establishment of the seedlings, talc based formulation of the promising *P. flourescens* isolates Pft 18, Pft 20 and Pft 25 and standard Pf 1 and carbofuran were applied to the soil @2.5 gm/pot. Six replications were maintained. The treatments were compared with untreated control.

Enzyme extraction: Leaves were collected from bacterized and control tomato plants 45 days after planting. The samples were immediately homogenized with liquid nitrogen. One g of powdered sample was homogenized with 2 ml of 0.1 M sodium phosphate buffer (pH 7.0) at 4 º C. The homogenate was centrifuged for 20 min at 10000 rpm. Protein extract prepared from leaves and roots were used for estimation of peroxidase (PO), polyphenol oxidase (PPO) and phenylalanine ammonia - lyase (PAL).

Spectrophotometric assay: Assay of peroxidise (PO) Peroxidase activity was assayed spectrophotometrically (Hartee, 1955). The reaction mixture consisted of 1.5 ml of 0.05 M pyrogallol, 0.5 ml of the enzyme extract and 0.5 ml of 1 percent H_2O_2. The reaction mixture was incubated at room temperature (28 ± 1 ° C). The change in absorbance was recorded at 30 sec. interval for 3 min. The boiled enzyme preparation served as blank. The enzyme activity was expressed as change in the absorbance at 420 ηM min $^{-1}$ g^{-1} on fresh weight basis (Hammerschmidt *et al*., 1982).

Assay of polyphenol oxidase (PPO): One g of leaf was used for polyphenol oxidase estimation. The reaction mixture consisted of 1.5 ml of 0.1 M sodium phosphate buffer (pH 6.5) and 200 µl of the enzyme extract. To start the reaction, 200 µl of 0.01 M catechol was added and the activity was expressed as changes in absorbance at 495 ηM min $^{-1}$ g^{-1} fresh weight of tissue.

Assay of L -phenylalanine ammonia lyase (PAL) The phenylalanine ammonia lyase assay was conducted as per the method described by Ross and Sederoff (1992). The assay mixture containing 100 µl of enzyme, 500 µl of 50 mM Tris HCl (pH 8.8) and 600 µl of mM L -phenylalanine ammonia lyase was incubated for 60 min. The reaction was arrested by adding 2 N HCl. Later 1.5 ml of toluene fraction containing trans - cinnamic acid was separated. The toluene phase was measured against the blank of toluene. Standard curve was drawn with graded amounts of cinnamic acid in toluene as described earlier. The enzyme activity was expressed as ηM of cinnamic acid at 290 ηM min $^{-1}$ g^{-1} of fresh tissue.

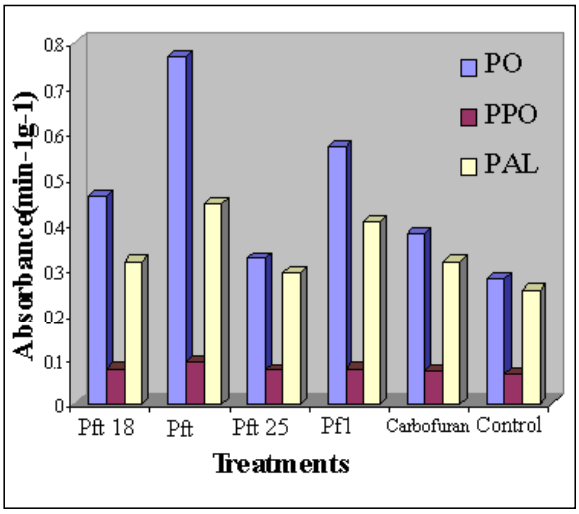
Nematode population Soil: Soil samples of 200 cm^3 were collected at random in each pot and the nematode population was estimated. The soil was processed by following Cobb's sieving and decanting method (Cobb, 1918) and Modified Baermann funnel technique (Schindler, 1961).

Root: Root samples were collected @ 5 g of roots per plant. The root samples were washed and stained with acid fuchsin-lactophenol for examining the females and egg masses under stereoscopic binocular microscope.

RESULTS AND DISCUSSION Significant increase in the activity of peroxidase was observed in the leaves of the tomato plants treated with the *P. fluorescens* isolate Pft 20, which accounted for 181.02 per cent increase over control. This was followed by Pf 1 with 151.45 per cent increase over control. There was an increase in the activity of polyphenol oxidase in all the *P. fluorescens* isolates treated plants and the highest activity was recorded in the Pft 20 treated plants with 27.69 which

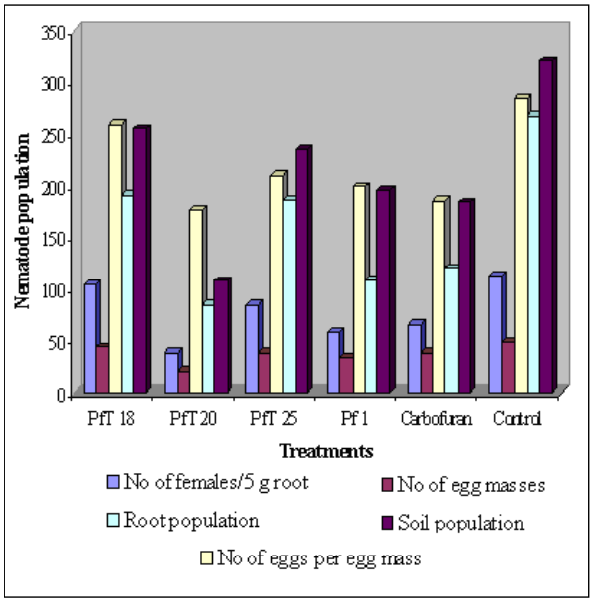
accounted for 39.13 per cent increase over control. There was relatively a lesser difference in the activity of PPO observed in the other *P. fluorescens* isolates treated tomato plants. Increase in the activity of phenylalanine ammonia lyase was observed in all the *P. fluorescens* treated tomato plants. However Pft 20 (0.443) and Pf 1 (0.434) treated plants recorded significant increase among the other isolates (Fig.1).

Figure 1. Efficacy of talc formulations of *P. fluorescens* isolates on enzyme activity of tomato leaves infested with *M. incognita*



Significant reduction in the soil and root population of nematodes observed in the Pft 20 treated tomato plants might be due to the production of toxic metabolites like antibiotics and cyanide (Voisand *et al.*, 1989), antibiotics like pyrolnitrin, pyoluteorine and 2,4-diacetyl phluroglucinol (Bangara and Thomashow, 1996) and hydrogen cyanide which degrade the toxin produced by the pathogen (Defafo *et al*., 1990) (Fig.2).

Figure 2. Efficacy of talc formulations of *P. fluorescens* isolates on *M. incognita* infestation in tomato under green house conditions



High activity of the oxidative enzymes was observed in the Pft 20 treated tomato plants. *P. fluorescens* isolate Pft 20 significantly reduced the infestation of root-knot nematode by altering the host physiology in terms of high accumulation of peroxidase, polyphenol oxidase and phenylalanine ammonia lyase. The increase in plant growth and reduction in nematode observed in the present study may be due to induced systemic resistance or multiple potential defense mechanisms (Wei *et al.*, 1996; Jonathan *et al*., 2000; Anita and Rajendran, 2002).

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Research in Resistance Management

Refugium Area as an Important Tool for B.t. Cotton to Delay Resistance and Improve Host/Prey Availability for Natural Enemies Specially in the Monocultures Agro-ecosystem

INTRODUCTION There is a continuing need to increase food production as the world population is expected to be more than 6 billion by 2050. The cost for achieving production has become very high because of the need to control insect pests that cause an estimated loss of 8-10$ billion annually. The difficulties experienced in controlling insect pests over the past 45 years have been largely due to the wide, unwise use of pesticides. Indiscriminate use of insecticides has resulted in the development of resistance in insects and the significant decrease of natural enemies.

Insect resistance to insecticides 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 cited from B. Fakrudin *et al* 2004).

In Egypt, cotton lost 50% of its cotton yield due to the cotton leaf worm (CLW) Spodoptera littoralis resistance to Toxaphene countrywide in 1961. The product was applied 4-5 times on the same pest in the same season without any rotation program .For that reason, Egypt is considered one of the first countries applied rotation program on cotton since 1979(Temerak 2002).In 1971, 3000 of baflo chronic toxicity were appeared in Koutour KaferSheik Governorate due to the airplane application of Leptophos (Phosvil)on cotton. In 1975/76; MOA imported Gusathion methyl which arrived Egypt as gusathion ethyl. The last showed severe toxicity to cotton labors. Also. the same was happened with Gelecron to kill CLW eggmasses (Temerak 2006 under publication).

In India, farmers even apply 36 to 40 rounds of pesticides to the cotton crop of a duration of 150-180 days in a single season i.e., one spray for every five days (Banerjee *et al*., 2000). However , Fakrudin et al 2003 reported one spray every 2-3 days interval although the recommended spray is 8-10 sprays in India. The last representing a very high environmental pollution. Applying different class of chemicals that has different mode of actions delay resistance, decrease number of sprays and better efficacy (Temerak 2003a).

Bio-pesticides such as Bt (*Bacillus thuringiensis*) products are widely regarded as being the least harmful to natural enemies. Because of its selectivity and environmental safety, usage of Bt is increasing, particularly in IPM programs. Foliar application of Bt breaks down quickly under field conditions due to UV sensitivity and rainfall. (Gopalaswamy *et al* 2003) Also, it does not have stable results from year to another. Plant incorporated protectants (PIP) utilizing B.t. offered the best solution to elongate the bio-residual activity of this group in the field.

The primary target pest of this technology in India and several other countries is the cotton bollworm (CBW), *Helicoverpa armigera* (Hubner) which causes economic losses up to about 250 billion in India (cited from Fakrudin et al 2003).

The introduction of Bt transgenic crops is an important addition to the existing components of Integrated Pest Management. There is a considerable increase in global area under transgenic crops from 1.7 million hectares in 1996 to 52.6 million hectares in 2001, in which the share of Bt crops was 15% of the total area (James 2001). The economic advantage gained during 1999 by Bt cotton alone has been estimated to be $213 million in the USA. Cultivation of transgenic crops has led to a reduction in pesticide use and significant increase in yield (Cannon 2000). Of the $8.1 billion (US dollars) spent annually on insecticides worldwide, it was estimated that nearly $2.7 billion could be substituted with Bt biotechnology applications (Krattiger 1997.cited from Gopalaswamy *et al* 2003)

The technology is perceived to be effective and eco-friendly for reducing the considerable amount used from conventional insecticides. When resistance to insecticides had 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 cited from B. Fakrudin etal 2004). Furthermore, the problems of pest management in B.t. cotton have been accompanied by the development of resistance to key CBW & Cotton leaf worm (CLW) pests (*H. armigera*, Kranthi *et al*. 2000; *Pectinophora gossypiella* Tabashnik *et al*. 2000a,b; *S. exigua* Moar *et al.* 1995; S. littorals, Muller-Cohn *et al.* 1996).

Moreover, the situation of beneficial arthropods is being disturbed because they are density dependant and Bt cotton areas do not harbour enough availability of host/prey for beneficials. No considerable work was done to quantify the effect of B.t. cotton areas on the abundance of natural enemies or beneficial arthropods.

Management of refugia as areas percentages versus free-toxin cotton areas and safety type of insecticides, will be play a significant role to dilute or decrease the two problems of resistance and conservation of beneficial arthropods

A -Refugia importance for natural enemies or beneficials

A.1 *-Positioning and size of refuge areas, and its effect on natural enemies*: No considerable work was done to find out the best size area of refugia and its relation to the abundance of beneficial arthropods. Refuge area as 20% treated with conventional insecticides (according to Matten 2000) may be not enough to serve and conserve natural enemies from insects, mites, true spiders and wild arthropods.

Refuge area as more than 20% may be recommended. May be 4% untreated non-B.t is better than 20% treated with conventional insecticides. Also, may be 20% as refuge areas is enough when use new safe insecticides instead of conventional insecticides

Moreover, mass release of some additional beneficials e.g. Trichogramma or coccinellids may be incorporated and needed especially in mono-crop systems that use only 20% refuge areas. No considerable published work was seen until now to study such effect. Furthermore, information on the effect of beneficial enemies (that currently presented or released in the agroecosystem) on B.t. resistant insects are urgently needed

A.2*- The need of new molecules to be applied in the refuge areas:* Therefore, care must be taken to ensure that refuges, particularly those sprayed with insecticides, produce adequate numbers of susceptible insects. Models and experimental data showed that separate but adjacent refuges might be superior to other strategies for insects, which can move between plants in their larval stage (Shelton *et al*. 2002).

The non-Bt.cotton areas must use highly safe insecticides in rotation to conserve natural enemies and also, not to produce resistant from using them. This can be done through alternate different class of relatively safe chemicals that are different in their mode of actions through IPM program (Temerak 2002).

One of the most important strategies suggested by several entomologists across the globe to manage and delay insecticide resistance is use of new molecules having novel modes of action. Recently some of the new molecules have entered the pesticide market, which can play a major role in resistant pest management (Viiaykumer *et al* 2004a)

A.2.1 - *Using safe new insecticides to control sucking in non- B.t. cotton:* With the introduction of novel eco-friendly insecticides in the past 4-5 years, cotton pest management now appears to be very promising. The chloronicotinyls (imidacloprid, acetamiprid and thiomethoxam) and the insect growth regulator diafenthiuron are selectively more effective on the sucking pests and less toxic to beneficial insects as compared to all the conventional insecticides . (Kranthi *et al* 2004).

A.2.2- *Using safe new insecticides to control CBW/CLW in non-Bt. Cotton:* More interestingly, apart from the introduction of Cry toxins in the form of transgenic technology, chemicals such as spinosad, indoxacarb, emamectin benzoate, novaluron and lufenuron ensure effective control of *H. armigera* while being less toxic to beneficial insects in the cotton ecosystem. Kranthi et al 2004

A.3- *Negative cross resistance of spinosad & non-Bt cotton of Spodoptera:* Temerak 2003b indicated that spinosad was not being affected by the existing CLW resistance to conventional insecticides. New molecules with new mode of action are generally costly in short view , but the added value of decreasing or not being affected by the existing resistance mechanism to conventional may be considered not costly. From the economic point of view, intelligent farmers do not compare cost /ha based on single product cost but they are compared the whole package of the season . Such package may achieve less no. of sprays , better efficacy of resistant insects and good yield.

B-Refuge importance to reduce or delay resistance

B.1*-Non-refuge:* Mono-cultivation of Bt transgenic crops is likely to select intensely for resistance because pests will be exposed to Bt even when they are not causing economic damage (Mallet & Porter 1992).

B.2 - *Examples of possible resistance:* 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 cited from B. Fakrudin *et al* 2004).

Helicoverpa armigera is capable of developing resistance to Cry1Ac in 7 to 8 generations (Kranthi *et al.* 2000). Highly mobile polyphagous pests such as *Helicoverpa* may develop resistance to Bt on one transgenic crop and then disperse, nullifying the effectiveness of a wide range of Bt transgenic crops expressing the same or similar Cry proteins. Pests with resistance to CryIA proteins in transgenic plants may also display significant resistance to Bt biopesticides.

Field collected pink bollworm , *Pectinophora gossypiella* quickly evolved resistance to Cry1Ac under laboratory selection (Patin *et al.* 1999, Simmons *et al.* 1998, Tabashnik *et al*. 2000b). *Pectinophora gossypiella* selected with Cry1Ac protoxin developed 300-fold resistance to Cry1Ac protoxin, and high levels of cross-resistance to Cry1Aa and Cry1Ab protoxin, and low levels of resistance for Cry1Bb protoxin (Tabashnik *et al*. 2000a). Three selections with Cry1Ac in artificial diet increased resistance of pink bollworm to >100-fold relative to a susceptible strain (Liu *et al.* 2001).

In general, Spodoptera spp. larvae are not very susceptible to the Cry toxins (Strizhov *et al.* 1996). However, Cry1C toxin had been reported to be toxic against S. exigua (Visser *et al* .1988) and *Spodoptera littoralis* (Van Rie *et al.* 1990). Selection to Cry1Ca caused 850-fold resistance to Cry1Ca and cross-resistance to Cry1Ab, Cry9C, and Cry2A, as well as to a recombinant Cry1E-Cry1C fusion protein in S. exigua (Moar *et al.* 1995), while in S. littoralis, 500-fold resistance to Cry1Ca and partial cross-resistance to Cry1D, Cry1E, and Cry1Ab has been recorded (Muller-Cohn *et al.* 1996).

This may undervalue the benefits of Bt in IPM approaches (Waage 1996), as it runs the risk of breakdown of resistance in the long-term.

B.3*-Toxin-free areas (refuge):* The primary strategy for delaying insect resistance to transgenic crops under large monocultures is to provide refuges of non-Bt crop plants that serve to maintain Bt-susceptible insects in the population. This potentially delays the development of insect resistance to Bt crops by providing susceptible insects for mating with resistant insects (Liu *et al.* 1999). (Roush 1997b ; Vijaykumar *et al* 2004a ; Vijaykumar *et al* 2005a)

Currently suggested refuge strategy is very relevant and practicable under situations of monocropping in countries like US, Australia, China etc. But in countries like India, multiple cropping systems having a strong mix of several alternative hosts for bollworms such as chickpea, pigeonpea, sunflower, sorghum, maize and chillies which are grown both as sole crop and as mixed crop in the same area and in the same season as that of cotton which occupies only 5% of the total cultivable area and these alternative hosts are known to support high susceptible populations of the pest, thereby serving as natural refugia (Vijaykumar *et al* 2004 b)

B.4 *- Refuge areas from non-cotton crops*: On the other hand, farmers may be reluctant to sacrifice a large number of refuge plants to insects just to maintain susceptible alleles. In China, *H. armigera* naturally possesses a vast refuge as it can feed on corn, soybean, peanut, and many other crops. Studies that have monitored the sensitivity of *H. armigera* field populations to Bt insecticidal protein Cry1Ac from 1998 to 2000 indicated that *H. armigera* is still susceptible to Cry1Ac protein (Wu *et al.* 2002b).

Although development of *H. armigera* on Bt cotton was much slower than on common cotton, there was a high probability of mating between populations from Bt cotton and other sources due to scattered emergence pattern of *H. armigera* adults and overlap of second and third generations. Thus, in a cotton, soybean, and peanut mix system, non-cotton crops provided a natural refuge (Wu *et al.* 2002a). As indicated earlier in the diverse cropping systems of the tropics (Sharma *et al.* 2001), where the insects have several alternative and wild hosts, there may not be any need to grow the refuge crops (Gopalaswamy *et al* 2003).

B.5 *-Positioning and size of refuge areas:* Bt cotton is planted shall be fully surrounded by a belt of land called 'refuge' in which the same non-Bt cotton shall be grown." The size of the refuge belt should be such as to take at least five rows of non-Bt cotton or shall be 20% of the total sown area, whichever is more. The refuge strategy in time and space would serve to decrease selection pressure to the Bt toxin, as intercrossing between the bollworms from Bt cotton and non Bt refuge will dilute the resistance to Bt gene product (Vijaykumar *et a*l 2004 b).

There is also a debate regarding the spatial design of the refuge system (separate/seed-mixture) to be adapted. Roush (1997a) pointed out that seed mixes can actually promote resistance development for insects that move from plant to plant.

Many researchers are concerned that cotton bollworm might become resistant to Bt toxins without a crop management strategy adapted to the farming systems. Considering the results obtained in resistance mechanism heritability as well as the role of natural and cultivated host plants in the dynamics of the bollworm populations, modelling is a tool already used to develop such a strategy in diverse situations . In the US, it led to the 'High Dose Refuge' strategy (HDR)

combining a high level of toxin expression in the cotton plant associated with a toxin-free refuge inside the cotton area (Gould 1998).

The high dose strategy, combined with the use of refuges, is widely agreed to be the best technical approach for managing resistance, and evidence is accumulating that 'separate' refuges are more effective at conserving pest susceptibility than 'mixed' refuges (Cannon 2000).

Therefore, care must be taken to ensure that refuges, particularly those sprayed with insecticides, produce adequate numbers of susceptible insects. Models and experimental data showed that separate but adjacent refuges might be superior to other strategies for insects that can move between plants in their larval stage (Shelton *et al.* 2002).

Increasing the size of the refuge delays the development of resistance. Some workers have called for refuges as large as 50%, if farmers are allowed to spray them, which may present a dilemma and reduce farm profitability (Gould & Tabashnik 1998 cited from Gopalaswamy *et al* 2003).

Theoretical models suggest that pyramiding two dissimilar toxin genes in the same plant may require smaller refuges (Roush 1997a).The refuge fields must be within 0.8 km of their Bt fields (EPA/ USDA 1999).

B.6 - *EPA opinion*: The US Environmental Protection Agency (EPA), which regulates transgenic pesticidal crops, believes that scientifically sound long-term insect resistance management (IRM) strategies are essential to the protection of Bt microbial pesticides, transgenics, and reduction in the risks from the use of pesticides. The EPA has imposed mandatory IRM requirements for Bt cotton. Two structured refuge requirements have been imposed: 4% unsprayed or 20% sprayed crops (Matten 2000), and the refuge fields must be within 0.8 km of their Bt fields (EPA/ USDA 1999). Obviously, enforcing a similar system for small holding farmers will not be possible in most parts of Asia.

B.6 - *Parallel development of Bt resistant insect & and the susceptible same insect from non Bt cotton:* Although Bt cotton that produces Cry1Ac toxin has been effective against pink bollworm (Patin et al. 1999, Tabashnik *et al*. 2000b), the slower development of resistant larvae on Bt cotton as compared to susceptible larvae on non-Bt cotton could reduce the probability of mating between susceptible and resistant insects, and this asynchrony could reduce the expected benefits of the refuge strategy (Liu *et al*. 1999, Liu *et al.* 2001, Storer *et al*. 2001).

C --Additional ways to reduce resistance

C.1 - *Other abiotic factors:* Vijaykumar *et al* 2005b indicated that the efficacy of Bt cotton plants in killing *H. armigera* larvae is higher in irrigated condition compared to the Bt plants grown under rainfed conditions

C.2 - *Pyramiding two dissimilar toxin genes in the same plant*: Theoretical models suggest that pyramiding two dissimilar toxin genes in the same plant has the potential to delay the onset of resistance much more effectively than single-toxin plants released spatially or temporally, (Roush 1997b cited from Gopalaswamy *et al* 2003.

The basis for this strategy is sometimes referred to as "redundant killing" because insects adapted to one toxin may be susceptible to the second toxin. If the plants contain two Bt toxins at a high dose, insects that are able to survive on a plant with one high-dose toxin are rare, and insects that are able to survive on plants with two high-dose toxins will be very rare. (Roush 1997b and Adamczyk *et al.* 200).

Dual toxin (Cry1Ac and Cry2 Ac) Bt cottons will provide substantially better control of *H. zea, S. frugiperda,* and *S. exigua* compared with the existing single toxin (Cry1Ac) Bt cultivars, and may not require supplemental insecticidal applications (Stewart *et al.* 2001).

The strategy of "pyramiding," i.e., combining two toxins in a single transgenic plant will, at best, substantially reduce the size of the needed refuge and at worst, produce resistance to both toxins in the same amount of time as for a single toxin (Roush 1997b). Cross-resistance among toxins and the ability of insects to develop resistance to multiple toxins will limit the success of this approach (Roush 1998).

C.3 - *Application of insecticides in B.t cotton when it is needed:* Vijaykumar *et al* 2005c in their Study on the pattern of cross-resistance of Cry 1Ac toxin selected (for seven generations) *H.armigera* to chemical insecticides (*viz*., cypermethrin, fenvalerate, endosulfan, quinalphos, chlorpyriphos, methomyl and spinosad) conducted under laboratory conditions using discriminating doses of insecticides revealed negative cross resistance as Cry1Ac toxin selected *H. armigera* individuals were more susceptible to all the chemical insecticides tested irrespective of the group, compared to the unselected larvae from non-Bt cotton fields. The study strengthens the concept of "using chemical insecticides" as one of the tools in Bt resistance management strategy to increase the life of Bt technology.

It is generally recommended that the conventional insecticides specially the new generation of PYs to be only used in B.t. cotton when it is needed and keep the new safe molecules for refuge areas. Such PYS have to be sprayed once per season. This differentiation will help to maintain considerable

amount of beneficial in non-Bt plus may delay Bt resistance.

The new generation of PYs are Lambda cyhalothrin & Gamma cyhalothrin and Fenpropathrin . These groups are photo-stable pyrethroid insecticide for the control of both chewing and sucking insect pests in agriculture. It kills the insects by contact and stomach action. It offers knock down and residual control, and antifeeding and repellency properties extend the biological effect against both sucking and lepidopterous larvae.

SUMMARY The above is dealing with refuge size and positioning of old conventional and new molecules insecticides in the Bt cotton and free-toxin areas in relation to the conservation of beneficial arthropods

1-It is clear that refuge areas have to be accompanied Bt cotton specially in mono-crop system

2- Size of refuge area % will depend greatly on, single toxin or double toxin in the same plant, safe new insecticides or old conventional insecticides, diversity of crops or monoculture .For time being, it is generally assumed between 20-50%

2-It is generally assumed that using only safe new molecules in refuge areas will help in double ways the resistance and the availability of beneficial arthropods. Under this situation ,size of refuge should be 20%

4- It is generally assumed that using old conventional insecticides in rotation in refuge areas may help provided that refuge areas should not less that 50%

3- It is generally proposed that using only conventional insecticides specially the new generation of PYs in Bt areas when its needed, will help to combat CBW as well as sucking insects as well. It is also recommended to be used once per season.

There is a continuing need for a combined team of ecologists, geneticists, entomologist and plant breeders in determining system-wide impacts and devising optimal ways of deploying insect-resistant crops and reserve/conserve beneficial arthropods

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Abstracts

Cross Resistance of Bromopropylate in the Two Spotted Mite *Tetranychus urticae* Koch (Acari: Tetranychidae)

ABSTRACT Bromopropylate resistant strain (R) *Tetranychus urticae* showed strong positive cross resistance to Dicofol and a mixture of Dicofol and Tetradifon (Neotox Super), moderate positive cross resistance toward Amitraz and low negative cross resistance toward Chlorpyrifos. No cross resistance has been observed to Abamectin, Dinobuton, or Triazophos. These results suggest that Dicofol, Neotox Super, and Amitraz should not be rotated with Bromopropylate in a resistance management program. Chlorpyrifos used in this program will reduce Bromopropylate resistance gene in the two spotted spider mite population and will restore the activity of this pesticide.

KEYWORDS: *Tetranychus urticae*, Bromopropylate, Cross Resistance

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Effect of Consecutive and Alternate Application in the Development of Bromopropylate Resistance in the Two Spotted Spider Mite *Tetranychus urticae* Koch in the Field

ABSTRACT The development of the two spotted spider mite *Tetranychus urticae* Koch resistance to Bromopropylate was evaluated in two application programs: First, consecutive Bromopropylate spray and second, alternative Bromopropylate with abamectin. These results showed a significant increase in LC_{50} values in the first program treatments which were 10.60 (95% CL, 8.34-13.74), 22.76 (95% CL, 16.67-31.08), 14.04 (95% CL, 11.20-17.58) and > 320 mg/l compared with the initial value before spray, which was 5.67 mg/l (95% CL, 4.38-7.34). In the second program a significant increase was observed after the first spray treatment, which was 9.96 (95% CL, 7.39-12.69) compared to the value before spray. Whereas for other spraying treatments, no significant difference was observed in this program. Low resistance ratio (2.58 fold) in the alternative program, indicated that, this program was better for reducing the development of resistance, compared with high value (56.4 fold) in consecutive program.

KEYWORDS: *Tetranychus urticae*, Bromopropylate, resistance, consecutive, alternative.

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Susceptibility of Imidacloprid-Resistant Colorado Potato Beetles to Alternative Insecticides

ABSTRACT Imidacloprid, and, to a lesser extent, other neonicotinoid insecticides have been widely used to control the Colorado potato beetle, *Leptinotarsa decemlineata* (Say). Although an overwhelming majority of beetle populations are still susceptible to neonicotinoid insecticides, high selection pressure exerted by repeated use of these compounds has resulted in geographically isolated resistance hotspots, including a population in southern Maine. At the time of the study, that population was about 30-fold resistant to imidacloprid and could not be effectively controlled by its applications. Because Colorado potato beetles are commonly resistant to multiple chemicals belonging to different insecticide classes, we conducted a series of laboratory and field experiments determining susceptibility of the imidacloprid-resistant Colorado potato beetles to other insecticides currently registered for use on potato.

In the laboratory, we determined susceptibility of the imidacloprid-resistant Colorado potato beetles from a population in Southern Maine to other insecticides currently registered for use on potato. In the absence of exposure to insecticides, mortality was significantly higher for the imidacloprid-resistant larvae than for the susceptible larvae from laboratory-reared reference strain. This suggested possible fitness disadvantages associated with the resistance trait. On the opposite, resistant larvae exhibited significantly less mortality than susceptible larvae when exposed to cyfluthrin, carbaryl, azinphosmethyl, and

methamidophos. Their susceptibility to oxamyl was also somewhat reduced, although it did provide nearly 100% mortality at the highest concentration tested. Disulfoton was highly toxic to the resistant larvae.

To determine if oxamyl and disulfoton could be used in place of neonicotinoids to provide early season protection against overwintered adult beetles, we conducted a greenhouse study with potted potato plants. Oxamyl killed about 40% of the adults confined on potted potato plants, altered their feeding behavior (fewer adults positioning themselves on plants), and reduced defoliation by more than 90%. Disulfoton was not lethal to adults. However, it significantly suppressed their feeding, leading to 87% reduction in plant defoliation.

In field trials with the imidacloprid-resistant population, oxamyl and spinosad provided the best beetle control, distantly followed by disulfoton. Novaluron was somewhat of a disappointment, although it did reduce the number of large larvae. There was little difference between the plots treated with imidacloprid or thiamethoxam and untreated control, except for a 1-2 week delay in peak beetle abundance on the treated plots. There was a good consistency between the results of the Petri dish, greenhouse, and field experiments.

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Pyrethroid resistance in citrus thrips, *Scirtothrips aurantii*

ABSTRACT Pyrethroid resistance in the South African citrus thrips *Scirtothrips aurantii* has been known to occur in populations on citrus in certain production areas since 1995. However, reversion has generally allowed for the continued use of a single pyrethroid spray per annum. During the last two years, reversion does not seem to be occurring in populations on citrus near Burgersfort and Hoedspruit, due possibly to multiple sprays of pyrethroids on other crops grown in the vicinity. Multiple resistance between fluvalinate and formetanate was also found near Burgersfort where both products had a minimal effect on citrus thrips populations in a small trial (mean pre-spray fruit infestation by larvae was 47%; 6 days after treatment, fruit infestation by larvae after formetanate (0.0125% a.i.) plus sugar was 35% and after fluvalinate (0.0072% a.i.) was 28%). *Scirtothrips aurantii* is becoming increasingly common on macadamias where pyrethroids are used for the control of pentatomid bugs. Where macadamias are grown adjacent to citrus, populations of citrus thrips in both crops will most likely be resistant to pyrethroids.

KEYWORDS Scirtothrips aurantii, citrus, pyrethroid, carbamate, resistance

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Cost and Mitigation of Insecticide Resistance in the Maize Weevil, *Sitophilus zeamais*

ABSTRACT A common assumption in models of insecticide resistance evolution is the association between resistance and fitness costs in the absence of insecticides. There is empirical evidence of such associations, but their physiological basis (and mitigation) is little investigated. Pyrethroid-resistant populations of the maize weevil (*Sitophilus zeamais* (Coleoptera: Curculionidae)) offer this opportunity. Pyrethroid resistance in this species was initially observed in five Brazilian states by 1995, but the phenomenon apparently decreased and did not spread to other regions probably due to the occurrence of a fitness disadvantage in resistant individuals in the absence of insecticides. The present investigation aimed to verify if differences in respiration rate and fat body morphology are related to differences in rate of development in Brazilian populations of *S. zeamais* resistant to insecticides and thereby provide evidence of the existence (or not) of a physiological fitness cost acting against insecticide resistance in maize weevils. This may occur due to a possible energy trade-off between insecticide resistance and other physiological processes associated with development and reproduction. To achieve this, studies of the rate of development, respiration and fat body cytomorphology were carried out in one insecticide-susceptible (from Sete Lagoas) and two resistant populations (from Jacarezinho and Juiz de Fora) of *S. zeamais*. The

resistant population from Jacarezinho showed higher body mass associated with higher energy reserves (higher trophocyte area) for development and reproduction, as well as for insecticide resistance. The resistant population from Juiz de Fora however, does not appear to have large enough energy allocation for insecticide resistance expression and development and/or reproductive performance suggesting a trade-off between resistance and other life history traits.

KEYWORDS Adaptive cost, adult respiration rate, body mass, carbon dioxide production, grain beetles, pyrethroid resistance, rate of development, trophocyte area.

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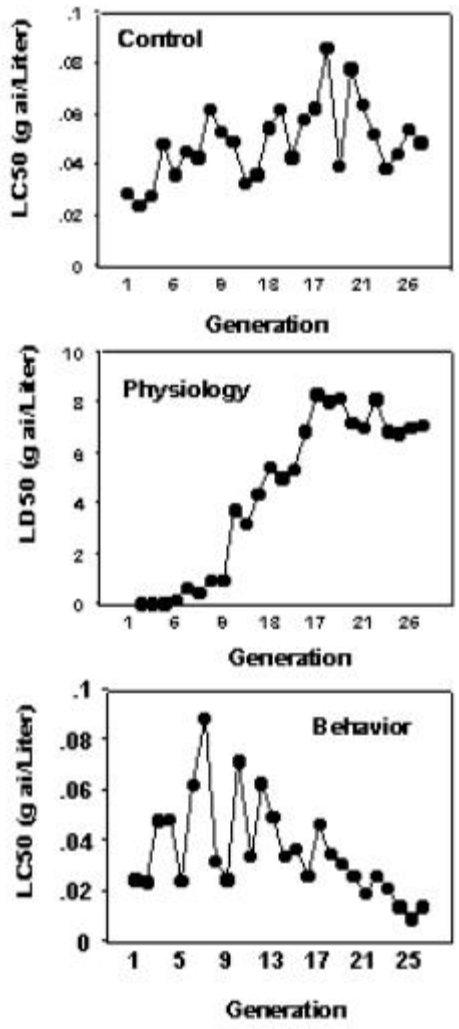
Selection for Increased Susceptibility or Increased Resistance to Permethrin in the Diamondback Moth.

ABSTRACT We have been exploring the behavioral responses of insect pests to insecticides and its genetic correlation with physiological tolerance to these toxins. The unique aspect of our approach has been a focus on the interaction between behavioral and physiological responses to toxins, rather than either form of adaptation by itself. In previous studies, we documented negative genetic correlation between behavioral response and physiological tolerance to permethrin in *P.xylostella* regardless of whether the measurement of the behavioral response was on the larval or adult stage. The practical significance of a genetic correlation between two traits is that selection on one trait can cause a change in both simultaneously. Selection experiments were conducted to test the hypothesis that low heterogeneous doses could lead to increased susceptibility to an insecticide by selecting on behavior. The population examined was originally from Celeryville, Ohio, a population that consistently has shown large genetic variance for both traits and significant negative correlation between them. Twenty-six generations of *P.xylostella* were maintained under selection in relatively large cages in a glasshouse. Two replicates were maintained of each of three selection regimes: selection for physiological tolerance, selection for behavioral avoidance, and a no selection control. The line selected for physiological tolerance was exposed to a relatively high and uniform concentration, whereas the line selected for avoidance was exposed to a relatively low and heterogeneous concentration. The concentration chosen for the first generation for the line selected for physiological tolerance was the LC_{50} (0.109 g AI/liter of permethrin) of the parental generation. For lines selected for behavioral avoidance, the first generation was exposed to the LC_5 (0.00436 g AI/liter of permethrin) of the parental generation. Subsequent generations were selected with the LC_{50} and LC_5 of the preceding generation for lines selected for physiological tolerance and behavioral responsiveness, respectively. Each generation began

Figure 1.



with approximately 200 pupae harvested from the previous generation, and new sets of treated plants. The extra pupae that are removed from each cage in each generation were reared to the adult stage and the

offspring were tested for tolerance to a topically applied dose and for behavioral responsiveness. The population did respond as expected to physiological selection (Figure 1). For generations 1-20, lines selected for behavioral response remained slightly lower in LC_{50}, but not significantly different from the unselected control lines (Figure 1). Based on confidence intervals from the probit analyses, the LC_{50} of the lines selected for avoidance, however, were significantly lower than that of the control lines in generations 21-26 (Figure 1). These results demonstrate that selection on behavioral responses can result in greater susceptibility than a population that has not been selected at all, despite ample genetic variation and potential for increased physiological tolerance. The importance of this result is that it indicates an alternative means of preventing resistance, as well as a means of reversing resistance while still using the insecticide, by exploiting behavioral responses and their genetic relationship with physiological tolerance.

KEY WORDS *Plutella xylostella*, pyrethroids, behavioral avoidance, physiological tolerance, heritability, genetic correlation, selection.

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First Report of Organophosphate Resistant *Boophilus microplus* (Acari: Ixodidae) within the United States of America

ABSTRACT *Boophilus microplus*, collected from Starr County, Texas, were determined to be resistant to the organophosphorous acaricides coumaphos and diazinon. Initial bioassay results from collected ticks produced a slope (SE) of 3.96 (0.22) which was different from that obtained from a susceptible reference population 6.97 (0.38). Resistance ratios (RR) (95% C.I.) indicated that the population was resistant to coumaphos 3.6 (3.4-3.8), 5.0 (4.5-5.5), and 6.5 (5.4- 7.7) at the LC_{50}, LC_{90}, and LC_{99}, respectively. Bioassay results generated from a second collection of ticks made 12 d after all cattle in the infested pasture were treated with coumaphos, confirmed coumaphos resistance, but produced a slope (SE) that was not significantly different from a susceptible laboratory reference population. The ticks were also resistant to diazinon, RR (95% C.I.) = 7.1 (6.5-7.7), 11.7 (10.3-13.3), 17.7 (14.5-21.5) at the LC_{50}, LC_{90}, and LC_{99}, respectively. The slope (SE) generated from the diazinon bioassay was different than that of a reference strain, 2.98 (0.12) and 6.09 (0.35), respectively. The high dose strategy used by the Cattle Fever Tick Eradication Program was able to eradicate coumaphos resistant *B. microplus* after just two treatments of coumaphos, 12 d apart.

KEYWORDS *Rhipicephalus (Boophilus) microplus*, pesticide resistance, cattle tick, acaricide, coumaphos, diazinon

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Posttranscriptional Regulation of Sodium Channel Gene Expression is Associated with Insecticide Resistance in Insects

ABSTRACT The voltage-gated sodium channels of the insect nervous system are the primary target of DDT and pyrethroid insecticides, which are known to exert their insecticidal effects by altering the function of voltage-gated sodium channels in the nerve membranes of insects and preventing the repolarization phase of action potentials. The loss of target site sensitivity to insecticides resulting from a substitution of leucine to phenylalanine, termed the *kdr* mutation, in the voltage-gated sodium channel of the insect nervous system is documented to be of importance in insecticide resistance of medically, agriculturally and economically important insects, including mosquitoes, house flies and german cockroaches. Yet, little is known about the molecular basis underlying the genotype and *kdr*-mediated resistance phenotype relationship. We conducted a systematic study of resistance-associated *kdr* allelic expression within and among populations of intra- and inter-species of insects, including the mosquitoes *Culex quinquefasciatus* and *Aedes albopictus,* the house fly *Musca domestica*, and the German cockroach *Blattella*

germanica, which bear different phenotypes ranging from susceptible to highly resistant. We compared genomic DNA and RNA expression levels within the same individuals from different insect strains. We found no correlation for the *kdr* allele at the genomic DNA level with levels of susceptibility or resistance to insecticide. We, however, identified a strong correlation between the *kdr* allele expression and the levels of insecticide resistance through RNA allelic variation and RNA editing. Our work clearly suggests the role of posttranscriptional regulation in the connection of the sodium channel genotype and its mutation-mediated resistance phenotype.

KEYWORDS Sodium channels; *kdr* mutation; RNA allelic variation; RNA editing

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Symposia

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