Resistant Pest Management Newsletter

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

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Table of Contents

Letter from the Editors _____ 2

Resistance Management from Around the Globe

A 2010 Response of the Tobacco Budworm *Heliothis virescens* (Fabricius), and the Cotton Boll Weevil *Anthonomus grandis* (Boheman), to Insecticides in Northeast Mexico – Antonio Palemon Teran-Vargas, Jesus Loera-Gallardo and Marco Antonio Reyes-Rosas _____ 3

Fungicide Sensitivity of Cucurbit Powdery Mildew Fungus (*Podosphaera xanthii*) on Long Island, NY in 2009 – Margaret T. McGrath _____ 5

Fungicide Sensitivity of Cucurbit Powdery Mildew Fungus (*Podosphaera xanthii*) on Long Island, NY in 2010 – Margaret T. McGrath _____ 8

Susceptibility of *Pectinophora gossypiella* (Lepidoptera: Gelechiidae) and *Cryptophlebia leucotreta* (Lepidoptera: Tortricidae) to Insecticides Used on Cotton Crops in Côte d'Ivoire, West Africa. Implications in Insecticide Resistance Pest Management Strategies – Doffou N. M., Ochou O. G. and Kouassi P. _____ 10

White Grubs (*Cyclocephala comate*) Susceptibility to Different Insecticides in San Martin Hidalgo Jalisco, Mexico – Pedro Posos Ponce, Aurelio Perez Gonzalez, Benito Monroy Reyes, Enrique Pimienta Barrios, Vicente Antonio Aceves Nuñez and Gustavo Enciso Cabral _____ 15

Bioefficacy of Newer Insecticides Against Groundnut Leaf miner *Aproaerema modicella Deventer* in Northern Dry Zone in Karnataka – Praveen, Y.V., Y.B. Kotikal, L. Ranjith kumar and Vijaykumar Ghante _____ 18

Insecticide Resistance in Chilli thrips, *Scirtothrips dorsalis* (Hood) in Andhra Pradesh – K. Vanisree, P. Rajasekhar, S. Upendhar, G. Ramachandra Rao and V. Srinivasa Rao _____ 22

Research in Resistance Management

Phenoloxidase Dependence of *Leptinotarsa decemlineata* Say. Cellular Immune Response Under the Action of High Derivatives of Chitin – L.R. Gaifullina, E.S. Saltykova and A.G. Nikolenko _____ 26

Susceptibility of Different Colour Morphs in *Helicoverpa armigera* (Hubner) (Lepidoptera, Noctuidae) to Chemicals – V. N. Ghante, L. Rankithkumar, A.M. Benki, L. Rajesh Chowdhary and Krishi Vigyan Kendra _____ 29

Baseline Sensitivity of Cucurbit Powdery Mildew Fungus (*Podosphaera xanthii*) to the Fungicide Metrafenone – Margaret T. McGrath _____ 31

Cytotoxic Effect of Potential Pesticide on the Cerebral Neurosecretory Cells (CNSC) Pink Bollworm, *Pectinophora gossypiella* (Saund.) – Magdy A. Massoud, Magdy M. K. Shekeban, A. S. A. Saad, Ahmed K. Mourad and Sultan R. Meharb _____ 34

Role of Monooxygenases in the Resistance Forming to Insecticides in Housefly (*Musca Domestica*) – M.P. Sokolyanskaya _____ 39

The Problem of the Insect and Mite Resistance to Insectoacaricides; Resistance to Modern Insectoacaricides – M.P. Sokolyanskaya _____ 44

Abstracts in Resistance Management

Comparative Efficacy of Single (*Cry1*Ac) and Stacked Bt genes (*Cry1*Ac+*Cry2*Ab) in Interspecific Bt Cotton Hybrids Against Tobacco Caterpillar, *Spodoptera litura* (Fab.) – Hareesha K. Badigar, Dr. S. B. Patil and Dr. S. S. Udikeri _____ 48
Pest Management and Contemporary Entomology – Galina Benkovskaya, Konstantin Kitaev, Elena Surina and Maxim Udalov _____ 49
Research Progress in Understanding Insecticide Resistance in Oriental Migratory Locust, *Locusta migratoria manilensis* – Jianzhen Zhang, Jianqin Zhang, Meiling Yang, Guohua Qin, Daqi Li, Yaping Guo, Enbo Ma and Kun Yan Zhu _____ 51

Letter from the Editor

Dear Subscribers,

I would first like to start off by thanking everyone that helped contribute to the Spring 2011 edition of the Resistant Pest Management Newsletter. Without your submissions this edition would not have been a success. Thank you everyone for your continued support and article submissions.

To make ourselves more readily available, the newsletter has created a Facebook page. The page can be found under the display name Brittany Harrison and is always accepting friend requests. The purpose of this page is to connect with our subscribers about updates regarding the newsletter as well as updates regarding the Whalon lab. This is also a forum for which you can speak with someone directly and ask any questions you might have in a quicker fashion. If no one is available via instant message then please feel free to leave a message in our inbox and we will get back to you as soon as possible. Through this social network we hope to gain a closer relationship to our subscribers as well as converse about the developing discoveries in the world of pesticide management. So please stop by our page and add us as a friend. We might already be friends with some of the people you know.

Sincerely,

Brittany Harrison
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Resistance Management from Around the Globe

A 2010 response of the tobacco budworm *Heliothis virescens* (Fabricius), and the cotton boll weevil *Anthonomus grandis* (Boheman), to insecticides, in Northeast Mexico

INTRODUCTION

In the 70´s, the tobacco budworm caused the breakdown of the cotton area in south Tamaulipas, Mexico (Adkisson, 1972; Bottrell and Adkisson 1977), where 230,000 ha of cotton were grown at the beginning of the 60´s (Vargas *et al.* 1979); at that time, the chemical control of *H. virescens*, depended exclusively on conventional pesticides, mostly methyl parathion, to which, high levels of resistance were developed (Bujanos-Muñiz, 1983; Wolfenbarger *et al*., 1984; Martinez-Carrillo *et al*., 1991). Pest resistance to insecticides along to low prices of cotton lint caused farmers to grow other crops as soybean, corn and sorghum. During the 80's, an increase in the cotton fiber price, along with the appearance of the pirethroids insecticides, returned farmers to grow cotton again, however the intensive use and abuse of pirethroids originated a rapid development of pest resistance to these insecticides, and in 1995, a new crisis was detected for *H. virescens* control(Terán-Vargas, 1996); causing a reduction of the 94.7% of the cotton planted in 1996.

Along with the tobacco budworm, *A. grandis* is the other most important pest in cotton; it can totally destroy 100% of the fructifications if no control measures are applied (Salgado, 2001). In spite of eradication programs in progress in some areas of Tamaulipas, and the knowledge that has been accumulated over the years concerning its control, the boll weevil continues to cause big losses to the cotton farmers.Many efforts have been made over the years to reduce the use of insecticides to control this pest, but chemical control is still a critical component in the management of the cotton boll weevil. An efficient use of insecticides is essential to reduce the economic damage caused by the weevils and maintain the profitability of cotton production.

Present study was aimed to review the toxicological situation for the most important cotton pests in Mexico, *H. virescens* and *A. grandis*, after 20 years that cotton has not been extensively planted in Tamaulipas.

METHODS AND MATERIALS

The tests were conducted in the lab of the experiment station Las Huastecas from INIFAP (The Nationa Institution for Agricultural, Livestock and Forestry Research) located in the south of the Tamaulipas state, at northeast Mexico. The response of two *H. virescens* populations and one of *A. grandis* to endosulfan, methyl parathion, chlorpyrifos, methomyl, permethrin, deltamethrin, malathion and azinphosmethyl, was evaluated.

The population of *A. grandis* was obtained from the north of the Tamaulipas state, by collecting oviposited cotton squares and sending them to the experiment station Las Huastecas, where were maintained in cages (40X40X60 cm). When the adult boll weevils emerged and reached a weight of 12 to 15 mg, were kept in petri cages to be ready for the bioassays.

The two populations of *H. virescens* larvaewere collected fromChickpeaand Malva (*Malva parviflora*), and reared in artificial diet (Southland Products Incorporated, Lake Village, Arkansas); they were kept in a plastic 30 ml container where 15 ml of diet were added. After pupation, the adults were used to produce more larvae, and when these larvae reached the third instar (25± 3mg), then they were utilized in the bioassays.

The bioassays were conducted by the topical application method as proposed by the Entomological Society of America (Brazzel, 1970), using a microliter ofacetone plus a known amount of the toxic, and applied on the pronotum. In these testsan electric microapplicator (Model M Instrumentation Specialites Company - ISCO, Inc. Lincoln, Nebraska) was utilized, plus a 500µl microsyringe (Hamilton Company, Reno, Nevada).

The response data were statistically analyzed to obtain the LD_{50} through the statistical package Polo Plus-Probit and Logit Analisis 2.0 (LeOra Software Company®, Petaluma, California).

RESULTS AND DISCUSSION

For *A. grandis*, the LD_{50} values, the confidence limits, the slope of the regression line, and the regression equation, for the insecticides evaluated, are indicated in table 1. Results showed that endosulfan was the most toxic insecticide with an LD_{50} of 7.5292 x 10^{-10} ug/weevil, followed, in order of toxicity, by methyl parathion and deltamethrin showing LD_{50} of .00000000250552 and .00000000559981 ug/weevil, respectively, and then azinphosmethyl with an LD_{50} of .00000000854767 ug/weevil. The least toxic insecticides were permethrin and malathion with LD_{50} of .00000601303, and .00279 ug/weevil, respectively.

Table 1: Values of the LD50, confidence limits and slope of the regression line for different insecticides applied to adults of the boll weevil *Anthonomus grandis* from northeast Mexico.

Active Ingredient	LD_{50} µg/weevil	Confidence limits (95%)		Slope
		Lower	Upper	
Deltamethrin	.00000000559981	.00000000295908	.00000000986118	0.44322
Malathion	.00279	.00118	.00574	0.44404
Azinphosmethyl	.00000000854767	.00000000328943	.0000000176399	0.49707
Permethrin	.00000601303	.0000025122	.0000127	0.39515
Endosulfan	.00000000075292	.000000000042227	.00000000320798	0.96050
Methyl parathion	.00000000250552	.00000000094973	.00000000474609	0.70914

*48 h afterapplication

According to the LD_{50} for this insect, obtained in previous years, the actual results indicate that this population tested in our bioassays is highly susceptible to the insecticides used. This susceptibility may probablybe caused by the boll weevil eradication program that is conducted in both, the north of Tamaulipas Mexico and the south of Texas USA; activity that may have eliminated *A. grandis* individuals carryng genes of resistance, and leavingonly susceptible individuals; by the other hand, malathion was the least toxic insecticide, and this may be probably caused by the selection pressure acting over the *A. grandis* population.

Loera-Gallardo *et al.* (1997) showed an LD_{50} of 0.081-0.0091 µg/weevil in Lower Rio Grande Valley TX, USA and North of the Tamaulipas state, in 1990 and 1991, respectively. Another study (Loera-Gallardo and Wolfenbarger, 1999), showed that LD_{50} values of Methyl parathion in *A. grandis* populations from Tamaulipas, Mexico and the Lower Rio Grande Valley TX, USA, were 0.0091 and 0.01mg/weevil, respectively, both values indicated susceptibility. In our study the response of *A. grandis* to Methyl parathion showed an LD_{50} of .00000000250552 that indicates to be even more susceptible.

For the *H virescens* colonies from Chickpea and Malva, plus a susceptible colony collected in 1982 in Obregon, Sonora, Mexico, the LD_{50} values, the confidence limits, the slope of the regression line, and the regression equation, for the insecticides evaluated, are indicated in table 2. The susceptible *H. virescens* colony has been maintained free of selection pressure since 1982 (Martínez-Carrillo 1991).

Table 2: Values of the LD50, confidence limits and slope of the regression line for different insecticides applied to larvae of *Heliothis virescens* from different hosts at northeast Mexico.

Active Ingredient	Colony	LD_{50} (µg/larva)	Confidence limits (95%)	Slope	RR
Endosulfan	*H. virescens* (Garbanzo)	0.31	0.27-0.36	2.911	7.5
	H. virescens (Malva)	1.043	0.857-1.327	1.788	2.2
	Susceptible (Obregón, Mexico)	2.33	1.95-2.77	2.64	
Methyl Parathión	*H. virescens* (Chickpea)	0.124	0.086-0.178	1.939	--
	H. virescens (Malva)	0.061	0.041-0.091	2.072	--
	Susceptible (Obregón, Mexico)	1.46	1.19-1.75	2.28	
Deltametrina	*H. virescens* (Chickpea)	0.00054	0.00020-0.00114	1.690	--
	H. virescens (Malva)	0.00028	0.00017-	1.155	--
	Susceptible (Obregón, Mexico)	0.004	0.00042 0.003-0.007	3.15	
Permetrina	*H. virescens* (Chickpea)	0.0062	0.0050-0.0078	1.994	--
	H. virescens (Malva)	0.0094	0.0075-0.0116	2.094	--
	Susceptible (Obregón, Mexico)	0.18	0.15-0.21	2.79	
Methomyl	*H. virescens* (Chickpea)	0.409	0.277-0.620	1.262	
	H. virescens (Malva)	0.034	0.019-0.060	2.140	
Chlorpyrifos	*H. virescens* (Chickpea)	0.113	0.090-0.142	1.953	
	H. virescens (Malva)	0.125	0.101-0.154	1.951	

The response of *H. virescens* Chickpea or Malva colonies to endosulfan was different; the population from Malva was 3.1 times more tolerant than the Chickpea population. When compared to the *H. virescens* susceptible population, the Chickpea and the Malva populations were 7.7 and 2.2 times, respectively, more tolerant.

The response to Methyl parathion did not show differences between the *H. virescens* Chickpea or Malva populations since the C. I. overlap, and they were more susceptible than the susceptible population showing a lower and different LD_{50}.

As with Methyl parathion, the response to Deltamethrin and Permethrin was similar in both, Chickpea or Malva populations, and both colonies showed to be more susceptible than the susceptible colony.

The response to Methomyl showed that the *H. virescens* Chickpea population was 12.0 times more tolerant than the Malva population; for this case, a base line of response does not exist.

The response to Chlorpyrifos did not exhibit difference in the LD_{50} for both, the *H. virescens* Chickpea or

Malva colonies. Also, for this case it does not exist a base line of response.

It is important to mention that *H. virescens* colonies from Chickpea or Malva have not been exposed to any insecticide spraying since 2001, at this year was when cotton was planted for the last time in South Tamaulipas. From results in our study, we assume that these two colonies may be utilized in the future as a base line of response because always showed to be more susceptible than the susceptible colony from Obregon Sonora, Mexico.

During 1996, the transgenic cotton Bollgard® (δ-endotoxin Cry1Ac of *Bacillus thuringiensis* var.) was introduced to Mexico, and as a consequence, the levels of resistance were reduced in such a way that in 2001 the LD_{50} for Methyl parathion was 0.32 (0.26-0.38) µg/larva; for Permethrin 0.01 (0.01-0.02) µg/larva, and for Deltamethrin in 1999 was 0.012 (0.009-0.015) µg/larva.

CONCLUSIONS

Recently, in Mexico, we started to grow transgenic cotton, and a notable reduction of the *H. virescens* population has been observed, however, a continuos monitoring for these pests, periodically, is needed because *H. virescens* may develop resistance to Bt and *A. grandis* is still under a frequent and intensive sprayings which may also cause this insect to develop resistance to insecticides.

Actual populations of *A. grandis* and *H. virescens* in Tamaulipas are considered susceptible to the insecticides tested.

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Fungicide Sensitivity of Cucurbit Powdery Mildew Fungus (*Podosphaera xanthii*) on Long Island, NY, in 2009

ABSTRACT

Fungicide resistance can be a major constraint to effectively managing powdery mildew in cucurbit crops. This project goal was to obtain information needed to guide fungicide recommendations in 2009 and 2010 by conducting fungicide sensitivity bioassays in spring squash plantings at the start of powdery mildew development and later in pumpkin crops. Resistance to fungicides in FRAC code 1 (e.g. Topsin M) and Code 11 (e.g. Flint, Cabrio, and Quadris) were

detected in all squash plantings where the bioassay was conducted. Proportion of the pathogen population estimated to have resistance varied among the plantings from 28-100% for Code 1 fungicides (average of 80%) and 20-100% for Code 11 (62%). Fungicides in these groups therefore were not recommended in 2009. *Podosphaera xanthii* exhibited greatest sensitivity to the Code 13 fungicide (Quintec) in 2009 with an average of 9% of the population in squash plantings and 6% in pumpkin crops tolerating 1 ppm active ingredient (quinoxyfen). While 21% and 4%, respectively, tolerated 80 ppm myclobutanil (a.i. in Code 3 fungicide Rally) and 20% and 11% tolerated 175 ppm boscalid (Code 7 fungicide in Pristine). Quintec was recommended as the most important fungicide to use in programs. A bioassay conducted at the season end (24 Sep) in research and commercial plantings of pumpkin revealed presence of pathogen strains completely resistant to Code 7 fungicide and documented that sole use of a fungicide with resistance risk can select for resistant strains during one growing season: proportion of the pathogen population tolerating 500 ppm boscalid was 100% for a plot treated every week with Pristine and 17-20% for a plot and commercial pumpkin fields receiving a recommended fungicide program (mobile fungicides applied in alternation and tank-mixed with a protectant fungicide). Strains able to tolerate this concentration would not be controlled with Pristine. Control of powdery mildew in commercial pumpkin crops was moderate to excellent in 2009.

INTRODUCTION

Cucurbit crops, in particular pumpkins, are important in Suffolk Co., NY, on Long Island. Powdery mildew is the most important disease in this crop group, occurring every year throughout the area. Control is necessary to avoid a reduction in yield and/or fruit quality.

Fungicide application continues to be the principal management practice, but successful control is challenged by resistance development. The pathogen develops best on lower surface (underside) of leaves. It is difficult to directly deliver fungicide to the lower surface; therefore, an important component of fungicide programs has been mobile fungicides. Unfortunately they are prone to resistance development because they have single site mode of action. This pathogen has demonstrated high potential to develop resistance.

Presently there are five chemical classes of fungicides registered for cucurbit powdery mildew: FRAC Code 1 (Topsin M), 3 (including Rally, Procure, Inspire, and Tebuzol), 11 (including Quadris, Flint, Cabrio, and Pristine), 7 (second active ingredient in Pristine) and 13 (Quintec).

Resistance to Code 1 and 11 fungicides is qualitative, thus pathogen strains are either sensitive or completely resistant, and fungicides are either effective or ineffective, respectively against these strains. Use of Topsin M (Code 1) is thought to have been extremely limited, if used at all, for many years, thus there was a possibility that resistant strains may have disappeared from the pathogen population in absence of selection pressure to maintain this trait. Bioassays conducted in 2008 however revealed that this is not the case, and in fact this resistance remains common in the population. On the other hand, resistance to Code 11 fungicides (the QoIs or strobilurins) was expected to be more common than found to be in 2008. A goal of bioassays conducted in 2009 was determining if the level of resistance to either Code 11 or 1 fungicides was sufficiently low that either could be recommended.Resistance to Code 3, 7, and 13 fungicides is known (or thought) to be quantitative, which means pathogen strains exhibit a range in sensitivity to them. With this type of resistance it is much more difficult to determine when pathogen strains have reached a level of insensitivity corresponding to control failure. In 2008 on LI, pathogen strains were detected tolerating relatively high concentrations of Code 3 and 7 fungicides. While these concentrations are below that in a spray tank, concentrations on sprayed leaves will decrease over time before the next application. Control was variable and often below expectation in pumpkin fields where Procure and Pristine were used, perhaps reflecting field-to-field variation in pathogen sensitivity. However, control was excellent with each in research plots where they were used alone on a strict weekly schedule.

MATERIALS AND METHODS

A seedling bioassay was used to determine the sensitivity to fungicides of populations of *Podosphaera xanthii* in commercial cucurbit crops in Suffolk Co., NY, as well as in research plantings during the 2009 growing season. Pumpkin seedlings cv 'Sorcerer' produced in a growth chamber were treated with fungicides at several concentrations using a backpack sprayer, the next day put in cucurbit plantings with powdery mildew for about 4 hours (Figure 1), and then kept in a greenhouse at LIHREC for about 10 days until powdery mildew was visible and could be assessed. Amount of mildew on leaves of treated plants was compared with leaves on non-treated plants to estimate the proportion of the pathogen population able to tolerate each fungicide concentration. The bioassay was conducted in spring-planted summer squash crops where powdery mildew starts to develop first in order to obtain a measure of pathogen sensitivity before fungicides were used (spring squash generally is not treated with fungicides specific for powdery mildew due to how late the disease develops in crop production). The seedling bioassay was conducted twice in September in commercial pumpkin crops and in research plots. Powdery mildew severity was assessed.

Figure 1: Fungicide-treated seedlings for bioassay in a commercial squash crop.

RESULTS AND DISCUSSION

The first bioassay was conducted on 30 July in 6 commercial fields and a research field of spring-planted summer squash. This crop was chosen because it is where powdery mildew starts to develop each season. Resistance to Topsin M and Flint were detected in all squash plantings where the bioassay was conducted (Table 1). Therefore for managing powdery mildew in 2009 fungicides were not recommended in these chemical classes, which are the MBC (FRAC Code 1) and QoI (Code 11) fungicides, respectively. Proportion of the pathogen population estimated to have resistance varied among the plantings from 28-100% for Topsin M (average of 80%) and 20-100% for Flint (62%). Similar results were obtained in 2008. Strains of the pathogen were detected with low sensitivity to Code 3 and 7 fungicides: 2-36% (18% average) of the pathogen populations was estimated to be able to tolerate 120 ppm myclobutanil (active ingredient in Rally) and 4-48% (19%) tolerated 175 ppm boscalid (a.i. in Endura and also in Pristine). The pathogen exhibited similar sensitivity to the four DMI fungicides: average proportion of the pathogen populations tolerating 80 ppm active ingredient was 11% for difenconazole (Inspire), 16% for tebuconazole (Tebuzol), 19% for myclobutanil, and 32% for triflumizole (Procure). The pathogen exhibited high sensitivity to Quintec (FRAC Code 13) with an estimated average of only 1% and 8% of the pathogen population tolerating 10 and 1 ppm quinoxyfen, respectively. Based on these results, Quintec was recommended as the main fungicide to use in non-edible-peel cucurbit crops with applications alternated with Pristine and a DMI fungicide.

Table 1: Percentage of powdery mildew populations estimated tolerant to different concentrations (ppm) of fungicides in commercial spring plantings of zucchini and a research planting in Long Island on 30 July 2009.

Assay	Thiophanate-	Trifloxy-	Quinoxyfen		Boscalid		Myclobutanil		Triflumizole	Difenconazole	Tebuconazole
location	methyl 50	strobin 50	1	10	50	175	80	120	80	80	80
Farm 1	20.2	28.1	2.0	0.1	14.7	4.3	7.3	11.5	8.6	1.9	4.2
Farm 2	99.9	92.6	6.0	0.9	35.1	48.4	17.7	2.1	27.9	1.7	6.9
Farm 3	50.8	64.9	11.5	0.0	33.8	16.5	10.7	35.8	49.8	22.1	1.6
Farm 4	76.7	100.0	11.1	0.5	92.2	12.0	28.0	20.6	35.8	18.3	21.7
Farm 5	54.8	72.6	5.3	1.5	28.1	23.0	28.6	21.1	39.0	1.9	23.8
Farm 6	45.6	100.0	17.1	1.3	16.4	27.7	8.6	13.4	25.5	3.4	4.4
LIHREC	73.7	69.4	2.6	5.2	34.0	15.0	22.5	17.0	31.3	14.0	35.3
Average	**59.4**	**79.9**	**8.8**	**1.7**	**38.7**	**19.5**	**21.2**	**21.5**	**36.8**	**10.3**	**18.4**

Another bioassay was conducted on the 3rd of Sept in 7 commercial pumpkin crops. As for the squash plantings, QoI resistance was detected in all pumpkin fields (Table 2). Proportion of the pathogen population estimated to have resistance varied among the plantings from 24-100% and averaged 77%. Strains of the pathogen were detected with low sensitivity to Code 3 and 7 fungicides, but they were at lower frequencies: average of 0.4% and 11% tolerated 120 ppm myclobutanil and 175 ppm boscalid, respectively. Sensitivity to Quintec also was similar with an estimated average of only 0.5% and 6% of the pathogen population tolerating 10 and 1 ppm quinoxyfen, respectively. These results document the utility of conducting bioassays in spring summer squash crops for predicting pathogen sensitivity in main season crops.

Table 2: Percentage of powdery mildew populations estimated tolerant to different concentrations (ppm) of fungicides in commercial plantings of pumpkin and a research planting in Long Island on 3 September 2009.

Assay	Trifloxy-	Quinoxyfen		Boscalid		Myclobutanil		Triflumizole	Difenconazole	Tebuconazole
location	strobin 50	1	10	50	175	80	120	80	80	80
Farm 5	24.3	1.0	0.6	2.2	3.2	0.0	0.1	14.7	0.4	1.8
Farm 6, Field 1	100.0	7.3	1.2	13.4	4.2	0.0	0.8	0.4	0.0	0.4
Farm 6, Field 2	66.0	1.5	0.4	2.5	0.7	0.2	0.1	0.0	0.0	0.4
Farm 7	73.6	0.7	0.4	1.2	0.3	0.1	0.0	7.8	0.0	0.4
Farm 8	90.2	11.4	0.2	46.5	41.0	5.5	0.4	1.2	0.8	0.8
Farm 9, Field 1	100.0	15.2	0.7	39.1	23.9	22.0	1.1	1.1	0.4	2.3
Farm 9, Field 2	87.7	7.0	0.3	16.4	2.7	0.8	0.4	7.0	0.2	0.7
LIHREC	100.0	20.2	0.1	63.1	53.0	1.7	1.3	2.6	0.1	16.4
Farm average	77.4	6.3	0.5	17.3	10.9	4.1	0.4	4.6	0.3	1.0

On the 24th of Sept a bioassay was conducted in two commercial pumpkin crops and research plots of pumpkin that had been treated every week with the same mobile fungicide or with a recommended fungicide resistance management program consisting of the mobile fungicides applied in alternation and tank-mixed with a protectant fungicide. Results from this bioassay document that sole use of a fungicide

with resistance risk can select for tolerant strains during one growing season: proportion of the pathogen population tolerating 500 ppm boscalid was 100% for a plot treated with Pristine, <1% for plots treated with Procure or Quintec, and 17-20% for a plot and the commercial pumpkin fields receiving a fungicide program (Table 3). Strains tolerating this concentration would be difficult to control with Pristine because this is similar to the concentration in a spray tank (411 and 698 ppm with Pristine at the highest label rate applied at 85 and 50 gpa). Proportion of the pathogen population tolerating 1-10 ppm quinoxyfen or 40 ppm triflumizole (a.i. in Procure) in plots treated with only Quintec or Procure was similar or slightly greater than in the other plots.

Table 3: Percentage of powdery mildew populations estimated tolerant to different concentrations (ppm) of fungicides in commercial plantings of pumpkin and a research planting in Long Island on 24 September 2009.

Assay	Quinoxyfen		Boscalid		Myclobutanil	Triflumizole	Difenconazole	Tebuconazole
location	1	10	175	500	40	40	40	40
Fungicide program	11.5	0.1	20.0	17.1	1.8	19.4	0.1	1.5
Procure	1.1	1.1	0.7	0.4	0.7	35.6	1.8	0.0
Quintec	1.6	1.0	3.9	0.3	0.3	12.3	0.0	1.9
Pristine	0.6	0.2	100.0	100.0	0.2	15.5	10.0	1.0
Farm 9, Field 1	32.9	0.3	20.3	20.3	2.8	40.5	5.1	40.5
Farm 9, Field 2	0.3	0.0	40.0	18.4	2.9	31.6	2.8	5.5

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Fungicide Sensitivity of Cucurbit Powdery Mildew Fungus (*Podosphaera xanthii*) on Long Island, NY, in 2010

ABSTRACT
Fungicide resistance can be a major constraint to effectively managing powdery mildew in cucurbit crops. The goal of this study was to obtain information needed to guide fungicide recommendations in 2010 and 2011 by conducting a bioassay in spring squash plantings at the start of powdery mildew development on Long Island, NY, and additional bioassays in pumpkin crops and research plantings. These bioassays provided an indication of which fungicides were likely to be most effective in 2010 and how different fungicide programs being used in commercial fields are affecting pathogen sensitivity to fungicides. Resistance was at a very high level to both FRAC Code 1 and 11 fungicides in 2010; therefore, neither chemistrywas recommended. Boscalid resistance was more common than in 2009 and is the most probable explanation for Pristine, the fungicide containing this active ingredient, being ineffective in 2010 when tested alone at LIHREC and for poor suppression of powdery mildew in some commercial crops on LI. All of the cucurbit powdery mildew populations assayed on Long Island exhibited a very high level of sensitivity to quinoxyfen, the active ingredient in Quintec.

INTRODUCTION

Fungicide resistance can be a major constraint to effectively managing powdery mildew in cucurbit crops. Presently there are five chemical classes of fungicides registered for powdery mildew in cucurbits. They are FRAC Code 1 (Topsin M), 3 (including Rally, Procure, Inspire, and Tebuzol), 11 (including Quadris, Flint, Cabrio, and Pristine), 7 (second active ingredient in Pristine) and 13 (Quintec). Bioassays conducted in previous years on Long Island have provided valuable information to guide fungicide recommendations. Resistance to FRAC Code 1 fungicides was found to be common in both 2008 and 2009. Use of Topsin M on cucurbit crops is thought to have been extremely limited, if used at all, for many years, thus there was a possibility that resistant strains may have disappeared from the pathogen population without selection pressure to maintain this trait. However, resistance remains common in the population. Thus this fungicide class will likely never again be effective for powdery mildew. On the other hand, resistance to Code 11 fungicides was expected to be more common than it was found to be in 2008. Resistance was more common in 2009. According to the bioassay conducted in spring squash crops in 2009 the proportion of the pathogen population estimated to have resistance varied among the plantings from 28-100% for Code 1 fungicides (average of 80%) and 20-100% for Code 11 (62%). Resistance to Code 1 and 11 fungicides is qualitative, which means pathogen strains are either sensitive or completely resistant, and consequently the fungicides are either effective or ineffective, respectively against these strains. Neither fungicide group was recommended in 2009 based on the bioassay results.

Resistance to Code 3, 7, and 13 fungicides is known (or thought) to be quantitative, which means pathogen strains exhibit a range in sensitivity to these fungicides. With this type of resistance it is much more difficult to determine when pathogen strains have reached a level of insensitivity to these fungicides which corresponds to control failure. In 2009 the powdery mildew pathogen exhibited the greatest sensitivity to the Code 13 fungicide (Quintec) with an average of 9% of the population in spring squash plantings and 6% in commercial pumpkin crops tolerating 1 ppm. While 21% and 4%, respectively, tolerated 80 ppm myclobutanil (Code 3 fungicide) and 20% and 11% tolerated 175 ppm boscalid (Code 7). Quintec was recommended as the most important fungicide to use in programs. A third bioassay was conducted on 24 Sep in two commercial pumpkin fields and in research plots of pumpkin that had been treated every week with the same mobile fungicide or with a recommended fungicide resistance management program consisting of the mobile fungicides applied in alternation and tank-mixed with a protectant fungicide. Results from this bioassay document that sole use of a fungicide with resistance risk can select for tolerant strains during one growing season: proportion of the pathogen population tolerating 500 ppm boscalid was 100% for a plot treated with Pristine and 17-20% for a plot and the commercial pumpkin fields receiving a fungicide program. Strains able to tolerate this high concentration would not be controlled with Pristine. Control of powdery mildew in commercial pumpkin crops was moderate to excellent. Monitoring fungicide sensitivity in 2010 was needed to determine if pathogen strains resistant to Code 7 fungicides are present early in powdery mildew development and to determine if changes occur in sensitivity to Code 3 and 13 fungicides.

MATERIALS AND METHODS

A seedling bioassay was used to determine the sensitivity to fungicides of populations of *Podosphaera xanthii* in commercial cucurbit crops in Suffolk Co., NY, as well as in research plantings during the 2010 growing season. Pumpkin seedlings cv 'Sorcerer' produced in a growth chamber were treated with fungicides at several concentrations using a backpack sprayer, the next day put in cucurbit plantings with powdery mildew for about 4 hours (Figure 1), and then kept in a greenhouse at LIHREC for about 10 days until powdery mildew was visible and could be assessed. Amount of mildew on leaves of treated plants was compared with leaves on non-treated plants to estimate the proportion of the pathogen population able to tolerate each fungicide concentration. The bioassay was conducted on 6 Aug in spring-planted summer squash crops where powdery mildew starts to develop first in order to obtain a measure of pathogen sensitivity before fungicides were used (spring squash generally is not treated with fungicides specific for powdery mildew due to how late the disease develops in crop production). It was also conducted on 31 Aug and 21 Sep in pumpkin crops and research fields at LIHREC. Fungicides used in the bioassays represent all of the major chemical classes at risk for resistance currently registered for managing powdery mildew: FRAC Code 1 (Topsin M), Code 11 (Flint), Code 3 (Nova, Procure, Inspire, Tebuzol), Code 13 (Quintec) and Code 7 (Pristine). Farm locations where the bioassays were conducted were designated using a numbering system established for previous projects.

Figure 1: Fungicide-treated seedlings for bioassay in a commercial squash crop.

RESULTS AND DISCUSSION:

Resistance to FRAC Code 1 and Code 11 fungicides were detected at a high level in all spring squash plantings where the bioassay was conducted (Table 1). Resistance to these chemistries is qualitative and cross resistance occurs amongst all fungicides in each group; thus a pathogen strain able to tolerate 50 ppm of any fungicide in each group is completely resistant and would not be controllable with any fungicide in the group. The bioassay results supported not recommending fungicides in these groups in 2010; similar results were obtained in 2009. Surprisingly, resistance to Code 1 fungicides was not detected in pumpkin crops at four of seven farms examined and the proportion of the pathogen population that was resistant was substantially lower than in the spring squash crops (Table 2). Resistance to Code 11 fungicides was high in all crops examined at both assay times.

Table 1: Percentage of powdery mildew populations estimated tolerant to different concentrations (ppm active ingredient) of fungicides in commercial spring plantings of summer squash and zucchini in Long Island on 6 Aug 2010. FRAC Code and product name included for the fungicides tested.

	Code 1	Code 11	Code 7			Code 3			Code 3	Code 3	Code 3	Code 13	
	Topsin M	Flint	Endura			Nova			Procure	Inspire	Tebuzol	Quintec	
Bioassay	Thiophanate-	Trifloxy-	Boscalid			Myclobutanil			Triflumizole	Difenconazole	Tebuconazole	Quinoxyfen	
location	methyl 50	strobin 50	50	175	500	40	80	120	40	40	40	1	10
Farm 4	100	100	100	51	100	100	100	64	100	24	52	0	0
Farm 5	81	83	88	50	32	69	56	28	75	32	92	0	1
Farm 6	100	100	93	8	4	75	34	1	100	0	100	0	1
Farm 11	100	91	91	50	100	100	95	95	100	32	55	1	0
Average	**95**	**94**	**93**	**40**	**59**	**86**	**71**	**47**	**94**	**22**	**75**	**0**	**0**

Strains of the cucurbit powdery mildew pathogen were detected and able to tolerate 500 ppm boscalid (FRAC Code 7), the active ingredient in Endura and an ingredient in Pristine (Tables 1-2). They were detected in most crops. Strains able to tolerate this concentration would be fully resistance to this chemistry because this is in the range of the concentration that would be present when Pristine is applied at the highest label rate to cucurbit crops. Considering that resistance was also found to be high for FRAC Code 11 fungicides, which is the group that the other active ingredient in Pristine belongs to, the potential existed for efficacy of Pristine to be affected by resistance in 2010.

Table 2: Percentage of powdery mildew populations estimated tolerant to different concentrations (ppm) of fungicides in commercial plantings of pumpkin and research plantings of squash and pumpkin on Long Island on 31 Aug 2010.

The cucurbit powdery mildew pathogen populations on Long Island exhibited some tolerance of FRAC Code 3 fungicides. Strains able to tolerate 120 ppm were detected at a high percentage in the spring crops assayed (Table 1). They were less common in pumpkin crops. Based on these assay results, this pathogen exhibits some variation in sensitivity to Code 3 fungicides, and is most sensitive to difenconazole.

All of the cucurbit powdery mildew populations assayed on Long Island exhibited a very high level of sensitivity to quinoxyfen, the active ingredient in Quintec. There was an extremely low level of detection of strains able to tolerate 1 and 10 ppm (Tables 1-3). The frequency of the pathogen population able to tolerate 1 ppm increased from an average of 0.2% on 6 Aug to 6.2% on 21 Sept. Only 0.7% tolerated 10 ppm.

Sensitivity of the pathogen population at the end of the season was often related to fungicide use and fungicide efficacy. This was especially evident for the LIHREC research fields. Proportion of the population able to tolerate 500 ppm boscalid was 70% where Pristine was applied weekly at the lowest label rate and control was ineffective versus 19% in another experiment where no powdery mildew fungicides were applied, and for 1 ppm quinoxyfen the proportions were 75% and 1% (Table 3). These results document why it is important to alternate amongst fungicides at risk for resistance development. Shifts in the pathogen population to higher frequency of resistant strains in response to fungicide use were also detected in commercial fields and associated with efficacy of powdery mildew control. For example, proportion of the population tolerating 500 ppm boscalid was 0% on 31 Aug and 11% on 21 Sep in a pumpkin crop at Farm 6 where control was good while these values in two fields where control was poor were 14% and 70% for Field 1 at Farm 9 and 11% and 52% for Farm 10.

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Susceptibility of *Pectinophora gossypiella* (Lepidoptera: Gelechiidae) and *Cryptophlebia leucotreta* (Lepidoptera: Tortricidae) to insecticides used on cotton crops in Côte d'Ivoire, West Africa. Implications in insecticide resistance pest management strategies

ABSTRACT
The pink bollworm *Pectinophora gossypiella* and the false codling moth *Cryptophlebia leucotreta* are two major cotton pests in Côte d'Ivoire, West Africa. However, unlike the cotton bollworm *Helicoverpa armigera* which was shown to be resistant topyrethroids, there is a lack of knowledge on the status of the susceptibility to insecticides in *P. gossypiella* and *C. leucotreta*.

Bioassays were carried out on adults of these two cotton endocarpic species in order to establish their susceptibility to major insecticides used on cotton crops. With regard to single insecticides, the LD_{50} values in *P. gossypiella* and *C. leucotreta* were respectively, 505.98 µg/g *vs* 41.36µg/g for cypermethrin, 215.87µg/g *vs* 103.41µg/g for Triazophos and 11049.06 µg/g *vs* 3798.23µg/g for acetamiprid. Concerning the mixtures, the LD_{50} values in both insects were respectively 147.40µg/g *vs* 29.40 µg/g for cypermethrin-triazophos and 487.51 µg/g *vs* 157.45 µg/g for cypermethrin-acetamiprid. Data indicated that *P.gossypiella* was 2 to 12-fold more resistant than *C. leucotreta* according to the nature of the single or the mixture insecticides; a synergistic effect was obtained with the association cypermethrin-triazophos in both species while this effect was not clearly determined with cypermethrin-acetamiprid. The relative resistance of *P. gossypiella* compared to *C. leucotreta* recommends a resistance monitoring plan especially in the former in order to anticipate or to prevent cases of resistance to the main insecticides used on cotton. In addition, the synergistic effect of the mixture triazophos-cypermethrin in both bollworm species shows the interest in keeping for the fruiting stage such pyrethroid-organophosphate associations in the current pyrethroid resistance management program enforced against *H. armigera* in Côte d'Ivoire since 1998.
Keywords: *Cryptophlebia leucotreta*, *Pectinophora gossypiella*, *Helicoverpa armigera*, insecticides, resistance, cotton, Côte d'Ivoire.

INTRODUCTION

In West Africa, the control of cotton pest by chemicals is an important yield determining factor as cotton crop is submitted to intense and diverse arthropod pest complex, susceptible to cause 40-60% yield losses depending on the cotton growing areas and years (Cauquil & Vaissayre, 1994; Vaissayre & Cauquil, 2000). The use of pyrethroid insecticides for the last 30 years on cotton crops in Côte d'Ivoire has evolved to a selection of resistant individuals in *Helicoverpa armigera* (Hübner) (Ochou &*al*., 1998). With this regard, the current pest control strategy in Côte d'Ivoire is based on pyrethroid resistance management in *H. armigera*. To reduce the selection pressure due to pyrethroids during the cotton growing period, the resistance management strategy recommends a restriction period in which pyrethroids are replaced by alternative chemicals with different mode of action such as endosulfan, profenofos, indoxacarb, spinosad, etc. (Ochou & Martin, 2001). Subsequently, the use of pyrethroid-organophosphorus synergistic associations is allowed only after August 10th during the fruiting stage of cotton. With this respect, while this strategy has proved very effective on *H. armigera*, it does not allow an effective control of other important pests, such as the Pink Bollworm *Pectinophora gossypiella* (Saunders) and the False Codling Moth *Cryptophlebia leucotreta* (Meyrick). These two cotton bollworms, behaving as endocarpic species, have shown for years a regular predominance in cotton fields in Côte d'Ivoire (Ochou, 1994; Doffou, 2005). The increasing field populations of these bollworms for the last decades raise questions about their resistance status to insecticides currently used on cotton. Indeed, unlike the cotton bollworm *H. armigera*, there is a lack of knowledge on the status of susceptibility to insecticides in *P. gossypiella* and *C. leucotreta*. The uncertainty on the insecticide resistance status in these two pests requires more research investigations in order to strengthen or to improve the current cotton resistance management program developed for years in Côte d'Ivoire. Accordingly, determining the insecticide susceptibility in *P. gossypiella* and *C. leucotreta* is essential. In fact, while it is known that these two endocarpic bollworm species are similar in their feeding habits, there is no clear evidence that they have the same response facing insecticides as each species presents a very different biology.

As monitoring pest susceptibility to insecticides is a key component for any resistance prevention program (Alaux, 1994), the objective of the present study is to establish the susceptibility of two emerging cotton pests, *C. leucotreta* and *P. gossypiella*, to three broadly used chemical classes (pyrethroids, organophosphorus, neonicotinoids). The data will be used as reference data to which future observations will be compared in order to elucidate or to anticipate the phenomenon of resistance in pests other than *H. armigera* on cotton crops in West-Africa. Bearing in mindthe prescription of a more appropriate strategy option to tackle actual pest problems, this study aims at improving the current pyrethroid resistance management program developed in Côte d'Ivoire.

MATERIALS AND METHODS

Insects. Strains of the Pink Bollworm *P. gossypiella* and the False Codling Moth *C. leucotret*a were collected on untreated cotton plots grown in Adiopodoumé, Abidjan. Cotton bolls were regularly collected and sent to the laboratory. Larvae were extracted from bolls and reared in a climatic cell, until the stage of butterfly is reached. Insects were maintained in cells at 25°C and 75% Relative Humidity. Moths are fed on 10% honey solution until the bioassays were performed.

Insecticides. Three technical grade insecticides of different classes were used for bioassays purposes: a pyrethroid insecticide (cypermethrin 93.4%), an organophosphorus (triazophos 71.94%) and a neonicotinoid insecticide (acetamiprid 97.7%). The technical grade samples were graciously provided by Bayer CropScience, Côte d'Ivoire.

Topical bioassays. The technical active ingredients are diluted in acetone. The mixtures of these insecticides were made at the following ratios as used in the field: 1: 4.16 for the association cypermethrin-triazophos, referring to 36 g/ha of cypermethrin and 150 g/ha triazophos; and 4.5: 1 for the association cypermethrin-acetamiprid, corresponding to 36 g/ha of cypermethrin and 8 g/ha acetamiprid. Five to six doses expressed in µg a.i/g insect have been tested for each insecticide

tested. The range of doses used in laboratory bioassays was set to cause a range in mortality between 0% and 100% after 48-h of exposure to insecticides. The tests were carried out on one-day old first generation individual moths weighing between 10 and 20 grams, regardless of gender. Insects were kept in a state of sleep with an ether solvent as an anesthetic. Batches of thirty (30) insects were treated per insecticide and per dose. Insects were placed individually in vials of 40 ml and held at 25°C and 75% RH. Acetone or water alone was used in the control treatments. Three replicates were tested for each insecticide dosage comprising acetone treated controls. The topical tests were performed with a Burkard Arnold Microapplicator Type LV 65, by applying 1µl of insecticide solution directly on the thorax of individual moths held between the thumb and the forefinger. Mortality was recorded after 48 h, and insects were considered dead if they did not move after prodding or were unable to right themselves 5 s after being turned upside down.

Analysis of dose-response data. The mortality data were subjected to probit analysis, using the software Win DL_{50} version 2 (CIRAD-CA Montpellier, France, 1999). The LD_{50} and LD_{90} values of single and mixtures insecticides and the slopes of dose-mortality regression lines were generated. Pearson's chi-square test was used to determine the fit of the statistical model (Finney, 1971). Data from all bioassays were corrected using Abbott's formula (1925). Trials were removed from analysis when the mortality in the controls exceeded 10%. The resistance ratio ($RR_{PBW/FCM}$) between the two species was calculated by dividing the LD_{50} value of the pink bollworm *P. gossypiella* by the LD_{50} value of the false codling moth *C. leucotreta*.

Synergism analysis. The combination-index method of Chou and Talalay (1984) was used here to determine cases of synergism or antagonism between cypermethrin and triazophos on the one hand, or between cypermethrin and acetamiprid on the other hand, according to the strains of *C. leucotreta* and *P. gossypiella.* This method is based on the doseeffect of each product used alone and in a mixture. The combination-index (*CI*) for quantifying synergism was calculated as follows:

$$CI_x = \frac{LD_x^{A(m)}}{LD_x^A} + \frac{LD_x^{B(m)}}{LD_x^B} + \frac{LD_x^{A(m)}}{LD_x^A} \times \frac{LD_x^{B(m)}}{LD_x^B}$$

- $LD_x^{A(m)}$ and $LD_x^{B(m)}$ are lethal doses of A and B used in the mixture m giving the mortality x ;
- LD_x^A and LD_x^B are lethal doses of products A and B required to produce the same mortality x when used alone.
- When two insecticides have an additive effect, the combination index value *CI*=1
- When two insecticides are synergistic, the lethal dose of the mixture is lower than expected and *CI*< 1.
- When two insecticides are antagonistic, toxicity of the mixture is higher than expected and *CI*> 1.

RESULTS

Susceptibility of *P. gossypiella* and *C. leucotreta* to single insecticides: cypermethrin, triazophos and acetamiprid

Data on Table 1 indicated that the LD_{50} values for the specific toxicity of each product in *P.gossypiella* and *C. leucotreta* were respectively, 505.98µg/g *vs* 41.36µg/g for cypermethrin, 215.87µg/g *vs* 103.41µg/g for triazophos and 11049.06 µg/g *vs* 3798.23µg/g for acetamiprid.

Cypermethrin appeared to be the most toxic insecticide in *C. leucotreta* while triazophos showed the highest toxic effect in *P. gossypiella*. Besides, cypermethrin was twice more toxic than triazophos and 90 more toxic than acetamiprid in *C. leucotreta*; meanwhile, triazophos was twice more toxic than cypermethrin and 50 more toxic than acetamiprid in *P. gossypiella*.

Table 1: Toxicological response of C. leucotreta and P. gossypiella strains to single insecticides of different chemical classes: cypermethrin (pyrethroid), triazophos (organophosphate) and acetamiprid (neonicotinoid)

Insecticides	Strains	N	χ^2	df	Slope	LD_{50}	$RR_{PBW/FCM}$	CI	LD_{90}
Cypermethrin	*P. gossypiella*	240	9.27	7	1.13	$5.06x10^2$	12.22	$3.26x10^2$-$7.86x10^2$	$6.90x10^3$
	C. leucotreta	330	13.18	9	0.92	41.36	-	23.94-69.84	$1.02x10^3$
Triazophos	*P. gossypiella*	210	3.63	7	3.31	$2.16x10^2$	2.09	$1.81x10^2$-$2.57x10^2$	$5.26x10^2$
	C. leucotreta	240	4.59	6	2.62	$1.03x10^2$	-	57.40-217.54	$3.18x10^2$
Acetamiprid	*P. gossypiella*	330	13.99	10	1.63	$1.10x10^4$	2.90	$8.72x10^3$-$1.43x10^4$	$6.73x10^4$
	C. leucotreta	240	6.31	7	2.21	$3.79x10^3$	-	$2.23x10^3$-$6.24x10^3$	$1.44x10^4$

N : number of adults tested χ^2 : chi2 **df** : degree of freedom **LD_{50}** and **LD_{90}** : lethal doses for 50% et 90% of adults **CI** : confidence interval **$RR_{PBW/FCM}$**: resistance ratio of PBW/FCM at LD_{50}

With regard to comparative reaction between the two insects to each product, *P.gossypiella* appeared 2-12 more resistant than *C. leucotreta*. According to insecticide classes, estimate resistance ratios between the two species were 12.22 for cypermethrin, 2.90 for acetamiprid and 2.09 for triazophos. With respect to each product, the similar slope values of regression lines shown in both pests, suggests the same mode of action for each chemical class tested.

Susceptibility of *P. gossypiella* and *C. leucotreta* to insecticide associations: cypermethrin-triazophos and cypermethrin-acetamiprid

Data on Table 2 indicated that the LD_{50} values for the specific toxicity of each mixture in *P.gossypiella* and *C. leucotreta* were, respectively, 147.40 µg/g *vs* 29.40 µg/g for cypermethrin-triazophos and 487.51 µg/g *vs* 157.45 µg/g for cypermethrin-acetamiprid.

Table 2: Toxicological response of *C. leucotreta* and *P. gossypiella* strains to two types of insecticide mixtures: cypermethrin-triazophos (pyrethroid-organophosphate) and cypermethrin-acetamiprid (pyrethroid-neonicotinoid).

Insecticides	Ratio	Strains	N	χ^2	df	Slope	LD_{50}	CI_{50}	$RR_{PBW/FCM}$	LD_{90}	CI_{90}
Cypermethrin + triazophos	1:4.2	*P. gossypiella*	210	2.05	7	3.15	147.40	0.64	5.06	$3.75\ 10^2$	0.60
		C. leucotreta	210	3.16	6	1.25	29.40	0.39	-	$3.08\ 10^2$	1.73
Cypermethrin + acetamiprid	4.5:1	*P. gossypiella*	270	15.57	9	1.01	487.51	0.80	3.10	$8.94\ 10^3$	1.10
		C. leucotreta	210	2.99	6	1.50	157.45	3.14	-	$1.05\ 10^3$	0.85

N : number of adults tested χ^2 : chi2 **df** : degree of freedom **LD_{50}** and **LD_{90}** : lethal doses for 50% et 90% of adults ***CI_{50}***and ***CI_{90}*** : Combination index at lethal doses for 50% and 90% of adults
$RR_{PBW/FCM}$: resistance ratio of PBW/FCM at LD_{50}

In *P. gossypiella*, the toxicity of the mixtures cypermethrin-triazophos and cypermethrin-acetamiprid based on their LD_{50} was significantly higher than the toxicity obtained for single products. The values of the combination indexes CI_{50} (0.64 and 0.80) and CI_{90} (0.60 and 1.10), obtained respectively for cypermethrin-triazophos and cypermethrin-acetamiprid mixtures in *P. gossypiella*, were close to 1 indicating an additive effect between the two products.

In *C. leucotreta*, the mixture cypermethrin-triazophos showed higher toxicity than the single products; in contrast, the mixture cyperméthrine-acetamiprid appeared 10 fold less toxic than cypermethrin alone (157.45µg/g against 41.36µg/g). The values of the combination indexes CI_{50} (0.39 and 3.14) and CI_{90} (1.73 and 0.85) obtained respectively for cypermethrin-triazophos and cypermethrin-acetamiprid mixtures in *C. leucotreta*, appeared very conflictual, indicating by the same time at lower dosages some additive effect for the mixture cypermethrin-triazophos and some antagonistic effect for the mixture cypermethrin-acetamiprid.

DISCUSSION

The toxicity of cypermethrin (pyrethroid insecticide), triazophos (organophosphate insecticide) and acetamiprid (neonicotinoid insecticide) based on their LD_{50} and LD_{90}, showed different susceptibility levels in each of the two endocarpic species. In addition, the parallelism observed in the regression lines for each insecticide confirms that each class of insecticide acts by the same mode of action in both pests*C. leucotreta* and *P. gossypiella*.

The LD_{50} and LD_{90} results showed that the pyrethroid cypermethrin has been very effective on *C. leucotreta* and to a lesser extent on *P. gossypiella*. According to Sweet and Hollings (1983), cypermethrin is very effective on the *C. leucotreta* strain in South Africa. These results confirm the pyrethroid action (Martin & Renou, 1995). The relative resistance of *P. gossypiella* as compared to *C. leucotreta* may be explained by the feeding habit of the two pests. In fact, *P. gossypiella* is specifically limited to the family Malvaceae, unlike *C. leucotreta*, a highly polyphagous species, having a large number of host plants. The former insect tied mostly to cotton crops has been likely submitted for years to regular insecticide treatments in cotton, acquiring therefore some relatively specific resistance, while the polyphagous feeding habit of the latter would enable the dilution of its resistance through diverse non treated host plants such as maize or carambola fruits.

Triazophos was less toxic than cypermethrin in *C. leucotreta* and more toxic to *P. gossypiella* than cypermethrin. This high toxicity of triazophos on *P. gossypiella* showed that this strain of *P. gossypiella* would be tolerant to pyrethroids, because according to Martin (2003), triazophos would be 3 fold more toxic to a resistant strain of *H. armigera* selected with deltamethrin. However, another explanation could arise from the mechanism of toxicity of triazophos. Indeed, the toxicity of triazophos is mainly explained by the formation of triazophos-oxon by oxidative desulfurization (Champ, 1985). Several factors influence the toxicity of such an organophosphate, like all phosphorothioates (Chambers & Carr, 1995; El-Merhibi &*al.*, 2004). This suggests that the high susceptibility of *P. gossypiella* to triazophos as compared to *C. leucotreta* may be due to a large number of target sites available for the metabolism of triazophos in its oxon shape in *P. gossypiella*.

Cypermethrin and triazophos were more toxic than acetamiprid in both *C. leucotreta* and *P. gossypiella*. This can be explained by the insecticidal specificity of acetamiprid which is known as more aphicide and aleurodicide than lepidoptericide (Ochou &*al.*, 1998). The neonicotinoid acetamiprid, appeared on the market since 1990 (Nauen, 2006), are shown to be selective with the specificity of the nicotinic receptors in insects (Tomisawa & Casida, 2002) and therefore the insecticide acts at the postsynaptic nicotinic receptors (Regnault-Roger, 2005).

Furthermore, it has been noticed during testing that under the effect of ether anesthesia, *C.leucotreta* spend more time asleep than *P. gossypiella*. This hyper

susceptibility of *C. leucotreta* with ether could also explain its high susceptibility facing toxic products. According to Nicolas and Sillans (1989), immobilization of bees by anesthesia before the topical application of toxic, intensifies the action of active ingredients. Anesthesia would thus cause directly on the nervous system, an effect of depolarization of the neuron.

The fact that the combination indexes (*CI*) for the mixture cypermethrin-triazophos in *C. leucotreta* and *P. gossypiella* are below 1 (CI_{50} = 0.39 and 0.64 respectively) suggests a synergism between the two products involved. In fact, the LD_{50} values of the mixture cypermethrin-triazophos in *C. leucotreta* and *P. gossypiella* are lower than the LD_{50} of the two active ingredients taken individually. Similar results were obtained on other species such as *H. armigera* (Vaissayre & Lucas-Chauvelon, 1989; Martin & Jacquemard, 1991) and *Spodoptera littoralis* (Martin & Jacquemard, 1991). This synergy could be explained either by competition for the organophosphate oxidase (Feyereisen, 1999; Martin, 2003) or by inhibition of esterases (Gunning &*al*., 1999) that would cause a slower degradation of pyrethroids. However, the CI_{90} values obtained indicate that the effectiveness of this mixture is interesting to moderate doses.

As for the mixture cypermethrin-acetamiprid, the value of CI_{50} (3.14) in *C. leucotreta* indicates some antagonism while in *P. gossypiella* the value of CI_{50} (0.80) shows an additive effect. The antagonist effect between the two products in *C. leucotreta* could be detrimental to cypermethrin as its toxicity was 4-fold reduced whereas acetamiprid was 24-fold magnified. These data suggest that such insecticide association would likely be effective on pyrethroid resistant individuals. In addition, they would be more effective at high doses with regard to the calculated CI_{90} (0.85) expressing an additive effect.

The lack of susceptible strains of *P. gossypiella* and *C. leucotreta*, and the missing of reference LD_{50} data in these two bollworm pests in Côte d'Ivoire, represent a true handicap in analyzing data. Nevertheless, the interest of present study is to serve as a baseline data which will help clarify in the coming years the trend in the change of the pest susceptibility to major insecticides used on cotton crops in Côte d'Ivoire.

CONCLUSION

This study shows that *P. gossypiella* is more resistant than *C. leucotreta* to the three insecticides tested (cypermethrin, triazophos, acetamiprid) and to their binary associations with cypermethrin. Meanwhile, *P. gossypiella* appeared to be more susceptible to triazophos than cypermethrin. In fact, the triazophos-cypermethrin mixture is more toxic in both endocarpic bollworms, as it has been observed on *H. armigera*. Accordingly, the addition of an organophosphate such as triazophos synergizes the effect of the pyrethroid insecticide and increases its effectiveness in bollworm species. In contrast, the association of cypermethrin-acetamiprid appears to be more effective against *P. gossypiella*, as it happen to be more efficient against insect strains resistant to pyrethroids. The two types of insecticide mixture should be part of the current insecticide resistance management strategy with a window program advising, the use of pyrethroid based insecticides only during the fruiting stage of cotton crops. The baseline data established for these major insecticides used in cotton crop in West-Africa are a fundamental step in further resistance study, as these data will serve as a basis for future comparison which will allow researchers to detect any changes in insecticide resistance levels in emerging pests. In addition, baseline data permit the selection of diagnostic doses, which can be used more conveniently to detect or monitor insecticide resistance in the field. Further investigation need to be done in order to elucidate the type of mechanism that could be involved in insecticide resistance in these two major pests in Côte d'Ivoire. The use of synergists such as piperonyl butoxide (Pbo) and S, S, S-Tributyl phosphorotrithioate (DEF) will help reach this objective.

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WHITE GRUBS (*Cyclocephala comate*) SUSCEPTIBILITY TO DIFFERENT INSECTICIDES IN SAN MARTIN HIDALGO JALISCO, MEXICO

ABSTRACT

In the state of Jalisco soil pests infest an area of approximately 200,000 ha, mainly concentrated in the downtown area, which are temporary in most these areas are most affected by soil insects, due to weather conditions (Felix, 1978, 1988). The main forms of pest management using the farmer is the cultural control and chemical control, usually used to protect the root area of cultivation of this plague, because the above are in the market new and different insecticides, which have been used indiscriminately without taking into account the allowable minimum dose producing resistance in pest management (PLM, 2003).

Key words: Infestation, larvae, damage, insect pests

INTRODUCTION

The group of pests that attack the root consists of the following categories: Colaspis chapalensis, Diabrotica virguifera and grub complex consists mainly of the genera: Colaspis chapalensis, Diabrotica virguifera and grub complex consists mainly of the genera: Cyclocephala comata Bates and Phyllophaga spp. (Morón, 1986, 1988, 1990, 2001). Phyllophaga larvae represent major plant health problems in different crops grown in the state such as, corn, grasses, vegetables; it can attack any crop at different phenological stages, and its critical period of 0 to 60 days. It is considered that if this pest is not controlled effectively can lead to losses of approximately 70% of normal production (Pérez, 1987). At first the new insecticides showed acceptable efficiency and through the years, performance levels have decreased significantly, therefore, recommended doses have increased consecutively without obtaining favorable results on the control of these insects. Recent years have showed that soil pests and Cyclocephala Phyllophaga present high levels of resistance to various insecticides used for their management (Posos, 1993). The above situation gave room to the completion of this work to ascertain the levels of this pest susceptibility to different insecticides used for control. The objective is to determine the susceptibility of white grubs at different insecticides used for control.

MATERIALS AND METHODS

The biological material of the population of larvae were obtained from commercial fields of crops grown in San Martin Hidalgo, Jalisco, during the cycle P / V 2009, which were placed in polyethylene bags, which were previously added proportions mixed with ground corn sprouts, while similar conditions were provided to their environment to be transported to the laboratory. Once collected the larvae were transferred to the laboratory and placed in plastic boxes with soil and organic matter, which provides food, selecting the third instar larvae according to size so that they were as evenly as possible. They were weighed and separated into groups of 20 larvae and were placed in disposable polypropylene boxes with a mixture of soil and organic matter that collected during several consecutive days an average of 1500 larvae. To determine the median lethal dose (LD50) of larval grubs, a topical application technique proposed by the FAO was used which consisted of applying a microliter of insecticide solution in acetone on the dorsal side of the larva using a micropipette and mortality was assessed at 24 h after treatment. The insects were placed in polypropylene boxes and provided with fresh food. Determining the criterion of death primarily consists of a group of bioassays with 10 larvae and four observation units to which they were given a dose of insecticide above a high dose.

These groups of larvae were observed for 48 h to establish the symptoms of intoxication that they show during the death process.

Treatments evaluated

For each treatment, insecticides extracted active ingredients of commercial products by dilution with acetone 99% pure reagent according to the weight ratio of 5 volumes using dose ranges, preparing 10 ml of each dose for each treatment. The products were diluted in acetone with 99% technical grade reagent grade putting on a shaker for 24 h.

Bioassays with acetone. Bioassays were run with ten larvae in four observation units with 99% acetone reagent grade purity. Each larva was treated with a microliter and mortality was taken after 24 h treated. In this way you can determine any negative effect of solvent on the insect.

Determination of median lethal dose (LD50). To determine the LD50 of each of insecticides involved and identified in the above table, tests were run using 5 ranges of doses and 20 larvae for each dose with 2 units of observation.

Experimental model. In each of the bioassays, we ran 5 doses discriminatory and an untreated. To determine each of the doses in question we used 20 larvae taken at random (individuals observed). When mortality occurred in the control with acetone, it was corrected by Abbott's formula (1925) that is:

$$\% \text{ Mortality} = \frac{\text{larvae live in the witness - live larvae in the treatment}}{\text{live larvae in the control}} \times 100$$

Once the bioassays and calculated the mortality data, these were transformed LC_{50} median lethal concentration expressed in ppm to LD_{50} median lethal dose expressed in mg / g body weight olive larvae in the control of the larva. Once the data plotted on a logarithmic scale mortality was observed if the dispersion cloud corresponds to a linear model, and then analyzed using the statistical method of Maximum Likelihood Probit analysis proposed by Finney (1971) cited by (and Vázquez Lagunes , 1994). These Probit analyses were performed using the computer program (SPSS, 2001).

RESULTS

Description of symptoms of death of the larvae. The symptoms presented by the larvae of C. comata by which established the criteria killed 24 h after the application of insecticides is:

Loss of mobility of the larva at 3 hours after applying the poison; After 6 hours after applying the insecticide

lost its larva form of "C" and took a yellow color hyaline; At 12 h the larvae lost turgor and movement, becoming more intense yellow color; After 24 h, the movement of the larvae was almost nil, became limp and light brown; After 48 h the larvae reached death and it became dark, tending to black and disintegrated easily in the environment in which it was, the criterion of death which was evaluated taking the symptoms appeared after 24 h of applied insecticides. Regarding the reliability of the results, Table 2 shows that the regression equations are positive and the coefficients of determination and probability. All treatments had acceptable coefficients of determination as they were above the 0.80 except terbufos and carbofuran remaining in 0.70. Note that the confidence level was high, with probability above 95% reliability which means that if you repeat the work of a hundred times we would get similar results for 92% of cases.

Table 2: Statistical estimates of the regression lines dose-mortality of different insecticides used for control white grubs (Phyllophaga sp) in San Martin Hidalgo, Jalisco. Mexico.

Treatment	Regression Equation	R^2	GL	P
1. Terbufos	Y= 54.5 + 3.66X	0.70	4	92
2. Clorpirifos	Y= 23.9 + 5.31X	0.83	4	97
3. Fipronil	Y= 60.0 + 3.39X	0.97	4	99
4. Carbofuran	Y= 45.83 + 3.48X	0.77	4	95
5.Tebupirimipos	Y= 23.7 + 6.60X	0.93	4	99

R^2 = coefficient of determination, GL = degrees of freedom, P = Probability (%)

Table 3 shows the median lethal doses (LD50) of the insecticides evaluated in the town of San Martin Hidalgo, to estimate the susceptibility of Phyllophaga larvae, showing that the population was very sensitive to presenting Fipronil LD50 of 0.48 mg / g of body weight. We followed the treatments with terbufos and carbofuran LD50 and that was 1.8 mg / g of body weight. The treatments on the larvae showed that little grubs were sensitive to treatment with chlorpyrifos and Tebupirimifos with an LD50 of 3.0 micrograms per gram of body weight, these being the least sensitive. In the case of the insecticide terbufos, the LD95 was the lowest with 13.5 mg / g of body weight, followed by treatment with 24.0 mg chlorpyrifos / g of body weight. On the other side with a very similar LD95 were Fipronil and Tebupirimifos with 32 mg / g body weight, and finally treatment with carbofuran was the one that required the highest dose of 42 mg / g of body weight.

Table 3: Response of third instar larvae of Phyllophaga sp to different insecticides in populations exposed to chemical control in San Martin Hidalgo, Jalisco.

Treatment	DL_{50}*	Fiducial limits 95% of confidence	DL_{95}*	Slope
1. Terbufos	1.0396	(0.7547-1.3168)	13.57	3.662±1.390
2. Clorpirifos	3.1861	(2.7001-3.7583)	24.45	5.314±1.388
3. Fipronil	0.4886	(0.1798-0.8182)	34.86	3.3885±0.329
4. Carbofuran	1.4937	(1.059-1.9312)	42.70	3.489±1.099
5.Tebupirimipos	2.7597	(2.2975-3.2971)	32.41	6.604±1.003

DISCUSSION AND CONCLUSION

It should be noted that insecticides such as carbofuran, chlorpyrifos, terbufos and Tebupirimifos are products that have on the market for several years, so their level of susceptibility has decreased sharply and its behavior is very similar in all four cases, however in the case of Fipronil the trend line is very different from the above treatments where you can see that the dose range is a wider basis of very small doses. This response may be due to the fact that the population of grubs has had little exposure to chemical control Fipronil therefore is very sensitive.

Conclusions

Concentrations of 6.000 and 8.000 ppm were observed over control of 85%. The favorable response to Probit analysis was obtained from 500 ppm, with mortalities of 15 to 40%, with 6000 and 8000 ppm mortality was 85% to 100%. The fipronil-based products in the towns of San Martin Hidalgo represent one of the best options for soil pest control because in that town the population of white grubs was very sensitive.

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Bioefficacy of Newer Insecticides against Groundnut Leaf miner *Aproaerema modicella Deventer* in Northern Dry Zone of Karnataka

ABSTRACT
The study was conducted during Summer-2009 at ARS, Bagalkotin Randomized Block Design (RBD) with 16 treatments including an untreated check. The highest mean larval mortality was noticed in the case of monocrotophos, followed by dichlorvos and quinalphos in both first and second round of spray applications. Lowest mortality was noticed in the case of neem oil. Significantly higher yields were obtained in all the insecticidal treatments ranging from 25.60 q per hectare to 32.26 q per ha and 2.00 t per ha to 6.00 t per ha of pod and fodder yield respectively, except NSKE and neem oil (18 q/ha, 1.33 t/ha and 20.00 q/ha, 0.70 t/ha respectively) which were on par with untreated check. Maximum benefit, for every rupee spent on plant protection was obtained by spraying with monocrotophos 36 SL @ 1ml/l (5.43). However quinolphos 25 EC @ 2ml/l (5.41) or fenvalerate 20 EC @ 0.5 ml/l or thiodicarb 75 WP @ 0.6 gm/l (4.77) or spinosad 48 SC @ 0.1 ml/l (4.71) were the next best treatments and were found to be effective and economic for the management of groundnut leaf miner.

Key words: *Aproaerema modicella*, Insecticides, Groundnut, Northern Dry Zone

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is a leading oilseed crop in India and an important oilseed crop of tropical and subtropical regions of the world. Groundnut is a native of Brazil and was introduced into India in the sixteenth century. The most important groundnut growing countries are India, China, Nigeria, Sudan and USA. It is grown over an area of 24.7 million hectares with a total production of 33 million tonnes in the world. In India, groundnut occupies an area of 6.9 million ha. with total production of 5.3 million tonnes. Its cultivation is mostly confined to the southern Indian states, viz., Gujarath, Andra Pradesh, Karnataka, Tamil nadu and Maharashtra accounting more than 80 per cent of the total production and acreage. The other important states where it is grown are Madhya Pradesh, Rajasthan, Uttara Pradesh and Punjab. In Karnataka the major growing districts are Chitradurga, Dharwad, Kolar and parts of Bellary, Raichur, Koppal, Yadgir and Gulbarga. In recent days more area is occupied by groundnut in upper Krishna area project of Raichur, Gulbarga and Bagalkot districts.

The low level of groundnut productivity in India is largely because the crop is raised under rainfed /dry land conditions. Groundnut is considered by farmers as the most remunerative crop with relatively low chance

of crop failures despite an unpredictable monsoon. But the insect pests and diseases form the important constraints in its production. Collor rot and tikka are important diseases, while white grubs (*Holotrichia* spp.), thrips and leaf miner (*Aproaerema modicella*) are the important insect pests of groundnut.

The groundnut leaf miner, *Aproaerima modicella* Deventer, belongs to family Gelechiidae, order Lepidoptera. It is an oligophagous pest and feeds only on leguminous host plants and a serious pest of groundnut in both rainy and post rainy season in India and of groundnut and soybean in South and South East Asia.

A wide range of insecticides belonging to all the groups of commercial insecticides such as chlorinated hydrocarbons, carbamates, organophosphates, pyrethroids, antifeedants and plant products have been evaluated and found effective against GLM in groundnut.

MATERIALS AND METHODS

A field experiment was laid out during January 2009 in Randomized Block Design (RBD) with 16 treatments including an untreated check. Each treatment was replicated thrice. Variety TMV-2 of groundnut was sown in plots of 5.0 x 3.0 m size maintaining the spacing of 30 cm between rows and 10 cm from plant to plant. Maize was grown as a barrier crop to avoid drift of insecticides from one treatment plot to another. The recommended package of practice was followed except the plant protection measures. The treatment details are presented in Table 1. High volume knapsack sprayer was used for application of spray fluid.

Table 1: Treatment Details of insecticides used in the management of groundnut leaf miner

Treatments	Chemical name	Trade name	Concentration
T_1	Monocrotophos	Nuvacron 36 SL	1 ml/l
T_2	Dichlorvos	Nuvan 100 EC	0.5 ml/l
T_3	Profenophos	Curacron 50 EC	2 ml/l
T_4	NSKE	Neem guard	5%
T_5	Neem oil	Neemegon (10,000 ppm)	2 ml/l
T_6	Methyl parathion	Metacid 50 EC	1 ml/l
T_7	Spinosad	Tracer 45 EC	0.1 ml/l
T_8	Indoxicarb	Avant 14.5 SC	0.3 ml/l
T_9	Acephate	Starthane 75 SP	1 g/l
T_{10}	Flubendiamide	Fame 480 SC	0.2 ml/l
T_{11}	Thiodicarb	Larvin 75 SP	0.6 gm/l
T_{12}	Methomyl	Lannate 75 SP	1 g/l
T_{13}	Buprofezin	Applaud 25 SC	1 ml/l
T_{14}	Fenvalerate	Sumicidin 20 EC	0.5 ml/l
T_{15}	Quinalphos	Ekalux 25 EC	2 m l/l
T_{16}	Untreated check	-	-

Observation on larval counts was made on 10 randomly selected plants from each treatment at 1 day before and 1, 3, 5, 7 & 15 days after each treatment spray. Based on the larval survival number at each spray application, per cent reduction in larval population was calculated by using the formula

= [(initial larval population – live larval population after every observation) /initial population x 100].

After the per cent reduction larval population was transformed into arcsine values and subjected for statistical analysis. The per cent reduction in larval mortality over untreated check was calculated by using the mean of the 1,3,5,7 and 15 days after spray application – mean of the untreated check which were calculated in both first and second round of spray application.

After the crop attained maturity it was harvested, the pods and fodder were kept separated from each treatment, dried properly and pod and fodder weight was recorded. Further, the plot wise yield was computed on hectare basis for statistical interpretations. Additional pod and fodder yield were worked out over untreated check.

The cost of cultivation was worked out as per the recommended package of practices. The economics of different treatments was worked out based on the pod and fodder yield and cost of protection. Treatment cost

was added to each treatment, then sale price of the pod and fodder was also considered to work out gross profit. Based on the cost of cultivation and the gross profit of economy of different treatments, the CBR was also calculated.

$$CBR = \frac{NR \text{ (Net returns)}}{COC \text{ (Cost of cultivation)}}$$

RESULTS AND DISCUSSION

The results of this investigation revealed that of the 15 insecticides tested thirteen insecticides have registered fairly high level of larval mortality ranging from 19.47 to 64.10 per cent excepting the neem oil and NSKE at 1 day after first round of application (Table 2 and 3). However, after 3 days application consistently the higher level of mortality (65.12 to 100%) was again with the insecticides monocrotophos, dichlorvos, profenophos, spinosad, methyl parathion, thiodicarb, indoxicarb, acephate and fenvalerate. The IGR's (Flubendeamide and Buprofezin) and botanicals (NSKE and neemoil) could not establish their efficacy after 3 days of application and at 7 days after application also same trend was followed in all the insecticides show in a higher larval mortality (29.58 to 100% and 34 to 100%) except IGR's and botanicals. After 15 days the larval mortality slowly decreases (30.70 to 90.60%) in all the treatments. The present findings are in support with the findings of Abdul Kareem *et al.* (1974), Abdul Kareem and Subramanian (1976), Sangappa and Ali (1977), Peter and Sundararajan (1991) and Mishra (1995).

Table 2: Bioefficacy of different insecticides on the larval mortality of groundnut leaf miner

Sl No.	Treatments	Initial population/10 plants (45DAS)	First application					Mean Per cent larval mortality	Percent reduction over untreated control
			Per cent larval mortality after						
			1 DAT	3 DAT	5 DAT	7 DAT	15 DAT		
1	Monocrotophos 36 SL @ 1.00 ml/l	11.70 (20.00)a	64.10 (53.17)a	100.00 (89.96)a	100.00 (89.96)a	100.00 (89.96)a	90.60 (72.12)a	90.94 (72.45)a	74.67
2	Dichlorvos 100 EC @ 0.5 ml/l	11.20 (19.54) a	48.21 (43.96)f	100.00 (89.96)a	100.00 (89.96)a	100.00 (89.96)a	85.71 (67.77)b	86.78 (68.65)b	70.51
3	Profenophos 50 EC @ 2 ml/l	11.30 (19.65) a	46.90 (43.21)g	100.00 (89.96)a	100.00 (89.96)a	100.00 (89.96)a	82.30 (65.09)c	86.84 (67.87)c	69.67
4	NSKE @ 5%	11.30 (19.73) a	17.70 (24.87)i	33.33 (35.25)h	35.48 (16.49)i	42.50 (40.67)d	38.94 (38.59)j	33.89 (35.41)j	17.32
5	Neem oil @ 2 ml/l	11.40 (19.73) a	16.67 (24.09)m	28.26 (35.25)i	29.58 (11.86)j	34.00 (35.65)e	30.70 (33.63)k	27.24 (31.45)k	10.97
6	Methyl parathion @ 50 EC1 ml/l	11.30 (19.65) a	19.47 (26.17)k	80.22 (63.57)c	83.33 (13.63)c	100.00 (89.96)a	72.57 (58.39)fg	71.12 (57.47)fg	54.85
7	Spinosad 48 SC @ 0.1 ml/l	11.20 (19.54) a	23.21 (28.79)j	86.05 (68.04)b	91.67 (49.78)b	100.00 (89.96)a	71.43 (57.67)g	74.47 (59.63)g	58.20
8	Indoxacarb 14.5 SC @ 0.3 ml/l	11.20 (19.54) a	49.11 (44.47)e	66.67 (54.71)e	78.95 (43.47)d	100.00 (89.96)a	78.57 (62.40)d	74.66 (59.75)d	58.39
9	Acephate 75 SP @ 1 ml/l	11.20 (19.54) a	43.75 (41.39)h	66.67 (54.71)e	76.19 (43.62)e	100.00 (89.96)a	74.11 (59.39)ef	72.14 (58.12)ef	55.87
10	Flubendiamide 480 SC @ 0.2 ml/l	11.30 (19.65) a	23.01 (28.65)j	38.63 (36.64)g	69.64 (43.96)g	70.59 (57.13)b	66.37 (54.55)h	53.06 (46.73)h	36.78
11	Thiodicarb 75 WP @ 0.6 gm/l	11.20 (19.54) a	38.39 (38.27)i	81.16 (64.25)c	84.62 (22.20)c	100.00 (89.96)a	70.54 (57.10)g	74.94 (59.94)g	58.67
12	Methonyl 60 SP @ 1 g/l	11.20 (19.54) a	47.32 (43.45)g	74.58 (59.70)d	80.00 (53.88)d	100.00 (89.96)a	71.43 (57.67)g	74.67 (59.76)g	58.39
13	Buprofezin 25 SC @ 1 ml/l	11.40 (19.73) a	66.26 (48.00)d	43.14 (41.04)f	55.17 (54.02)h	61.84 (51.65)c	63.16 (52.61)i	55.65 (48.23)i	39.38
14	Fenvalerate 20 EC @ 0.5 ml/l	11.30 (19.62) a	61.95 (51.89)b	65.12 (53.78)e	73.33 (52.89)f	100.00 (89.96)a	75.22 (60.12)e	75.12 (60.06)e	58.85
15	Quinalphos 25 EC @ 2 ml/l	11.30 (19.62) a	57.52 (49.31)c	100.00 (89.96)a	100.00 (89.96)a	100.00 (89.96)a	87.61 (69.36)b	89.03 (70.63)b	72.75
16	Untreated check	11.40 (19.73) a	11.40 (19.71)n	12.87 (21.02)j	14.77 (22.59)k	16.00 (23.57)f	26.32 (30.85)l	16.27 (23.78)j	
	S.Em.±	0.95	1.38	2.06	1.97	2.55	2.02	1.82	
	C.D. at 5%	NS	4.01*	5.95*	5.69*	7.37*	5.83*	4.80*	
	CV	5.79	6.18	5.70	5.42	5.90	6.24	5.87	

NS- Non significant * - Significant at 5% level
DAS- Days after sowing **-Figures in the parenthesis are arcsine transformed values
Means followed by the same letters in the column are not significantly different by DMRT (P=0.05)
DAT-Days after treatment

The results of the present investigation on per cent mortality of leaf miner larvae at 3 days after 1st and 2nd round of application of different insecticides revealed that monocrotophos, dichlorvos, and quinalphos were found highly toxic to the leaf miner larvae. After 5 and 7 days after applications, a similar trend was noticed by recording cent per cent mortality of the target pest. The superiority of monocorotophos, dichlorvos, and quinalphos are in agreement with the findings of Abdul Kareem (1976), Sangappa and Ali (1977), Ghorpade and Thakur (1989), Somashekar *et al.* (1991), Peter and Sundararajan (1991) and Umesh (1996).

Irrespective of the treatments, the larval mortality ranged between 19.47 and 64.10 per cent and 31.33 and 72.09 at 1 day after first and second round of application, respectively, except the botanicals, which shows inferior level of larval mortality. Similar trend was observed at 5 and 7 days after first and second round of application where cent per cent mortality was observed indicating the toxicity of different insecticides tested against leaf miner.

The efficacy of monocrotophos, dichlorvos and quinolphos is in accordance with the findings of Muthaih and Hussain (1991), Somashekar *et al.* (1991), Peter and Sundararajan (1991) and Sahayaraj and Amalraj (2006).

Rajput *et al.* (1985) have also reported the efficacy of methomyl and acephate which are in agreement with the present findings. In the present investigations fenvalerate and quinalphos were also found very effective in reducing the leaf miner population on crop. These findings are similar with the reports of Muthaiah (1993).

Flubendiamide and Buprofezin were not effective in reducing the leaf miner population after 3 days up to 15 days after spraying. The findings are contrary to Kumar and Krishnayya (1999) who proved its effectiveness and highest pod yield also.

NSKE and neem oil, botanical insecticides tested in the present study did not suppress the leaf miner even after 3 days up to 15 days both in first and second round of spray. The reason for ineffectiveness against the pest might be attributed to slow antifeedant property; also to the nature of the leaf miner infestation is by mining into the leaves, where the chemical may not work efficiently against the pest. The results of this investigation are in support with the findings and reasoning by Sadakathulla *et al.* (1976). On contrary to present findings,Gopal *et al.* (1992), Prabhakar *et al.* (1994) and Patil *et al.*(2003) reported NSKE as effective against GLM.

Yield and cost benefit ratio

Most of the insecticides were found moderately toxic when compared to monocrotophos in reducing leaf miner larval population at 3 days after application. But as the time advanced the magnitude of the efficacy was also increased **(**Table 2 and 3**)** although, the rate of mortality was considerably low in the beginning in most of the treatments except NSKE and neem oil and untreated check. Ultimately these treatments have recorded least pod and fodder yield also **(**Table 4**).** These findings are in accordance with the Sadakathulla *et al.* (1976). The results of present investigation although indicate the treatment difference among themselves with respect to larval mortality and also crop yield. However, the neem oil and NSKE treatments differed significantly with rest of the treatments in accordance with larval mortality rate and crop yield.

Table 3: Efficacy of different insecticides on the larval mortality of leaf miner on groundnut

Sl No.	Treatments	Initial population /10 plants (75DAS)	Second application					Mean Per cent larval mortality	Per cent reduction over untreated control
			Per cent larval mortality after						
			1 DAT	3 DAT	5 DAT	7 DAT	15 DAT		
1	Monocrotophos 36 SL @ 1.00 ml/l	**8.90** (25.76)b	**72.09** (55.86)a	**100.00** (89.96)a	**100.00** (8.6.96)a	**100.00** (89.96)a	**91.01** (72.52)a	**78.67** (62.47)a	**71.80**
2	Dichlorvos 100 EC @ 0.5 ml/l	**8.10** (25.17)b	**54.32** (47.46)f	**100.00** (89.96)a	**100.00** (89.96)a	**100.00** (89.96)a	**87.65** (69.40)b	**75.01** (59.98)b	**67.58**
3	Profenophos 50 EC @ 2 ml/l	**8.40** (25.39)b	**51.19** (45.66)g	**100.00** (89.96)a	**100.00** (89.96)a	**100.00** (89.96)a	**84.52** (66.81)c	**74.02** (59.33)c	**66.32**
4	NSKE @ 5%	**9.70** (26.34)ab	**16.49** (23.95)l	**32.10** (34.50)i	**39.09** (32.63)g	**46.15** (42.78)d	**28.87** (32.48)j	**28.73** (32.40)j	**9.72**
5	Neem oil @ 2 ml/l	**10.00** (26.55)ab	**18.00** (25.09)l	**23.17** (28.76)j	**25.40** (30.25)h	**40.43** (39.46)e	**21.00** (27.26)k	**23.00** (28.65)k	**4.78**
6	Methyl parathion @ 50 EC1 ml/l	**8.30** (25.32)b	**31.33** (34.02)k	**84.21** (66.56)c	**88.89** (70.50)b	**100.00** (89.96)a	**77.11** (61.39)ef	**64.97** (53.69)ef	**55.49**
7	Spinosad 48 SC @ 0.1 ml/l	**8.60** (25.54)b	**37.21** (37.57)i	**90.74** (72.26)b	**100.00** (89.96)a	**100.00** (89.96)a	**75.58** (60.36)f	**68.69** (55.95)f	**59.89**
8	Indoxacarb 14.5 SC @ 0.3 ml/l	**8.20** (25.24)b	**64.63** (53.49)c	**72.41** (58.29)f	**87.50** (69.27)bc	**100.00** (89.96)a	**81.71** (64.65)d	**69.08** (56.19)d	**60.43**
9	Acephate 75 SP @ 1 ml/l	**8.50** (25.46)b	**54.12** (47.34)f	**71.79** (57.90)f	**81.82** (64.73)d	**100.00** (89.96)a	**78.82** (62.58)e	**65.84** (54.21)e	**56.49**
10	Flubendiamide 480 SC @ 0.2 ml/l	**8.40** (25.39)b	**34.52** (35.97)j	**43.64** (41.33)h	**74.19** (59.45)e	**75.00** (59.98)b	**69.05** (56.17)g	**50.80** (45.44)g	**38.46**
11	Thiodicarb 75 WP @ 0.6 gm/l	**8.80** (25.69)b	**46.59** (43.03)h	**87.23** (69.04)c	**100.00** (89.96)a	**100.00** (89.96)a	**75.00** (59.98)f	**69.60** (56.52)f	**60.95**
12	Methomyl 50 SP @ 1 g/l	**8.20** (25.24)b	**57.32** (49.19)e	**80.00** (63.41)e	**85.71** (67.77)c	**100.00** (89.96)a	**69.51** (56.46)g	**66.79** (54.79)g	**57.69**
13	Buprofezin 25 SC @ 1 ml/l	**8.60** (25.54)b	**61.63** (51.70)d	**51.52** (45.85)g	**62.50** (52.22)f	**66.67c** (54.71)c	**51.16** (45.65)h	**47.08** (43.31)h	**37.88**
14	Fenvalerate 20 EC @ 0.5 ml/l	**8.60** (25.54)b	**68.54** (58.09)b	**70.83f** (57.29)f	**85.71c** (67.77)c	**100.00** (89.96)a	**79.07** (62.75)e	**64.05** (53.14)e	**60.01**
15	Quinalphos 25 EC @ 2 ml/l	**8.90** (25.76)b	**65.17** (53.81)c	**100.00** (89.96)a	**100.00** (89.96)a	**100.00** (89.96)a	**89.89** (71.43)ab	**77.33** (61.54)ab	**70.19**
16	Untreated check	**11.00** (27.26)b	**12.73** (20.89)m	**14.58** (22.44)k	**19.51** (26.20)i	**22.73** (28.460f	**34.55** (35.98)i	**19.18** (25.97)i	
	S.E m.±	**1.12**	**1.29**	**1.87**	**1.97**	**2.19**	**1.97**	**1.74**	
	C.D. at 5%	**3.24***	**3.75***	**5.41***	**5.71***	**6.35***	**5.70***	**5.70***	
	CV	**5.79**	**5.26**	**5.31**	**5.07**	**5.01**	**6.03**	**5.41**	

Table 4: Effect of insecticidal protection against ground nut leafminer on the pod and fodder yield of groundnut

Sl No	Treatments	Dosage	Pod yield (q/ha)	Fodder yield (t/ha)	Gross income from pod yield (Rs.2600/q)	Gross income from fodder yield/ Acre (Rs.100/t)	Total gross income	*Total Cost of cultivation/ ha	Net profit (Rs)	Additional pod yield over untreated check (q/ha)	Additional fodder yield over untreated check (t/ha)	C:B Ratio
1	Monocrotophos 36 SL	1 ml	32.00a	6.00a	83200	600	83800	15438	67761	15.14	4.67	**5.43**
2	Dichlorvos 100 EC	0.5 ml	26.00de	5.33a	67600	533	68133	15229	52371	9.14	4.00	**4.47**
3	Profenophos 50 EC	2ml	27.00cde	4.00b	70200	400	70600	15883	54317	10.14	2.67	**4.45**
4	NSKE	5%	18.00g	1.33ef	46800	133	46933	14913	31887	1.14	0.30	**3.15**
5	Neem oil	2ml/lt	20.00f	0.70f	52000	70	52070	15115	36885	3.14	0.00	**3.45**
6	Methyl parathion 50 EC	1ml/lt	26.00de	5.30a	67600	530	68130	15091	52509	9.14	3.97	**4.51**
7	Spinosad 48 SC	0.1ml/lt	28.00bcd	2.60cd	72800	260	73060	15500	57300	11.14	1.27	**4.71**
8	Indoxacarb 14.5 SC	0.3ml/lt	27.46bcde	3.30bc	71396	330	71726	15518	55878	10.60	1.97	**4.62**
9	Acephate 75 SP	1ml/lt	27.93bcd	4.00b	72618	400	73018	15415	57203	11.07	2.67	**4.74**
10	Flubendiamide 480 SC	0.2ml/lt	25.60e	2.00de	66560	200	66760	16627	49933	8.74	0.67	**4.02**
11	Thiodicarb 75 WP	0.6gm/lt	28.26bc	5.30a	73476	530	74006	15518	57958	11.40	3.97	**4.77**
12	Methomyl 50 SP	1gm/lt	27.06cde	3.30bc	70356	330	70686	16207	54149	10.20	1.97	**4.36**
13	Buprofezin 25 SC	1ml/lt	26.80cde	2.60cd	69680	260	69940	16159	53521	9.94	1.27	**4.33**
14	Fenvelerate 20 EC	0.5ml/lt	29.13b	5.30a	75738	530	76268	15025	60713	12.27	3.97	**5.08**
15	Quinalphos 25 EC	2ml/lt	32.26a	6.00a	83876	600	84476	15619	68257	15.40	4.67	**5.41**
16	Untreated check	------	16.86g	1.33ef	43836	133	43969	14227	29609			**3.09**
	S.E m. =		**1.11**	**0.19**	------	------	------	------	------	------	------	------
	CD at 5%		**3.21**	**0.56**	------	------	------	------	------	------	------	------

Cost: Benefit Ratio = Net profit/Cost of cultivation

* Cost of cultivation - Rs. 14227 + treatment cost

Means followed by same letters in the column are not statistically different by DMRT (P=0.05)

Among the treatments quinalphos reduced the larval population and record highest pod and fodder yield (32.00 q/ha and 6 t/ha) in groundnut. The present findings are in line with Somashekar *et al.* (1991) and Muthaiah (1993).

Monocrotophos recorded 32.00 q/ha and 6 t/ha of pod and fodder yield which was on par with quinalphos followed by fenvalerate pod yield (29.13 q/ha) and dichlorvos (5.33 t/ha). The present findings are in line with Muthiah and Hussain (1991), Somashekar *et al.* (1991), Peter and Sundararajan (1991) and Muthiah (1993).

Lowest yield was recorded in plots treated with NSKE (18.00 q/ha and 1.33 t/ha) and neem oil (20.00 q/ha and 0.70 t/ha).

Among the different insecticide practices monocrotophos recorded higher CBR of (Rs.5.43) followed by quinolphos (5.41) and fenvalerate (5.08) and lowest CBR was recorded by neem oil (3.45), NSKE (3.15) and untreated check (3.09).

It is evident from the study that insecticides which were highly effective to leaf miner and resulted more yield *viz.*, monocrotophos 36SL @ 1 m/l, quinalphos 25 EC @ 2 ml/l and fenvalerate 20 EC @ 0.5 m/l may be recommended to the groundnut growers to apply at 45 and 75 days after sowing for the effective management of leaf miner.

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Insecticide Resistance in Chilli thrips, *Scirtothrips dorsalis* (Hood) in Andhra Pradesh

ABSTRACT

The thrips population from Guntur district on chillies has developed high degree of resistance to various insecticides *viz*., monocrotophos, acephate, dimethoate, phosalone, carbaryl and triazophos followed by the population of Vizianagaram district with low levels of resistance implicating selection pressure and consequent resistance

levels. The *S. dorsalis* population of Guntur district acquired resistance to the tune of 4.19 folds to monocrotophos, 5.62 folds to acephate, 5.59 folds to dimethoate, 6.80 folds to phosalone, 4.06 folds to carbaryl and 3.39 folds to triazophos at LC_{50} in comparison with the Vizianagaram district population.
Key words: Chilli thrips, Resistance, Conventional insecticides.

INTRODUCTION

India has emerged today as the foremost producer and exporter of chillies contributing to almost 1/4th of the world's production. In India, chilli is grown in an area of 8.06 lakh ha, with a production of 12.98 lakh tonnes (Agricultural Statistics at a glance, 2009). The important chilli growing states in India are Andhra Pradesh, Orissa, Maharashtra, Karnataka and also in a number of other states as a round the year crop. In Andhra Pradesh, chilli is cultivated in an area of 1.89 lakh hectares with a production of 2.08 lakh tonnes. Guntur district in Andhra Pradesh alone contributes to over 35 per cent in area under chilli crop in India.

The important pests are thrips, *Scirtothrips dorsalis* (Hood), white mite, *Polyphagotarsonemus latus* (Banks), aphids, *Aphis gossypii* Glover and *Myzus persicae* Sulzer as sucking complex and tobacco caterpillar, *Spodoptera litura* (Fabricius) and pod borer, *Helicoverpa armigera* (Hubner) as pod borers (Rao and Ahmed, 1985). Chilli thrips, *Scirtothrips dorsalis* (Hood) (Thysanoptera : Thripidae) is a serious pest of *Capsicum annuum* L. in India, responsible for leaf curling (Ananthakrishnan, 1971). It multiplies appreciably at a faster rate during dry weather periods and the yield loss caused by the thrips is reported to range from 30-90 per cent (Borah, 1987 and Varadharajan, 1994).

Guntur district in Andhra Pradesh is traditionally a chilli growing district with an area of 63,573 ha with high input usage under monocropping conditions. Further, intensive cultivation of input responsive high yielding varieties and hybrids and sole reliance on insecticides are the common features of chilli cultivation in Guntur district. The excessive dependence on insecticides, their overuse and abuse has accelerated insect control problems through development of insecticide resistance (Reddy *et al*., 1992), pest resurgence, pesticide residues (Joia *et al*., 2001), reduction in natural enemy population and environmental contamination. Moreover, several of the chilli consignments meant for export were rejected stating higher insecticide residues being the culprit, thus lots of foreign exchange lost by way of rejections.

MATERIALS AND METHODS

Bioassay Studies

Chilly thrips, *S. dorsalis* were collected from chilli fields of Guntur and Vizianagaram. They were exposed to graded concentrations of each test insecticide following leaf dip method recommended by FAO (1979). About 25 ml of an insecticidal emulsion prepared in a beaker. Chilli leaves with petioles were plucked from untreated chilli plants and were dipped in insecticidal emulsion taken in a beaker for about 30 seconds. The treated chilli leaves were taken out of the beaker and dried for 30 minutes. The treated leaves were kept in specimen tubes of 25 ml capacity at the rate of one or two leaves per tube, allowing the cut end of petiole outside the specimen tube. Second instar nymph of *S. dorsalis* were collected from chilli fields carefully in to the aspirator tubes containing the treated chilli leaf at the rate of 20 per tube. The open end of the tube was closed with a stopper (cotton swab wrapped in a polythene paper) taking care that the petiole is projected outside the tube. The cut end of the petiole was wrapped with moist cotton to maintain turgidity of chilli leaf. Three replications were maintained for each insecticidal concentration with 20 nymphs in each replication. After 24 hours of the treatment, the treated leaf was carefully taken out from the tube and the mortality of thrips was recorded. Control was maintained at each time of experimentation where the leaves were dipped in water. The treatment mortality data was corrected using Abbott's Formula (Abbott, 1925).

Abbott's Formula:

Corrected per cent mortality =

$$\frac{T\text{-}C}{100\text{-}C} \times 100$$

T = per cent mortality in treatment, C = per cent mortality in control

The corrected mortality data of each insecticide was subjected to probit analysis (Finney, 1971) using MLP 3.08 software (Ross, 1987) and LC_{50}, LC_{90}, slope (b), chi-square (x^2), regression equation and fiducial limits were calculated.

The LC_{50} and LC_{90} values of each test insecticide against thrips populations of Guntur and Vizianagaram districts were taken into consideration for knowing the relative degree of resistance among the two populations. The relative degree of resistance to insecticides by thrip populations was calculated by the formula given by FAO (1979).

$$\text{Resistance factor} = \frac{LC_{50} \text{ of resistant population}}{LC_{50} \text{ of susceptible population}}$$

RESULTS AND DISCISSION

The bioassay studies conducted in the laboratory revealed the following results.

Monocrotophos

The LC_{50} and LC_{90} values of monocrotophos for Guntur population of *S. dorsalis* were 0.1340 and 0.3570 per cent, respectively (**Table 1**) with a slope (b) of 3.023. The chi-square test revealed that there was no heterogeneity in the population. The LC_{50} and LC_{90} values of monocrotophos against *S. dorsalis* of Vizianagaram district were 0.0320 and 0.1680 per cent, respectively (**Table 2**). The slope (b) of the log concentration probit line was 1.793. The population of *S. dorsalis* used for the study was homogeneous. A comparison of LC_{50} and LC_{90} values of monocrotophos against the two populations of thrips revealed that the Guntur population developed 4.19 fold resistance at LC_{50} and 2.13 fold resistance at LC_{90} in comparison with Vizianagaram population (**Table 3**).

Table 1: Toxicity of different conventional insecticides to *S. dorsalis* population of Guntur district

S. No.	Insecticides	LC_{50} (%) (95%FL)	LC_{90} (%) (95%FL)	Slope ± S.E (b)	Heterogeneity (χ^2)	Regression equation
1	Monocrotophos	0.1340 (0.0790-0.1770)	0.3570 (0.2480-1.2390)	3.023 ± 0.93	1.141	Y = 7.635+ 3.023X
2	Acephate	0.1460 (0.1020-0.1870)	0.4360 (0.3080-0.9940)	2.702 ± 0.64	2.793	Y = 7.255 + 2.702X
3	Phosalone	0.1700 (0.1150-0.2080)	0.3550 (0.2760-0.7200)	3.985 ± 1.12	2.115	Y = 8.072 + 3.985X
4	Dimethoate	0.1790 (0.1290-0.2150)	0.3520 (0.2790-0.6650)	4.343 ± 1.18	1.412	Y = 8.248 + 4.343X
5	Carbaryl	0.1260 (0.0820-0.1650)	0.4640 (0.3090-1.2490)	2.259 ± 0.54	1.294	Y = 7.036 + 2.259X
6	Triazophos	0.1120 (0.0690-0.1440)	0.2780 (0.2070-0.5770)	3.233 ± 0.85	1.844	Y = 8.080 + 3.233X

Acephate

The LC_{50} and LC_{90} values of acephate (Table 1) against the *S. dorsalis* of Guntur district were 0.1460 and 0.4360 per cent, respectively. Population was homogeneous as indicated by the chi-square test. The log concentration probit line recorded a slope (b) of 2.702. The population of *S. dorsalis* from Vizianagaram district had LC_{50} and LC_{90} values of 0.0260 and 0.1530 per cent, respectively for acephate (Table 2) with a slope (b) value of 1.663. The chi-square test indicated that the population was homogeneous. A comparison of LC_{50} and LC_{90} values of acephate against the two populations of thrips revealed that the Guntur population developed 5.62 fold resistance at LC_{50} and 2.85 fold resistance at LC_{90} in comparison with Vizianagaram population (Table 3).

Table 2: Toxicity of different conventional insecticides to *S. dorsalis* population of Vizianagaram district

S. No.	Insecticides	LC_{50} (%) (95%FL)	LC_{90} (%) (95%FL)	Slope ± S.E (b)	Heterogeneity (χ^2)	Regression equation
1	Monocrotophos	0.0320 (0.0190-0.0470)	0.1680 (0.1010-0.5580)	1.793 ± 0.41	1.185	Y = 7.669+ 1.793X
2	Acephate	0.0260 (0.0130-0.0380)	0.1530 (0.0900-0.5270)	1.663 ± 0.39	0.617	Y = 7.640 + 1.663X
3	Phosalone	0.0250 (0.0120-0.0370)	0.1670 (0.0940-0.7350)	1.536 ± 0.39	1.209	Y = 7.474 + 1.536X
4	Dimethoate	0.0320 (0.0180-0.0470)	0.1840 (0.1060-0.7190)	1.690 ± 0.41	0.990	Y = 7.525 + 1.690X
5	Carbaryl	0.0310 (0.0160-0.0470)	0.2030 (0.1110-0.9970)	1.576 ± 0.40	0.550	Y = 7.373 + 1.576X
6	Triazophos	0.0330 (0.0180-0.0500)	0.2190 (0.1170-1.1680)	1.564 ± 0.40	0.854	Y = 7.314 + 1.564X

Phosalone

The LC_{50} and LC_{90} values of phosalone for Guntur population of *S. dorsalis* were 0.1700 and 0.3550 per cent, respectively (Table 1) with a slope (b) of 3.985. The chi-square test revealed that there was no heterogeneity in the population. The LC_{50} and LC_{90} values of monocrotophos against *S. dorsalis* of Vizianagaram district were 0.0250 and 0.1670 per cent, respectively (Table 2). The slope (b) of the log concentration probit line was 1.536. The population of *S. dorsalis* used for the study was homogeneous. A comparison of LC_{50} and LC_{90} values of phosalone against the two populations of thrips revealed that the Guntur population developed 6.80 fold resistance at LC_{50} and 2.13 fold resistance at LC_{90} in comparison with Vizianagaram population (Table 3).

Table 3: Relative degree of resistance among the two populations of *S. dorsalis* to conventional Insecticides

Insecticide	Thrips Population	LC_{50} (%)	LC_{90} (%)	Resistance factor in comparison with Vizianagaram population	
				LC_{50} (%)	LC_{90} (%)
Monocrotophos	Guntur	0.1340	0.3570	4.19	2.13
	Vizianagaram	0.0320	0.1680	-	-
Acephate	Guntur	0.1460	0.4360	5.62	2.85
	Vizianagaram	0.0260	0.1530	-	-
Phosalone	Guntur	0.1700	0.3550	6.80	2.13
	Vizianagaram	0.0250	0.1670	-	-
Dimethoate	Guntur	0.1790	0.3520	5.59	1.91
	Vizianagaram	0.0320	0.1840	-	-
Carbaryl	Guntur	0.1260	0.4640	4.06	2.29
	Vizianagaram	0.0310	0.2030	-	-
Triazophos	Guntur	0.1120	0.2780	3.39	1.27
	Vizianagaram	0.0330	0.2190	-	-

Dimethoate

It is clear from the data (Table 1) that the Guntur population of *S. dorsalis* has LC_{50} and LC_{90} values of 0.1790 and 0.3520 per cent, respectively to dimethoate. The slope (b) of the lcp line was 4.343 with non-significance of chi-square test indicating that the *S. dorsalis* population tested was homogeneous. The LC_{50} and LC_{90} values of dimethoate for Vizianagaram population of *S. dorsalis* were 0.0320 and 0.1840 per cent, respectively (Table 2) with a slope (b) of 1.690. The chi-square test revealed that there was no heterogeneity in the population. A comparison of LC_{50} and LC_{90} values of dimethoate against the two populations of thrips revealed that the Guntur population developed 5.59 fold resistance at LC_{50} and 1.91 fold resistance at LC_{90} in comparison with Vizianagaram population (Table 3).

Carbaryl

The LC_{50} and LC_{90} values of carbaryl for Guntur population of *S. dorsalis* were 0.1260 and 0.4640 per cent, respectively (Table 1) with a slope (b) of 2.259. The chi-square test revealed that there was no

heterogeneity in the population. The LC_{50} and LC_{90} values of carbaryl against *S. dorsalis* of Vizianagaram district were 0.0310 and 0.2030 per cent, respectively (Table 2). The slope (b) of the log concentration probit line was 1.576. The population of *S. dorsalis* used for the study was homogeneous. A comparison of LC_{50} and LC_{90} values of carbaryl against the two populations of thrips revealed that the Guntur population developed 4.06 fold resistance at LC_{50} and 2.29 fold resistance at LC_{90} in comparison with Vizianagaram population (Table 3).

Triazophos

The LC_{50} and LC_{90} values of triazophos (Table 1) against the *S. dorsalis* of Guntur district were 0.1120 and 0.2780 per cent, respectively. Population was homogeneous as indicated by the chi-square test. The log concentration probit line recorded a slope (b) of 3.233. The population of *S. dorsalis* from Vizianagaram district had LC_{50} and LC_{90} values of 0.0330 and 0.2190 per cent, respectively for triazophos (Table 2) with a slope (b) value of 1.564. The chi-square test indicated that the population was homogeneous. A comparison of LC_{50} and LC_{90} values of triazophos against the two populations of thrips revealed that the Guntur population developed 3.39 fold resistance at LC_{50} and 1.27 fold resistance at LC_{90} in comparison with Vizianagaram population (Table 3).

Guntur population of *S. dorsalis* showed higher resistance to all the chemicals tested compared to Vizianagaram population, which may be due to the consumption of higher quantity of insecticides in Guntur district. This might have led to development of more resistance in Guntur population. More intensive cultivation of chilli which started much earlier in Guntur district, must have contributed to the higher degree of resistance acquired by Guntur population to all the conventional insecticides. In general there was resistance development even though at varying levels in both Guntur and Vizianagaram populations for commonly used conventional insecticides implicating selection pressure over years. Such selection pressure induced resistance was well documented in several polyphagous pests like *Spodoptera litura* (Fab.), *Spodoptera exigua* Hubner, *Helicoverpa armigera* (Hubner) *etc*. Hence it is the right time to withdraw selection pressure with insecticides in chilli ecosystem such that normally may be regained over a period of time.

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Research in Resistance Management

Phenoloxidase dependence of *Leptinotarsa decemlineata* Say.cellular immune response under the action of high derivatives of chitin

ABSTRACT
The cellular reaction of *Leptinotarsa decemlineata* hemolymph under the action of high-molecular weigh chitin derivatives - chitosan and chitosan succinate – has been investigated. The processes of hemocyte activation are shown to include both phenoloxidase-dependent and -independent mechanisms. It was found that chitosan and chitosan succinate stimulate cellular reactions of Colorado potato beetle hemolymph through phenoloxidase-independent mechanisms, increasing the total number of circulating hemocytes, the proportion of active spindle-shaped phagocytes and cells synthesizing the components of humoral immunity.
Key words: *Leptinotarsa decemlineata*,cellular immunity, phenoloxidase, chitosan, chitosan succinate.

INTRODUCTION

Ability to develop effective immune responses is one of the main reasons of prosperity of the class Insecta as a whole and the formation of highly resistant populations of insect pests in particular. In this regard, studies of the mechanisms of insect immune responses remain relevant in solving problems of modern theoretical and practical entomology. This work is devoted to the study of the mechanisms of activation of Colorado potato beetle cellular immune system by the high-molecular weigh derivatives of chitin - chitosan and chitosan succinate. We have recently demonstrated the induction of immunorecognition factors in L. decemlineata by these polysaccharides (Gaifullina et al., 2010). According to published data, in insects the recognition process is carried out with the participation of phenoloxidase system (POS) and is accompanied by activation of immune cells (Wilson et al., 1999; Nappi et al., 2005). Moreover, insect cellular immune responses, phagocytosis and nodulation, are carried out with the participation of prophenoloxidase (proPO) activating system (Mavrouli et al., 2005). Therefore, we have focused our attention on the study of chitosan and chitosan succinate action on the hemocyte system of Colorado potato beetle under the inhibition of phenoloxidase and injection of micro-organisms.

MATERIALSANDMETHODS

L. decemlineata larvae of fourth instar were collected from potato fields and reared in glasses with a volume of 0.5 dm^3. Insects in experimental variants one day prior to the analysis were fed with potato foliage moistened with 0.01% water suspension of chitosan (M=200 κDa, deacetylation degree 82%) or 0.01% water solution of chitosan succinate (M=330 κDa, deacetylation degree 95%). Inhibition of phenoloxidase was performed by injecting in hemocoel of 2mkl of phenylthiourea (PTU) at concentration of 0.01% by syringe with a sterile needle. Cellular suspension of *Bacillus subtilis* (strain) in titer $2x10^8$ cells/µl was used as a foreign agent. This suspension was also injected into hemocoel by syringe with a sterile needle. Measurement of phenoloxidase activity of haemolymph and slides preparation for hematological analysis was performed one hour after injection.

Phenoloxidase (PO) activity was determined in trys-HCl buffer (pH 7.5) with respect to substratum L-digydroxiphenilalanin using spectrophotometer Shimadsu at 475 nm 37ºC by optical density change during 5 min. Activity of this enzyme was evaluated in protein concentration, which was measured by Bradford method (Skopes, 1985). The enzyme activity was expressed in units/min/protein mg.

Hematological 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 by Romanovsky-Gimsa (Kuchel et al., 2010). Hematological analysis was carried with microscope AxioImager M1 (Carl Zeiss) at x630.

RESULTS AND DISCUSSION

In the hematological analysis prohemocytes, phagocytes - granulocytes and plasmatocytes – with amoeboid and spindle shape, spherulocytes and oenocytoids were identified. Under the action of chitosan and chitosan succinate on *L. decemlineata* larvae the total number of hemocytes in circulation and the proportion of plasmatocytes - proliferating and phagocytic cells - both amoeboid and spindle shape has been increased. Chitosan succinate also caused a significant increase of the percentage of spherulocytes secreting factors of humoral immunity. These changes in hemolymph cellular composition were accompanied by rise of phenoloxidase activity (Table 1).

Table 1: PO activity in *L. decemlineata* haemolymph by injection of PTU and *B. subtilis* under the action of chitosan and chitosan succinate

Variant	Diphenoloxidase activity, units/min/protein mg		
	Control	Chitosan	Succinate of chitosan
Without injection	0.842±0.034	1.012±0.009	1.041±0.0014
Injection of PTU	0.060±0.004	0.094±0.009	0.036±0.004
Injection of B. subtilis	0.106±0.003	0.049±0.002	0.059±0.009
Injection of PTU+B. subtilis	0.048±0.006	0.134±0.012	0.033±0.002

Thus, high-molecular weigh chitin derivatives-chitosan and chitosan succinate - stimulate hemocyte reaction *L. decemlineata*, as in the case with the development of intestinal infection by the action of bitoxibacillin (Gaifullina et al., 2006). At the same time, introduction of the chitin derivatives in the feed did not cause pathological changes in the cellular pattern of hemolymph of Colorado potato beetle, characteristic for infectious process, such as vacuolation, fragmentation and lysis of hemocyte cytoplasm, decentralization and pycnosis of nuclei, increased number of dead cells. Cells with spindle shape being an active form of phagocytes are formed from cells of amoeboid shape by drawing a pair of pseudopodia at the opposite poles. The increase in the total number of hemocytes may be a consequence of their increased neoformation in hemopoietic organs, mitotic division of circulating plasmatocytes and prohemocytes and further cytodifferentiating, the transition of hemocytes adjacent to the tissues and the spinal vessel to the free-circulating status. Whatever the case, increasing the number of hemocytes and their percentage change in the hemolymph of *L. decemlineata* suggests the presence of mediators that stimulate hemocyte morphogenesis and cell-mediated immunity of the insect as a whole. These mediators may be chitin derivatives or products of their degradation, as well as biogenic amines, some members of which have a regulatory effect on the hemocyte activity in insects. Thus, biogenic monoamines increase the total number of hemocytes (Kim GS and Kim Y., 2010), locomotor activity of phagocytes through the actin cytoskeleton, increasing the concentration of F-actin (Diehl-Jones et al., 1996), mediate hemocyte spreading and migration into a zone of bacterial infection (Merchant et al., 2008). Mediation of hemocyte reaction to the chitin derivatives action by biogenic monoamines is indirectly confirmed by an increase in the hemolymph phenoloxidase activity involved in the regulation of the biogenic amines titer.

The main source of phenoloxidase activity in the insect hemolymph is themselves hemocytes (Mavrouli et al., 2005; Ling and Yu, 2005), that completely explains the parallel increase in the activity of this enzyme and the count of immune cells. Increase of the level of phenoloxidase activity, which correlated with increased viability of the insect under the influence of the chitin monomer and oligomers was detected earlier and was associated with the action of these substances as signaling molecules inducing an immune response (Saltykova et al., 2001; Gaifullina et al., 2010). Obviously, a similar phenomenon occurs in the case of action of the high-molecular chitosan succinate and chitosan on the Colorado potato beetle.

Parallel increase in phenoloxidase and hemocyte activity of Colorado potato beetle hemolymph under the action of chitin derivatives, suggests a relationship in the functioning of these immunity factors. More significant presence of this relationship is demonstrated at the inhibition of phenoloxidase in the hemolymph of Colorado potato beetle by PTU injection (Table 1). After the injection of PTU in larvae, hemocoel percentage of spindle-shaped granulocytes decreased by 4-6 times in normal conditions and under the action of chitin derivatives, which indicates the PO-dependence of activation of these phagocytes (Fig.1). At the same time, increase in the percentage of spindle-shaped plasmatocytes and spherulocytes, as well as oenocytoids after joint injection of PTU and *B. subtilis*, is observed in this case which suggests a PO-independent functioning of these cells. Injection of *B. subtilis* caused a standard protective hemocyte reaction: increasing the number and vacuolization of active phagocytes, both granulocytes and plasmatocytes, increasing the number of spherulocytes as well as oenocytoids under the action of chitosan succinate. PO activity in this case, also significantly decreased, which may be associated with involvement of this enzyme in the recognition and binding of bacterial cells and the exhaustion of its active form (Table 1).

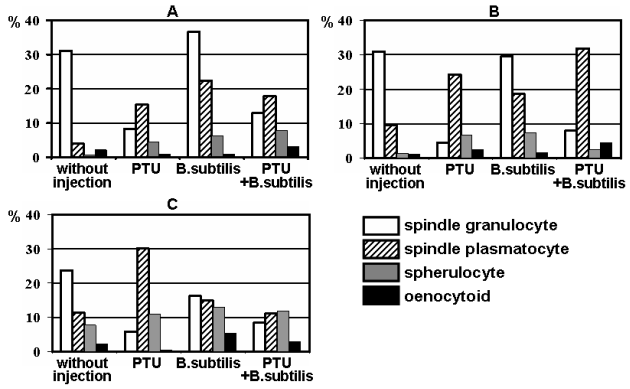

Figure 1: The cell reaction of *L. decemlineata* hemolymph in the inhibition of phenoloxidase and injection of B. subtilis under the action of chitosan and chitosan succinate. A – control, B – action of chitosan, C – action of chitosan succinate.

Thus, the processes of hemocyte activation include both PO-dependent and -independent mechanisms. Chitin derivatives stimulate PO-independent hemocyte reaction. Previously it has been shown that phagocytosis of bacteria by *Ceratitis capitata* hemocytes depends on proPO activating system, namely the RGD-binding receptors, focal adhesion kinase (FAK)/sarcoma and mitogen-activated protein kinase signaling pathways that induce secretion of PO activating peptides (Mavrouli et al., 2005). As well as the activity of dopa decarboxylase, multifunctional enzyme involved, particularly, in the defense reactions of insects (Sideri et al., 2007). It is noteworthy that phagocytosis of bacteria is a proPO-dependent process, whereas phagocytosis of latex beads and lipopolysaccharides is proPO-independent (Mavrouli et al., 2005; Sideri et al., 2007; Lamprou et al., 2007). The results of these studies confirm our hypothesis about the similarity of mechanisms of insect immunity stimulation by chitin derivatives and lipopolysaccharides (Gaifullina et al., 2010).

In conclusion, one can summarize that PO synthesizing in insect hemocytes in response to immune stimuli participates in the activation of phagocytic cells. Probably, the mechanism of this activation is associated with the process of synthesis and degradation of biogenic amines. Inductors of immunorecognition factors - chitosan succinate and chitosan - through PO-independent mechanisms stimulate cellular reactions of Colorado potato beetle hemolymph, increasing the total number of circulating hemocytes, the proportion of active spindle-shaped phagocytes and cells synthesizing the components of humoral immunity.

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Susceptibility of different colour morphs in *Helicoverpa armiger*a (Hubner) (Lepidoptera, Noctuidae) to chemicals

INTRODUCTION

American bollworm, *Helicoverpa armigera* (Hubner) is one of the most serious pests and has attained the status of national pest. It has been suggested that *H. armigera* populations occurring in different regions of the country may differ significantly in various biological parameters including response to pesticides (Lingren, 1982). Significant differences in expression levels of carboxyl esterase in the populations of *H.armigera* occurring in different locations in India has been well documented (Phokela and Mehrotra, 1985 and Mehrotra and Phokela, 1986). Among various morphological traits, the larval colour has been reported to be highly variable and found to vary from velvety black to yellowish green (Nagarajarao and Abraham, 1956). Considerable colour variation in the larvae ranging from green, fawn, pink, yellow or brown and very dark to light green or pink (Brodley, 1977; Uthamasamy *et al*., 1988), Association of larval colouration with nutrition has been known, however, genetic basis to same is obscure hitherto. Larvae exhibited distinct colours and markings when reared on cotton, corn, tomato and tobacco.

Though the genetic control of colouration in *H.armigera* is not known, variability within a basic colour is obvious. Complex set of modifiers must be in operation that can change in quantitative fashion (Jameson and Pequegnat, 1971). In view of the cryptic variations in colour, the exact mode of inheritance could not be understood and it is still a complex phenomenon in *H. armigera*. Specific pattern in frequency of occurrence of colour morphs in geographic population of cotton bollworm has been well documented in south Indian cotton ecosystem (Vijaykumar and Patil, 2005). There is general feeling that somehow, the colouration/pigmentation pattern on larval body segments has a correlation to resistance; however, no data exist for this hypothesis as of now. Moreover, recently pigeonpea growers in Gulbarga district complaining the loss of chemical efficacy against black coloured *Helicoverpa* larvae in their field led us investigate the response of different colour morphs prevailing in the pegionpea ecosystem against the xenobiotics currently in use in the district against pod borer *H. armigera* and the same is communicated in the present study.

MATERIALS AND METHODS

Early instar larvae of field populations of *H. armigera* were collected from pigeonpea ecosystems from Gulbarga district, Karnataka, India (pigeonpea bowl of Karnataka) which is endemic to pod borer infestation during two consecutive years (2004 and 2005). These locations supported large levels of pod borer infestation, which permitted collections of significant samples. More than 3000 larvae were collected for both the years, brought to the insectory in multicavity trays with a bit of plant parts and were sorted into different colours. Totally five different colour morphs viz., Blackish, whitish, greenish, brownish and yellowish were encountered in the field collections. Larvae of uniform size weighing approximately 30-40 mg were selected from the bulk collection for each colour morph and were bioassayed using discriminating dose of insecticides molecules by topical application technique as per Armes et al.(1993).

Seven insecticides representing five insecticide groups *viz.*, carbaryl (Carbamates), monocrotophos, quinalphos, chlorpyriphos (Organophosphates)

endosulfan (Cyclodine), cypermethrin and fenvalerate (Synthetic pyrethroids) and spinosad (New molecule) were chosen for discriminating dose bioassays. Except spinosad, technical grade insecticides were used in all the cases. In each treatment, 100 larvae were used in three replications and surviving larvae were counted 72 hours after treatment.

RESULTS AND DISCUSSION

The results of the bioassay using seven different xenobiotics, representing five different chemical groups against five different colour morphs, are presented in Table 1. Wide range of differences in the response was observed across the colour morphs. During 2004 season, the per cent survival of different colour morphs of *H. armigera* larvae at 0.75 ug/ul discriminating dose of quinalphos has ranged from 32.00 to 72.00 per cent. Whereas, the widely used OP chemical chlorpyriphos at 1 ug/ul caused a survival ranging from 16.00-59.00 per cent. The per cent survival with discriminating dose of 10 ug/ul of cyclodiene compound Endosulfan has ranged from 26.00 to 87.00 per cent. Resistance frequencies recorded with 1.5 ug/ul of spinosad a new insecticide molecule were ranged between 4.00 to 22.00 per cent. The carbamate insecticide methomyl when used at the discriminating dose of 1.2 ug/ul has recorded moderate survival of *H.armigera*. The resistance frequency recorded with methomyl ranged from 12.00 to 64.00 per cent. The resistance frequency exhibited against synthetic pyrethroide insecticide cypermethrin 0. 1 ug/ul per larva ranged from 20.00 to 92.00 per cent across different colour morphs. Whereas, the same was 13.00 to 84.00 per cent for fenvalerate another synthetic pyrethroid, at 0.2 ug/ul. The trend remained almost same for the second season of 2005 (Table 1.)

Table 1: Response of different colour morphs of *Helicoverpa armigera* to various xenobiotics

Xenobiotics	Year	Resistance frequency of different colour morphs (%)				
		Black	Whitish	Green	Brown	Yellow
Quinalphos (0.75ug / ml)	2004	72	32	47	55	62
	2005	68	35	50	50	59
	Pooled	70	33.5	48.5	52.5	60.5
Chlorpyriphos (1ug / ml)	2004	59	16	40	48	26
	2005	65	22	44	53	32
	Pooled	62	19	42	50.5	29
Endosulfan (10ug / ml)	2004	81	26	43	60	48
	2005	88	30	53	65	57
	Pooled	84.5	28	48	62.5	52.5
Spinosad (1.5ug / ml)	2004	22	4	14	18	12
	2005	18	7	16	24	17
	Pooled	20	5.5	15	21	14.5
Methomyl (1.2ug / ml)	2004	64	12	32	50	31
	2005	58	16	37	48	40
	Pooled	61	14	34.5	49	35.5
Cypermethrin (0.1ug / ml)	2004	92	20	54	68	65
	2005	88	25	48	70	55
	Pooled	90	22.5	51	69	60
Fenvalerate (0.2ug / ml)	2004	84	13	50	76	52
	2005	90	17	62	80	60
	Pooled	87	15	56	78	56

Resistance frequencies from the pooled data to seven xenobiotics for five different colour morphs of *H. armigera* suggested wide range of difference in the response even the larvae were collected from same ecosystem and in the same location. Among the five colour morphs, the black coloured larvae recorded higher resistance frequencies regardless of the xenobiotics followed by brown, green, yellow and white. Therefore, it may be inferred that black coloured morphs exhibited greater tolerance in contrast to least in white morphs. To our knowledge, there is no documented evidence on differential response of various colour morphs to different xenobiotics and hence the present study is first of its kind.

Padma kumari et al.(1995) reported poor permeability of larval cuticle and higher metabolism in the cuticle itself was important in imparting the resistance against deltamethrin in sirsa population of *H. armigera*. It is therefore likely that the ability of the black coloured morphs to survive almost all the xenobiotics compared to other morphs may be due to the higher pigmentation and melanin content and cuticular thickening which might have interfered the penetration of chemical by acting as a physical barrier in the study location, on the other hand white coloured morphs with no pigmentation were found to be highly susceptible as revealed by resistance frequencies. The colour morphs brown, yellow and green with moderate level of pigmentation found to have moderate level of

resistance frequencies. Quick excretion immediately (2hr after treatment) in black coloured morphs can also be regarded as one of the physiological mechanisms of resistance development. It is interesting to note that the abundance of black coloured morphs was high in pigeonpea ecosystem of Gulbarga district (unpublished) from where the reports on crop failure due to higher resistance level were available (Basavanagoud 1994; Vijaykumar *et al*.2003). Among the xenobiotics, resistance to pyrethroids was highest followed by OP's, endosulfan, methomyl and spinosad, which might be due to the inherent ability of *H. armigera* to develop resistance and also due to the continuous selection pressure in the pigeonpea ecosystem. Due to the lack of pertinent literature it is very difficult to explain the mechanism behind such response in different colour morphs. However, other resistance mechanisms like higher metabolic rates and enzyme activities cannot be ruled out and therefore demands future focus in this direction.

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Baseline Sensitivity of Cucurbit Powdery Mildew Fungus (*Podosphaera xanthii*) to the Fungicide Metrafenone

ABSTRACT

Metrafenone baseline sensitivity varied little among the 55 cucurbit powdery mildew fungal isolates tested using a leaf disk bioassay. All were insensitive (resistant) to 1 ppm metrafenone, 42 of 54 isolates (78%) tested at 10 ppm were resistant (able to grow and sporulate on at least half of the treated disks); 16 of 38 isolates (42%) tested at 20 ppm exhibited resistance to this concentration; 6 of 12 isolates (50%) tested at 40 ppm exhibited tolerance (limited growth on less than half of the treated disks); no isolates were resistant. There was no evident relationship between sensitivity to metrafenone and sensitivity to FRAC Group 1, 3, 7, 11, or 13 fungicides.

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 United

States. 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 mobile 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 most of the main fungicide classes at-risk for resistance currently registered in the US for its management: benzimidazole (MBC; FRAC Group 1), demethylation inhibitors (DMIs; Group 3), quinone outside inhibitors (QoI, aka strobilurins; Group 11), and carboxamide (Group 7).

Metrafenone is a novel active ingredient being developed by BASF Corporation. Vivando is the trade name of the product being developed with this active ingredient. The experimental number was BAS 560 F.

The goal of this study was to provide the first step in fungicide resistance management by determining the baseline sensitivity of *Podosphaera xanthii* to metrafenone, 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 research fields of pumpkin at LIHREC in Riverhead, NY, in 2008 and 2009, and a research field of pumpkin in NJ in 2009. Isolates also were collected from commercial fields of pumpkin in Suffolk County, NY in 2008 and 2009. Collections were made near the end of the crop production period after fungicides had been applied to manage powdery mildew. A sub-set of 55 isolates was used in this study. Sensitivity to other fungicides had been examined for some of these isolates as part of other studies.

A leaf disk bioassay was used to determine the baseline sensitivity of *Podosphaera xanthii* to metrafenone (McGrath et al 1996)(Figure 1). Pumpkin seedlings (*Cucurbita pepo* 'Sorcerer') at the cotyledon leaf stage (about seven-day-old) were sprayed with various concentrations of metrafenone using a DeVilbiss atomizer bottle attached to a compressed air source (30 psi). Treated plants were allowed to dry overnight and then disks were cut from the cotyledons using a #9 cork borer (9 mm diameter). Six disks of each treatment were placed on water agar in sectioned Petri plates. Each plate had four sections thus there were three treatments per plate plus a non-treated control. Disks were inoculated by transferring about 10-20 conidia to each disk center. Each disk in a plate was inoculated with each *P. xanthii* isolate, then plates were incubated for at least 10 days at room temperature on a laboratory shelf under constant light supplied by aquarium bulbs, at which time the control treatment showed good growth, with sporulating mildew covering an average of about 50% of leaf disk area. The percent leaf disk area colonized by sporulating mildew was recorded for each disk and averaged for each treatment. Where pathogen growth was not readily visible with the unaided eye, a dissecting microscope was used to determine if there was any growth, including just mycelium on the leaf surface, and to verify that inoculum was present.

A *P. xanthii* isolate was considered to be insensitive (resistant) to a particular fungicide concentration if it was able to grow and sporulate on at least half of the disks. Usually, isolates able to grow on at least half of the disks, exhibited no more than 80% reduction in growth.

An isolate was considered to be tolerant if there was growth on less than half of the disks or the quantity of growth was substantially less than on the non-treated disks (typically less than 10%). The ability to produce spores is considered an important attribute defining a resistant or tolerant isolate because it means the isolate can continue to spread. An isolate was considered to be sensitive if there was no growth on any disk. Disks with no evident growth at 10 days often were re-examined about 4 days later to determine if there was any change in isolate sensitivity. Extremely rarely only a very limited amount of mycelial growth has been observed with no sporulation at 10 days. In these situations sometimes conidial chains have formed several days later.

Three bioassays were conducted to test 55 isolates in total. Vivando (BAS 560 F formulated product) was used. Six concentrations of metrafenone were used ranging from 0.01 to 40 ppm. Concentrations tested were changed in each successive bioassay based on results obtained in the previous one. All isolates were not tested at all concentrations.

RESULTS AND DISCUSSION

Metrafenone baseline sensitivity varied little among the powdery mildew fungal isolates tested. All 55 isolates tested were insensitive (resistant) to 1 ppm metrafenone (Table 1). Most grew well on leaf disks

from cotyledons treated with this concentration, and also lower concentrations tested in the first bioassay. Five isolates did not grow as well on leaf disks treated with 1 ppm metrafenone as on non-treated disks. One isolate was not tested at a higher dose. Twelve of the 54 isolates (22%) tested at 10 ppm exhibited tolerance of this concentration; the other 42 (78%) were resistant to 10 ppm. Sixteen of the 38 isolates (42%) tested at 20 ppm exhibited resistance to this concentration (Figures 1 and 2); 16 were tolerant. Six of the 12 isolates (50%) tested at 40 ppm exhibited tolerance; no isolates were resistant; none of these were tested at 20 ppm.

Figure 1: Leaf disk bioassay plate with isolate NJ-1A tested at 1 ppm metrafenone (right section), 10 ppm metrafenone (top section) and 20 ppm metrafenone (left section). Compared to growth on the non-treated disks in the bottom section, growth of this isolate was not affected by a metrafenone concentration of 1 ppm, growth was moderate to good at 10 ppm, and it was poor to moderate at 20 ppm. This isolate was able to grow and sporulate on 4 of 6 disks treated with 20 ppm, therefore it was rated resistant to this concentration.

Figure 2: Leaf disks treated with 20 ppm metrafenone and inoculated with isolate NJ-1A in bioassay plate shown in Figure 1.

Table 1: Baseline sensitivity of 55 isolates of *Podosphaera xanthii* to metrafenone. Isolate name is in column 1, next 3 columns contain information about the isolate, then their sensitivity rating for metrafenone followed by results for each concentration tested, and lastly sensitivity to other fungicides tested in previous studies. R=resistant, T=tolerant, S=sensitive. Value following this letter is the concentration. Quality of isolate growth on treated disks compared to non-treated was rated G=good, M=moderate, P=poor, and S= sensitive (no growth). Percentage of disks with growth is noted.

						Metrafenone (ppm)												
Isolate	Year	Location	Crop	Metrafenone		1		10		20		40		MBC	QoI	Boscalid	Nova	Quintec
11-19A	2009	Research Field-NY	Pumpkin	R1	no 10+	G	100%								R	S 50	S 2	R 1
AP1-1	2009	LI Farm 3	Pumpkin	R1	T10	G	100%	M	33%			S	0%		R	S 50	S 2	S 10
11-8A	2009	Research Field-NY	Pumpkin	R1	T10	G	100%	P	50%			S	0%		R	R 150	S 20	
11-19C	2009	Research Field-NY	Pumpkin	R1	T10	G	100%	M	17%			S	0%		R	S 150	S 20	
I-5	2009	LI Farm 4	Pumpkin	R1	T10	G	100%	P	33%	S	0%				R	S 150	S 20	R 10
ZA-5	2009	LI Farm 5	Pumpkin	R1	T10	G	100%	P-M	17%			S	0%		R	S 50		T 10
ZA-8	2009	LI Farm 5	Pumpkin	R1	T10	G	100%	P	17%			S	0%		R	T 50		T 10
NJ-2C	2009	Research Field-NJ	Pumpkin	R1	T10	G	100%	P	50%					R		R 500	R 40	R 10
S-2	2009	LI Farm 2	Pumpkin	R1	T10	G	100%	P	50%					R		R 500	S 2	R 10
ZB-4	2009	LI Farm 5	Pumpkin	R1	T10	G	100%	P	67%					R	R	T 50	R 20	T 10
S-3	2009	LI Farm 2	Pumpkin	R1	T10/T40	G	100%	P	33%			P	17%			R 50	R 40	T 10
ZB-1	2009	LI Farm 5	Pumpkin	R1	T10/T40	G	100%	P	17%			P	33%		S	S 150	T 20	
AP2-6	2009	LI Farm 3	Pumpkin	R1	T20	G	100%	P	33%	P	17%			R	R	T 50	S 20	T 10
IKG	2008	Research Field-NY	Pumpkin	R10	S20	M	100%	P-M	75%	S	0%			R			S 2	
AP2-3	2009	LI Farm 3	Pumpkin	R10	S20	G	100%	M	67%	S	0%			R	R	R 500	T 20	T 10
11-8B	2009	Research Field-NY	Pumpkin	R10	S20	G	100%	M	67%	S	0%					R 500		
I-4	2009	LI Farm 4	Pumpkin	R10	S20	G	100%	M-P	50%	S	0%				R	S 150	S 20	S 10
11-6C	2008	Research Field-NY	Pumpkin	R10	T20	G	100%	M	100%	P	50%							
L1-B	2008	LI Farm 1	Pumpkin	R10	T20	G	100%	M	100%	P-M	50%							
L2-B	2008	LI Farm 1	Pumpkin	R10	T20	M	100%	M	50%	P	17%							
PA4-H	2008	PA Farm 1	Pumpkin	R10	T20	G	83%	M	100%	P	50%							
S-E	2008	LI Farm 2	Pumpkin	R10	T20	G	100%	P	80%	P	33%							
Z-B	2008	LI Farm 5	Pumpkin	R10	T20	G	100%	M	100%	P	40%							
AP1-4	2009	LI Farm 3	Pumpkin	R10	T20	G	100%	G	100%	P	50%				S? (little	S 150	S 20	R 10
AP1-5	2009	LI Farm 3	Pumpkin	R10	T20	M-P	83%	M	100%	P	17%			R		R 500	S 2	T 10
AZ-5	2009	LI Farm 3	Zucchini	R10	T20	G	100%	M	83%	P	50%			R		S 50	S 40	S 10
11-9A	2009	Research Field-NY	Pumpkin	R10	T20	G	83%	G	83%	P	80%				S	S 50	S 20	T 10
11-14B	2009	Research Field-NY	Pumpkin	R10	T20	G	100%	M	50%	P	33%				R	S 150	S 20	
I-1	2009	LI Farm 4	Pumpkin	R10	T20	G	100%	G	83%	P	40%				R	S 150	S 20	
I-3	2009	LI Farm 4	Pumpkin	R10	T20	G	83%	M-G	67%	P	33%			R	R	S 150	R 2	T 10
ZB-3	2009	LI Farm 5	Pumpkin	R10	T20	G	100%	M	100%	P	60%			R	R	S 50	S 2	S 10
ZB-6	2009	LI Farm 5	Pumpkin	R10	T20	G	100%	M	100%	P	33%			R		T 50		R 10
AP1-3	2009	LI Farm 3	Pumpkin	R10	T40	G	100%	M	83%			P	33%		R	S 50	R 20	T 1
AP2-1	2009	LI Farm 3	Pumpkin	R10	T40	G	100%	P-M	67%			P	17%		R	S 150	S 20	
11-32A	2009	Research Field-NY	Pumpkin	R10	T40	G	100%	M	100%			P	17%	R	R	R 150	T 20	
11-5A	2009	Research Field-NY	Pumpkin	R10	S20	G	100%	M	50%	S	0%				R	S 150	S 20	
11-32B	2009	Research Field-NY	Pumpkin	R10	S40	G	100%	P-M	67%			S	0%		R	S 50	S 2	R 1
11-24B	2009	Research Field-NY	Pumpkin	R10	T40	G	100%	P-M	50%			P	17%		R	S 150	T 20	
11-24A	2009	Research Field-NY	Pumpkin	R10		G	100%	P	50%					R	R	S 50	S 20	T 10
11-13B	2008	Research Field-NY	Pumpkin	R20		G	100%	M	100%	P-M	100%							
11-16B	2008	Research Field-NY	Pumpkin	R20		G	100%	M	100%	P-M	50%							
11-41A	2008	Research Field-NY	Pumpkin	R20		G	100%	M	100%	M	100%							
L2-D	2008	LI Farm 1	Pumpkin	R20		G	100%	M	83%	M	60%							
PA4-I	2008	PA Farm 1	Pumpkin	R20		G	100%	G	100%	M	67%							
S-A	2008	LI Farm 2	Pumpkin	R20		G	100%	G	100%	M	67%							
S-C	2008	LI Farm 2	Pumpkin	R20		G	100%	M	83%	M	83%							
I-2	2009	LI Farm 4	Pumpkin	R20		M	100%	M	75%	P-M	60%				R	S 50	S 2	T 10
NJ-1A	2009	Research Field-NJ	Pumpkin	R20		G	100%	G	100%	M-G	67%			R	R	R 500	R 2	S 10
NJ-2A	2009	Research Field-NJ	Pumpkin	R20		G	100%	P-M	100%	P	83%			R		R 500	T 2	T 10
NJ-3C	2009	Research Field-NJ	Pumpkin	R20		G	100%	M	100%	G	83%				R	R 150	T 20	
NJ-4C	2009	Research Field-NJ	Pumpkin	R20		G	100%	M-G	67%	M	50%			R	R	R 500	T 40	R 1
S-1	2009	LI Farm 2	Pumpkin	R20		G	100%	G-M	100%	M	67%			R	R	T 50	R 40	R 10
ZA-4	2009	LI Farm 5	Pumpkin	R20		M-G	100%			M	100%			R	R	T 150	T 20	R 10
ZB-5	2009	LI Farm 5	Pumpkin	R20		G	100%	M	100%	M	100%			R		T 50		T 10
ZB-2	2009	LI Farm 5	Pumpkin	R20		G	100%	M	83%	P-M	50%				R	S 50	R 20	

The isolates tested for their sensitivity to metrafenone exhibited a range in sensitivity to other fungicides (Table 1). Resistance to MBC (FRAC Group 1) and QoI (FRAC Group 11) fungicides is very common in this group of isolates based on those that were tested. Eight isolates exhibited a level of resistance to boscalid (500 ppm) expected to impart field resistance to fungicides with this active ingredient, which is in FRAC Group 7. There was no evident relationship between sensitivity to metrafenone and sensitivity to

any of the other fungicides tested. Sensitivity was also examined for FRAC Group 3 and 13 fungicides.

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Cytotoxic Effect of potential pesticides on the cerebral neurosecretory cells (CNSC) of pink bollworm, *Pectinophora gossypiella* (Saund.)

ABSTRACT

Toxicity of chlorpyrifos, profenofos, spinosad, emamectin benzoate and azadirachtin was determined against the newly hatched larvae of pink bollworms, *Pectinophora gossypiella* (Saund.) using film residue assay method. The data revealed that emamectin benzoate was a superior potent compound by LC_{50} 0.001 ppm followed by spinosad with LC_{50} of 0.0065 ppm while profenofos was the least toxic one with LC_{50} of 0.614 ppm.

The cytotoxic effect revealed certain deviations in the ultra structure of the cerebral neurosecretory cells (CNSC) of the treated pink bollworms larvae as compared by the untreated ones.

On the other hand, the chromatin in CNSC of the untreated larvae was centrally denser in the distribute than the cells of the treated pink bollworms larvae. Furthermore, the reduction in the ribosome regions was clearly shown in the cerebral neurosecretory cells of the treated larvae compared to the untreated larvae.

Lysosomes were completely inhibited and rarely observed in the CNSC of the treated larvae. In addition, the mitochondria were affected after the treatment by the above mentioned pesticides. They showed small size and hydrolyze as compared to the untreated larvae.
Moreover, the endoplasmic reticulum and Golgi bodies were absent in CNSC of the treated larvae.

INTRODUCTION

The importance of the insecticides used for the agriculture is to prevent the insect associated losses to the crop. In fact there is a great need to develop alternative insecticides or additional techniques, which would allow a rational use of pesticides and provides adequate crop protection for sustainable food, feed and fiber protection. Among the most promising alternative to the conventional insecticides is spinosad. The extensive worldwide testing has demonstrated that spinosad provides an effective control for key pests in numerous crops, including vegetables and cotton (Bret *et al.*, 1997; Nolting *et al.*, 1997; Temerak 2003 and 2006). The toxic effect of spinosad is the short lived due to its low persistence in the environment, which making it an ideal insect control product for use in glass house IPM programmes (Miles and Dutton 2000). Also the neem extracts has shown a great promising potential control agents against lepidopteron larvae (D'Andrea *et al.*, 2001; Rawale 2002; Sarafraz *et al.*, 2005).

Furthermore, the emamectin benzoate, the avermectin bio-insecticide, is one of the most recent introduced potential products to the control programme of cotton leafworm and bollworm complex in Egypt instead of the conventional old insecticides.

The histopathological studies were conducted to evaluate the cytotoxic effect of these potential pesticides on the cerebral neurosecretory cells (CNSC).

MATERIALS AND METHODS

1- *Pectinophora gossypiella* larvae:

The stock culture of PBW susceptible strain was supplied by the Bollworm Research Department, Plant Protection Institute, Agricultural Research Center, El-Dokki, Gize, Egypt, where it has been mass reared for several years under conditioned laboratory without exposure to insecticides. The rearing procedure was adopted as that described by Abdel –Hafez *et al* (1982).

2- Pesticides Used:

2.1. Organophosphorous Insecticides
a) Chlorpyrifos (Dursban 48% EC) ®
b) Profenofos (Selecron 72% EC) ®
2.2. Botanical insecticides
Neem Azadirachtin (Achook 0.15 % EC) ®
2.3. Bio insecticides
a) Emamectin benzoate (Proclaim 5% S.G) ®.
b) Spinosad (Spintor 24% S.G) ®

3- Experimental Method:

The film residue assay method was used where water concentrations were prepared and then 1 ml of each concentration was distributed inside Petri-dish of 10

cm diameter; then left for complete dryness. Ten of first instar larvae were introduced in each treated Petri-dish where each concentration was replicated three times. After 30 minutes from initiation the test, the semi-artificial diet was provided to feed the larvae on the internal edges of the previous used Petri-dish, and then they were kept under the same rearing conditions in the laboratory. For control, three Petri-dish replicates were treated only with water and were provided to the larvae. Mortality larvae were counted after 7 days and the mortality percentages were calculated for insecticides and control.

4- Cytological procedures and electron microscopic investigation of the cerebral neurosecretory cells (CNSC)

4.1. Dissection and fixation of *Pectinophora gossypiella's* brain tissues:

Having anesthetized the fourth instar larvae by immersing them into a cup filled with cold glutaraldehyde for 3 min., the front portion of the head capsule was cut using delicate sharp scissors while the larva was dipped in the fixative solution in Petri dish. The brain was removed using fine forceps under the dissecting microscope. The brain transferred to the first fixative solution glutaraldehyde 4% in 0.1 Mole PO. (Phosphate buffer), PH 7.2 (8% ampoules of glutaraldehyde, dilute 1:1 with 0.2 M PO_4 buffer), for 2-4 hours at 4°C. The samples washed with 0.2 M. sucrose in 0.1 M PO_4 (3 x 15 min. each) and the tissues were left to soak in the 4th change overnight. The samples were rinsed with 0.1 Mole PO. buffer. After that the samples were fixed with the second fixation using 1.% Osmium tetraoxide in 0.1 M PO_4 buffer, PH 7.2 for 1 hour at 4◦C, The samples were rinsed in 0.1 M PO, buffer (Mourad 1983).

4.2 Dehydration:

After fixation the tissues were dehydrated by soaking them in ethanol/water mixture of progressively inc-reasing concentrations starting with 30% and ending with absolute alcohol » according to the following time table:

30% ethanol in water	1 x	5-10 min
50% ethanol in water	1 x	10 min
70% ethanol in water	1 x	10 min
30% ethanol in water	1 x	10 min
96% ethanol in water	2 x	10 min
100% ethanol	1 x	10 min

Specimens that have to be stored overnight during dehydration are kept in 10% ethanol. There is a very little loss of lipid during long period of storage in 70% ethanol (Audrey I980). Continue dehydration with 1:1 (ethanol: propylene oxide as a transitional solvent) 5 rain, followed by 100% propylene oxide 4 x 10 min.

4.3. Infiltration:

Infiltration of dehydrated tissue had been initiated in 1:1 solution of propylene oxide and the embedding plastic mixture. Continue infiltration with 1:3 (Propylene oxide: embedding mixture) had been done overnight at room temperature.

4.4. Embedding media:

The following epoxies. Epon 812, polybed 812 have all given excellent results when used in these proportions:

Epon 812	5-0 ml
Hardener DDSA-HY	1.5 ml
MNA (methyl nadic anhydride)	3.5 ml
Accelerator (DMP-30)	0.2 ml

4.5. Sectioning and staining:

Thick sections of l m were stained for light microscopy examination. The sections examined to choose the right area for ultra sectioning and to complete trimming the block. By using the ultra microtome and glass knives, brain sections were cut at 1µm. They are removed from the water bath with a small glass rod and placed on a drop or distilled water on a glass slide. The slide was placed gently on a hot plate until the water was removed. Few drops of 5% methylene blue were placed on the slide on the hot plate (about 30 seconds). The excess of the staining solution were washed using distilled water. The slide dried again on the hot plate. The samples were examined with the light microscope after mounting.

4.6. Staining of thin sections (on the grids):

Thin sections (about 50 nm.) which are picked upon grids stained with Uranyl acetate 1.2# for 10-20 min. (1.2 g Uranyl acetate and 100 ml. H_2O) and washed with distilled water. Sections were stained with freshly prepared lead citrate 10-20 min. (0.4 g lead citrate and 100 ml (ION) NaOH. Then the sections were washed with (0.02 M) Na OH and finally with distilled water and dried.

4.7. Examining the sections:

After staining, the sections were examined by using the electron microscope type Joel 100 CX of the Faculty of Science, Alexandria University. The following cell organelle was examined in tissues of both treated and untreated control larvae:

Ribosomal density, Endoplasmic Reticulum, lysosomes, Mitochondria and Glycogen granules.

5. Statistical Analysis:

5.1. Regression equation and confidence limits:

Regression equation and confidence limits were calculated according to probit analysis computer program (Finney 1971). Regression equations are applied to predict mortality percentages (Y) for any

applied concentrations (X), whereas (a) is intercept from (Y) axis and (b) is slop of the linear regression.

5.2. Toxicity index (T.I):
The toxicity index values (T.I) were calculated according to Sun (1950) as follow:

$$\text{Toxicity Index (T.I)} = \frac{LC_{50} \text{ of the most toxic insecticide}}{LC_{50} \text{ of the tested insecticide}} \times 100$$

RESULTS AND DISCUSSION

Bioassay of certain insecticides against the newly hatched larvae of pink bollworm, *Pectinophora gossypiella* (Saunders):

The toxicities of three different groups of insecticides; organophosphate, bio-insecticides and plant origin insecticides; were compared against the newly hatched larvae of pink bollworm using film residue method. The two organophosphate insecticides (chlorpyrifos and profenofos) were involved in this study as a common standard control that being used as commercial insecticides against the pink bollworm and also they were recommended by the Egyptian Ministry of Agriculture. The results including the regression equations, the percent mortality, LC_{50} and LC_{95} values and its confidence limits, the slope values and the toxicity index were obtained and presented in Table (1).

Table (1): Toxicity parameters of certain insecticides against the newly hatched larvae of pink bollworm, *Pectinophora gossypiella* (Saunders).

Insecticides		Regression equation Y= a + b x	LC_{50} (Confidence limits)	LC_{95} (Confidence limits)	Slope	T.I*
OP's	Chlorpyrifos	Y=0.72+1.59X	0.355 (0.421-0.300)	3.84 (7.49-2.15)	1.59 ± 0.04	0.28
	Profenofos	Y=0.45+ 2.14X	0.614 (0.70 -0.532)	3.60 (5.42 - 2.66)	2.14 ± 0.048	0.16
Bio insecticides	Spinosad	Y=1.58+0.72X	0.0065 (0.011-0.004)	1.23 (4.29-0.38)	0.72 ± 0.01	15.38
	Emamectin Benzoate	Y=2.36 +0.79X	0.001 (0.0016 -0.0007)	0.125 (0.372 - 0.43)	0.79 ± 0.012	100
plant origin	Neem (Azadirachtin)	Y=0.946+0.77X	0.06 (0.091-0.038)	8.35 (33.52 - 2.52)	0.77 ± 0.013	1.67

***T.I = Toxicity index**

The results in this table show that, the LC_{50} values of chlorpyrifos, profenofos, spinosad, emamectin benzoate and neem (Azadirachtin) are 0.355, 0.614, 0.0065, 0.001 and 0.06 ppm; respectively, and the corresponding LC_{95} values were 3.84, 3.60, 1.23, 0.125 and 8.35 ppm for chlorpyrifos, profenofos, spinosad, emamectin benzoate and neem (Azadirachtin), respectively.

According to the LC_{50} values, the toxicity index (T.I) was calculated considering the most toxic compound having a figure equal to (100). The data show that, the most toxic insecticide is emamectin benzoate (T.I = 100) followed by spinosad (T.I = 15.38), neem (Azadirachtin) (T.I = 1.67), chlorpyrifos (T.I = 0.28), and then the least toxic one is profenofos (T.I = 0.28).

The finding of Abbassy *et al*. (1984) was in agreement with these results when reported that, according to the LD_{50}'s, chlorpyrifos was more toxic than profenofos on the 4th instar larvae of *P. gossypiella* by topical application in the laboratory. Also, Shekeban (1989) found chlorpyrifos was more toxic than profenofos and propoxur in laboratory studies against the 4th instar larvae of Egyptian cotton leafworm. In the same manner, the results of Shekeban (2002 a, b) were in harmony when tested three organophosphorous insecticides against the susceptible male moths strain using the attracticide resistance monitoring technique and the vial residue assay technique and cleared that chlorpyrifos was more toxic than profenofos. El-Arami (2008) disagree with the previous finding where he tested three organophosphorous insecticides against the susceptible male moths strain using the attracticide resistance monitoring technique in laboratory and found that, profenofos was toxic than chlorpyrifos.

Regarding to the toxicity of the recent compound, emamectin benzoate, which derived from the secondary metabolites of a fungus, the findings of Gupta *et al.* (2005) and Sontakke *et al.* (2007) were in harmony with the obtained result when tested against bollworm complex (American bollworm *H. armigera*, pink bollworm *P. gossypiella* and spotted boll worm *Earias* sp.) and reported that emamectin benzoate was the most potent treatment in reducing the damage of bollworms resulting in significantly higher yields. Also; Shekeban *et al* (2010) reported that emamectin benzoate was the most toxic insecticide between the tested insecticides and this result supported the foundation of this stud`y.

The results of many investigators (i.e. Swamy *et al* 2001; Radwan 2002; Temerak 2003; Dahawan *et al* 2006) were agreed with the present results about the superiority of spinosad and the ability to replace the old chemicals in the current official resistance rotation programme in Egypt.

Cytotoxicity studies with electron microscopy for the cerebral neuro-secretory cells (CNSC):

In order to obtain more detailed information on the mechanism of the effect of the used insecticides (profenofos, spinosad, chlorpyrifos, emamectin benzoate and neem (Azadirachtin) on the cell activity and ultra structure in the pink bollworm, *Pectinophora gossypiella*, some ultra microtomic preparations of the

cerebral neurosecretory cells (CNSC) of treated and untreated 4th instar larvae were prepared and examined with the electronic microscope.

These cells were chosen to study the effect of these insecticides because they exhibit some advantages as; they have a special shape which was well identified before, most of the all micro organelles can be stained and illustrated more easy than other types of cells and for it's important role upon the nervous system of the insect. (Toshio Ichikawa, 1991 and Homberg *et al.* 2004).

In general; the following illustrations of treated larvae were obtained as shown in the photos (3-8). The treated larvae showed some deviations of the resulted CNSC from the normal ones in their ultra structure.

Table (2): Comments on the obtained CNSC from the untreated and treated 4th instar larvae of the pink bollworm, *Pectinophora gossypiella*,

Picture number	Comment
1	Untreated cells showing general shape of the CNSC
2	Untreated cerebral neurosecretory cell show two adjacent cells close to each other. Notice the Golgi apparatus, Mitochondria and the rough endoplasmic reticulum (RER).
3	Treated cells with emamectin benzoate show cell malformation and disorder of the neurosecretory cells.
4	Treated cells with chlorpyrifos show the numerous numbers of lysosomes inside the cell with and without autolytic active foci.
5	Treated cells with chlorpyrifos show the spaces between the divided C.N.S.C. and the presence of cracks in the neural tissue.
6	Treated cells with spinosad show the beginning apolysis of the cell wall and as well apolysis of surrounded nucleus membrane .Notice the huge size of mitochondria.
7	Treated cells with Neem (Azadirachtin) show the apolysis of mitochondria and another irregular one on its way to disappear.
8	Treated cells with profenofos, show the active and non active lysosomes .Notice the presence of black dots (secretions) inside the cerebral neurosecretory cells which expected to be accumulation of Acetylcholine (Ach) and Adrenaline.

Photo: 1

Photo: 2

Photo: 3

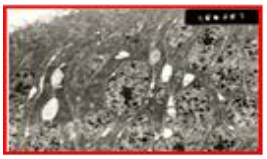

Photo: 4

Photo: 5

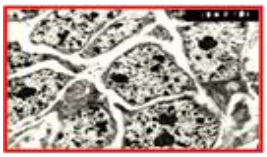

Photo: 6

Photo: 7

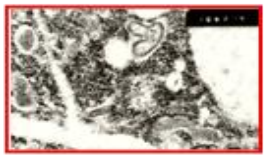

Photo: 8

1-Chromatin was denser and centrally distributed, in CNSC of untreated than treated cells. The decrease of chromatin dense in treated CNSC may be due to a blockage effect of the most selected insecticides on DNA synthesis in CNSC of *P. gossypiella* larvae.

2- There was reduction in ribosome regions in the preparations of treated CNSC in comparison with that of control. Such result may be due to an inhibition of the protein synthesis activity after treatment with the selected insecticides.

3- Lysosomes were rarely to be observed and in most cases they were completely inhibited in treated CNSC

4- Mitochondria were affected after treatment with the above mentioned insecticides. They were small in the size and hydrolyzed in the preparations of treated CNSC photos (6-7), while in check untreated were normal in size and structure. The endoplasmic reticulum and Golgi apparatus were found in untreated check C.N.S.C. while in treated cells were not visible.

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ROLE OF MONOOXYGENASES IN THE RESISTANCE FORMING TO INSECTICIDES IN HOUSEFLY (*MUSCA DOMESTICA*)

ABSTRACT: The monooxygenase mechanisms of the housefly resistance in the process of resistance formation to insecticides from three chemical classes: organophosphates, pyrethroids and derivate of benzylphenylurea were researched in this work. It was revealed that dependency on cytochrome P-450 microsomal monooxygenases play a part in formation of house fly resistance to insecticides, especially to pyrethroids. In a case with pyrethroids also correlation of increase in activity of this group of enzymes with increase in level of resistance of corresponding strains was observed. In house flies, selected with deltamethrin and ethophenprox, an increase of the monooxygenase activity correlated with increase in quantity of cytochrome P-450.

INTRODUCTION

Most insecticides are metabolized through a relatively small number of the basic types of enzymes, catalyzing several basic types of reactions. These enzyme systems give the chance to living organisms to transform potentially harmful connections into more polar hydrophilic substances which can be quickly removed from their bodies.

The basic types of reactions by means of which it is carried out primary detoxification are an oxidation, hydrolysis, dehydroclorination and transport of groups. Oxidation is carried out by means of microsomal mixed function oxidase (MO, MFO) which is in the gut, a fatty body, malpighian vessels and nervous system at insects. The oxidase system exhibits wide substrate specificity and can catalyze some types of bio-transformations.

Some researchers have shown that oxidative detoxification is the major metabolic way for the majority of pyretrines and pyrethroids (Chang, Kearns, 1964). Oxidation occurs at the terminal methyl groups on a lateral chain of chrysanthemum acid. This was first shown for allethrin, dymethrin, ftalthrin and pyretrin I, subjected to processing NADP•H homogenates abdomens of houseflies.

Monooxygenases and cytochrome P-450 take part in the mechanism of resistance of arthropods to organophosphates. Resistance of *Musca domestica* in many respects depends on activity of this enzyme to malaoxone (Fragoso et al., 2002) and *Leucoptera coffeella* to chlorpyrifos, disulfoton, ethion and methyl parathion (Welling et al., 1983). The indirect certificate on resistance communication to dichlorvos of the mushroom-infesting sciarid fly *Lycoriella mali* with activity MFO is received by using piperonyl butoxide (PBO) – the mixed function oxidase inhibitor (Brewer, Keil, 1989). At the same time it has not revealed considerable distinctions in activity MO between sensitive and resistant to malathion and chlorpyrifos individuals *Locusta migratoria manilensis* (Yang et al., 2009). Oxidase activity at malathion-resistant strain *Nilaparvata lugens* practically did not differ in comparison with sensitive (Liu, Han,. 2004).

By assumption F. Plapp (1976) detoxication of chitin synthesis inhibitors in particular diflubenzuron, goes through oxidizing processes, i.e. with the help of monooxygenases. This is confirmed by researches F. Ismail and D. Wright (1992) who found that natural populations *Plutella xylostella* from Malaysia, resistant to chlorfluazuron and teflubenzuron, possess higher monooxygenase activity, than a sensitive laboratory strain.

MATERIALS AND METHODS

Microsomal and postmicrosomal fractions from imago houseflies were obtained by method of differential centrifugation of L.L. Chang and E. Hodgson (1975). Abdomens imago were gomogenized with Potter

gomogenizator in 0.1 M K-phosphatic buffer pH=7.8, containing 1мМ sodium ethylenediaminetetraacetat (EDTA), 1 mM dithiothreitol (DDT), 10 % of glycerol at 200 abdomens per 10 ml of the buffer. Homogenat was filtered through a double layer of a gauze, and centrifuged for 15 minutes at 10,000 g in centrifuge K-24. Then a supernatant was centrifuged for 60 minutes at 100,000 g in Beckman ultracentrifuge. The microsomal deposit was suspended in 0.1 M K-phosphatic buffer pH=7.5. All operations were performed at temperature 0-4°C.

Activity of microsomal monooxygenases was defined by quantity formed as a result of reaction demethylation aminopirin formaldehyde (Nash, 1953). The incubation medium contained 1.6 ml of a solution gomogenat, 0.2 ml 1.5% aminopirin, 50 mcl $MgCl_2$ (160mM). Reaction was started by addition 0.2 ml 30mM NADP•H. In the control instead of NADP•H water (H_2O) was added. After incubation for 30 minutes in a water bath with shaking at 30°C to the incubatory environment it was added on 0.5 ml of solutions of 25% $ZnSO_4$ and $Ba(OH)_2$ (the sated solution). A mixture was centrifuged for 10 minutes at 7-8 000 rpm for sedimentation of proteins. Then for sedimentation of proteins to 1.5 ml supernatant 1.5 ml of a Nash reactive was added, incubated a mix of 30 minutes at 60°C, measured optical density of a solution at 412 nanometers on a spectrophotometer SF-46, the molar extinction coefficient of 8000 $M^{-1}cm^{-1}$ (Nash, 1953).

Quantity of cytochrome P-450 was defined by a differential spectrum of a complex restored by dithionite sodium of enzyme with CO (a method of Omura and Sato, 1964). Isolation of microsomes was carried out as described above. After resuspenzion microsomes were diluted by buffer to concentration of protein of 1 mg/ml, in control and skilled ditches some crystals dithionite sodium were added, then in skilled ditche for 1 minute CO was ditch which received mixing of the concentrated solutions of sulfuric and ant acids and cleared transmission through a pyrogallol solution in KOH solution (25% a pyrogallol solution and 60% a solution the KOH in the ratio 1:5). An absorption spectrum measured on differential spectrophotometer Spekord M40, using the molar extinction coefficient $91 \cdot 10$ $M^{-1}sm^{-1}$. An indicator of the maintenance of cytochrome P-450 in microsomes was the difference of the maximum absorption at 450 nanometers and the minimum absorption at 490 nanometers.

The protein concentration was defined by method Lowry (Lowry et al., 1951).

RESULTS AND DISCUSSION

From the six studied preparations, referring to three different chemical insecticide classes, resistance in the houseflies is formed quicker to pyrethrjids (fenvalerat and deltamethrin) and slower to derivate of benzylphenylurea chlorfluazuron (fig. 1). The intermediate position is occupied by the pyrethroid ethophenprox and organophosphates phoxim and phosmet.

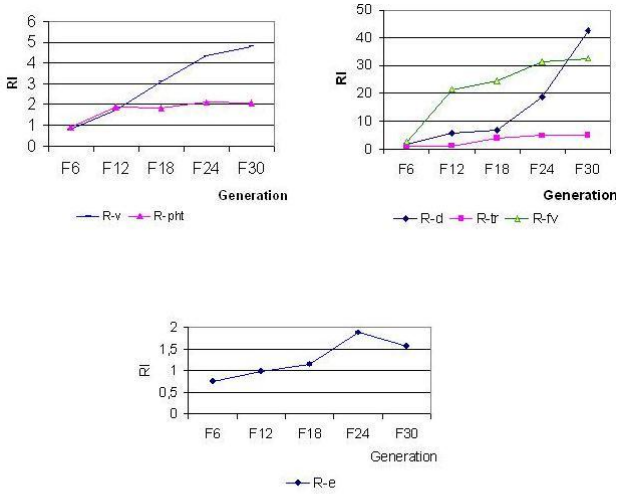

Fig. 1. The dinamic of resistanse formation to insecticides from three chemical classes:in the selected strains of housefly.

Results of researches by activity definition monooxygenases and to the maintenance of cytochrome P-450 in selected strains of houseflies are presented in tables 1 and 2 accordingly. In flies, selected by phoxim, monooxygenase activity increases a little in the sixth generation (less than in 1.5 times), and then remains throughout all selection practically at the same level. The content of cytochrome P-450 at these flies sharply increases in the sixth generation (more than in 3 times). In 12th generation it decreases in 2 times and then again increases to level of the sixth generation.

Table 1: Monooxygenase activity in house flies, selected by insecticides (nmol/min·mg protein)

Generation	Strain R-v	Strain R-pht	Strain R-tr	Strain R-d	Strain R-fv	Strain R-e
F_0 (strain S)	16.91±0.73					
F_6	21.34± 1.85	30.87± 5.76	17.49± 0.39	17.08± 0.77	18.89± 3.74	5.24± 0.98
F_{12}	23.53± 0.96	22.08± 6.18	35.36± 11.84	20.76± 4.43	21.3± 4.49	19.99± 0.96
F_{18}	23.4± 2.11	21.4± 1.39	27.0± 2.78	39.2± 0.97	25.7± 1.06	14.87± 1.96
F_{24}	22.94± 1.5	24.5± 2.16	36.98± 4.92	39.05± 2.94	25.51± 1.57	21.39± 1.66
F_{30}	27.34± 4.45	26.66± 5.12	38.96± 2.94	42.41± 3.29	28.8± 2.05	23.54± 1.67

Note –in dark checks difference of selected strain with sensitive is reliably, P>0.95.

Table 2: The maintenance of cytochrome P-450 in house flies, selected by insecticides (nmol/mg protein)

Generation	Strain R-v	Strain R-pht	Strain R-tr	Strain R-d	Strain R-fv	Strain R-e
F_0 (strain S)	0.057±0.012					
F_6	0.184± 0.02	0.110± 0.023	0.063± 0.0079	0.072± 0.0057	0.189± 0.046	0.089± 0.0043
F_{12}	0.084± 0.0037	0.147± 0.0078	0.150± 0.0096	0.116± 0.012	0.129± 0.0095	0.212± 0.0063
F_{18}	0.17± 0.010	0.149± 0.008	0.153± 0.012	0.142± 0.016	0.131± 0.0027	0.134± 0.0085
F_{24}	0.15± 0.011	0.128± 0.011	0.21± 0.006	0.186± 0.01	0.151± 0.0073	0.191± 0.003
F_{30}	0.18± 0.016	0.139± 0.014	0.176± 0.012	0.208± 0.013	0.179± 0.008	0.215± 0.006

In flies,-selected by phosmet, activity of microsomal monooxygenases in the sixth generation increases in a greater degree, than in the strain, selected by phoxim – almost 2 times. To 12th generation it decreases a little and remains at this level until the 24th generation. The content of cytochrome P-450 in treated by phosmet house flies to the sixth generation has increased in 2 times, to 12th and to 18th generations – almost in 3 times in comparison with a sensitive strain, to 24th generation it decreases a little. Despite fluctuations in the values of MFO activity and content of cytochrome P-450, these values are always higher than at a sensitive strain that, as a rule, is marked for resistant populations of insects. The raised content of cytochrome P-450 and its activity are shown at flies, middle-resistant to phosmet and ethaphos (Ivanova et al., 1989). Monooxygenase activity in resistant to fenitrothion strains of beetle *Oryzaephilus surinamensis* was in 14 (larvae)–50 (imago) time above, than at a sensitive strain, and the content of cytochrome P-450 – in 3 (larvae) – 10 (imago) time above (Rose, Wallbank, 1986). The contribution of these enzymes in detoxication of phosmet in highly resistant to this compound house flies (Zolotova, Roslavtseva, 1981) is considerable. At the same time, in middleresistant flies (RI=14) the role of these enzymes is insignificant. Apparently, the microsomal oxidase is activated with increasing levels of resistance and its contribution in this case the mechanism of resistance increases.

In strains selected with ethophenprox, MO activity and the content of cytochrome P-450 in 6th generation does not differ from those indicators in the sensitive strain. Then, in the process of selection, the quantity of cytochrome increases to 18th generation almost in 3 times, in 24th generation – in 3.7 times in comparison with initial value. In 30th generation the content of cytochrome decreases a little, but, nevertheless, it is in 3.1 times above, than in the sensitive strain. Monooxygenase activity also increased to 12th generation in 2 times, and then remains at that level until the 30th generation.

In strain, selected with deltamethrin, both described above indicators are in 6th generation level of the sensitive strain. Then the content of cytochrome P-450 has consistently increased: in 12th generation – 2 times, in 18th – 2.5, in 24th – 3.3 times and in 30th generation – 3.6 times. Monooxygenase activity also increased in the process of selection, but not uniformly. In the 12th generation it does not differ from initial level, and to 18th increases in 2 to 3 times and remains at this level to 30th generation.

The content of cytochrome P-450 in fenvalerat-selected strain is at a maximum in the 6th generation – in 3.3 times higher than the initial value for the sensitive strain. Then there is a decrease of this level, although the content of cytochrome P-450 is always above compared with the sensitive strain: in 12th and 18th generations in 2.3 times, in 24th generation – in 2.6 times. Activity of monooxygenases throughout all selection with fenvalerat practically varies very little and is rather insignificant – even to 24th generation it is only in 1.5 times exceeds activity of this enzyme in the sensitive strain.

C. Golenda and A. Forgash (1989) have informed about the role of monooxygenases in resistance of house flies to fenvalerat. Use of fenvalerat with sinergist PBO at the ratio 1:1 increases twice the amount of fenvalerat in the organism resistant to pyrethroids flies and decreases twice the quantity of fenvalerat hydroxylated metabolites. Similar experiences with PBO and permethrin have resulted K. Brewer and C. Keil (1989) to a conclusion about communication of the resistance of the mushroom sciarid with oxidase activity. The contribution of monooxygenases in detoxification of pyrethroids is considerable in multi-resistant *Helicoverpa armigera* from natural populations of China, India and Pakistan (Yang et al., 2004; Tan, McCaffery, 2007), permethrin in Colorado potato beetle (Nedelkina et al., 1988). At the same time monooxygenase activity did not correlate with resistance of *Heliothis virescens* to cypermethrin (Ottea et al., 2000).

Activity of microsomal monooxygenases in a strain of a house fly, selected with chlorfluazuron, in the sixth generation sharply falls – in 3.2 times in comparison with the sensitive strain, while the content of cytochrome P-450 a little above initial level. In 12th generation both indicators increased in comparison with the previous value – in 3.8 times the MO activity and in 2.4 times – the content of cytochrome P-450. In 18th generation again there is a decrease of enzyme activity and quantity of cytochrome P-450. In 24th and in 30th generations both indicators increased.

T. Sparks and B. Hammock (1983) spent studies of biochemical mechanisms of resistance to chitin synthesis inhibitors on an example diflubenzuron with the help of sinergists on a house fly. The coefficient of sinergism, defined for this insecticide in sensitive and resistant strains of a housefly with the help piperonyl butoxide (PBO) and sesamex was 2.6 and 77.6; 14.8 and 36.3 accordingly. These results clearly show that oxidation ways are important in a metabolism of diflubenzuron. Activity microsomal oxidases in relation to 7-metoksirezofurinu, 7-etoksirezorufinu and 7-etoksikumarinu in 7-28 time lower in the sensitive strain *P. xylostella* in comparison with tolerant to teflubenzuron a strain of the wrecker and two strains which have been selected for resistance to this preparation in vitro (Lin et al., 1989).

CONCLUSION

Thus, it is possible to draw a conclusion that dependent on cytochrome P-450 microsomal onooxygenases play a part in formation of house fly resistance to insecticides, especially to pyrethroids that was repeatedly marked and in the literature. In a case with pyrethroids the activity increase is noted not simply, but also correlation of increase in activity of this group of enzymes with increase in level of resistance of corresponding strains (fig. 2) was observed. The similar fact resulted also by other researchers (El-Guindy et al., 1982) at use for selection of a house fly permethrin.

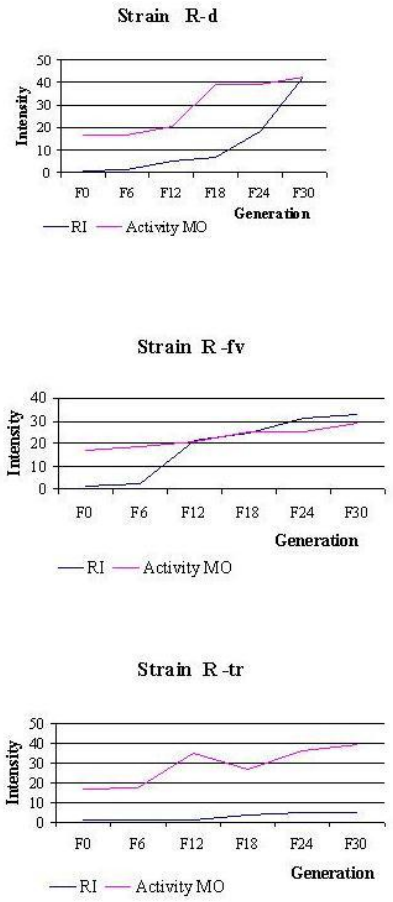

Fig. 1. Change of the monooxygenase activity and RI in the process of pyretriod selection.

In house flies, selected with deltamethrin and ethophenprox, an increase of the monooxygenase activity correlated with increase in quantity of cytochrome P-450. In other strains such clear relationship was not observed. A possible reason for this discrepancy is the fact that in the detoxification of insecticides may be involved and other components of the monooxygenase system (cytochrome b5, NADP H-cytochrome-c-reductase). As cytochrome P-450 exists in an organism of insects in the form of the several interconnected structural forms large number of endogenous and exogenous substances can serve the substrat for microsomal oxidases.

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THE PROBLEM OF THE INSECT AND MITE RESISTANCE TO INSECTOACARICIDES; RESISTANCE TO MODERN INSECTOACARICIDES

For struggle against the harmful arthropods resistant to organophosphates (OP), the preparations which are belonging to the class pyrethroids have been synthesized. They effectively operate on a wide range of agricultural pests, including resistant to other insecticides, are low toxic for warm-blooded animals, quickly break down to low toxic substances in soil and do not possess phytotoxicity. The advantage of pyrethroids before traditional insecticides is their high selective toxicity concerning insects and low norms of the expense on operating substance (Elliot et al., 1978). But wide and often unreasonable application of these insecticides has led to the occurrence of resistance to these preparations in populations of pests.

After application, within several years, of tetramethrin on the natural population of *Musca domestica* in Czechoslovakia the resistance index (RI) to this insecticide has grown 100 times (Rupes. 1982). On cattle-breeding farms of Japan after application of permethrin, house flies have a large resistance to it (RI =1117, as well as to a number of others pyrethroids (RI to resmethrin - 767, allethrin – 209, fenothrin– 115, fenvalerate – 305) (Motogama. 1984). Horn flies from populations of the State of Louisiana (USA) have shown resistance to fenvalerate (RI = 10-36) and permethrin (RI = 10-27) (Quisenberry et al., 1984). In two years after application fenvalerate has ceased to act on population of flies *Haematobia irritans exigua* and RI to this insecticide has increased to 45 (Schrifzerling et al., 1982). At pear deaf adder *Psylla pyricola* Foerster for 5 years resistance to fenvalerate has increased at 16-32 time. These insects have shown resistance to permethrin and flucytrinat, but have kept sensitivity to fenpropathrin (Burts et al., 1989).

In 1982, in the hothouses of England, resistance to pyrethroids in glasshouse whiteflies had been found for the first time. The resistance index to permethrin in various populations varied from 6 to 4000, in 15 populations it exceeded 1000 (Wardlow, 1985). In 1983, on cotton fields in the central Australia, populations of *Heliothis armigera* have been found out, steady to pyrethroids cypermethrin, deltamethrin and fenvalerate. Survivors after application of these preparations in cutting concentration of the individual have formed a strain which possessed 15, 20-30 and 20-fold resistance to these pyrethroids accordingly. Same year resistance of cotton-boll worm *Heliothis armigera* for the first time has been found irritans to fenvalerate and permethrin in the USA. In 1985 resistance to pyrethroids had been found in 15 states. Resistance index varied in various regions, and the part of individuals of the same population is steady against one pyrethroid, a part – to another (Gunning et al., 1986).

In Bashkiria (Russia) application within 10 years of deltamethrin against Colorado potato beetle has led to formation of 4-6- fold resistance of this pest (Leontjeva et al., 1996). The Nizhniy Novgorod population of Colorado potato beetle for some years (from the end 90 till 2001) has increased resistance to deltamethrin, a sumi-alpha, lambda-cyhalothrin and some other pyrethroids, actively applied in this area, at 8-12 time (Ivanov et al., 2002), and population of this pest in the Belgorod region for the five years' period (1993-98) on industrial landings of a potato has generated 25-fold resistance to permethrin, and in a private sector – 50-fold (Vasiljeva, 2000).

Along with harmful arthropods resistance to pyrethroids was shown also by some useful insects. O. J. Eremina and S. A. Roslavtseva (Eremina, Roslavtseva, 1987) studied the level of insecticide in several pyrethroids – permethrin, cypermethrin, deltamethrin, fenvalerate, cyfluthrin and alphamethrin – for a number of useful insects. During researches it was found out that larvae both imago podisus and imago strawberry seed beetle have appeared rather steady against action of these preparations.

In the past century, for almost 20 years of application of pyrethroids resistance to the different kinds of insects has increased from 10 to more than 70 (Farnham, 1985). However, a number of cases on the occurrence of resistance as a whole more than registered individual cases have decreased the economic efficiency of their application. It is connected by the resistance that is found not only in wreckers of agriculture, but also in entomofages or in the kinds which do not have practical value. Besides, the resistance indexes defined in vitro is not always adequately reflected in real-life stability.

The question was long enough discussed, whether resistance to chitin synthesis inhibitors (CSI) can develop. There is numerous literary data about efficiency of diflubenzuron against resistant to various insecticides insects. For example, this preparation was active against resistance to organophosphates mosquitoes *Aedes nigromaculis*,

A. melanimon, *Culex tarsalis* (Schaefer et al., 1975), rice weevil *Sitophilus oryzae*, red flour beetles *Tribolium castaneum* (Carter, 1975), and metopren mosquitoes *C. pipiens* (Brown, 1978). Moreover, even the six-fold application of diflubenzuron on one of farms of Denmark, and the subsequent selection of flies that were resistant to them (Keiding, 1977) in vitro have not led to occurrence.

Later, there was data about the formation of resistance to these insecticides. For example, there is data about existence naturally resistant (tolerant) to teflubenzuron strains of diamondback moth *Plutella xylostella*, and also about possibility of development of resistance at cabbage ask to it CSI in vitro (Lin et al., 1989). Selection of *T. confusum* during 8 generations has led to increase of resistance index in 2 times (Brown et al., 1978). Resistance to diflubenzuron in Egyptian cotton leafworm (Ahmed et al., 1987) has developed, and at a house fly under the influence of this preparation it was possible to develop resistance with more than 1000-fold resistance (Pimpriker, Georghiou, 1979). G-j. Gono et al. (1986) have found out in caterpillars *Agrotis ypsilon* high natural stability to this preparation. Preparations of this class do not find wide application in agriculture. The possible reasons of this are their manufacture expenses and the effect is shown not so quickly as at application insecticides in other classes.

High toxicity OP for warm-blooded animals and the many fast development of resistance in pests to synthetic pyrethroids:, expensive manufacture CSI was caused by necessity of working out and introduction of representatives of new classes of insecticides.

The greatest popularity now has received avermectins and neonicotinoids. Avermectins are a product with the ability to live in soil microorganism, *Streptomyces avermitilisu,* and are naturally synthesized in the form of a complex of closely related connections on a chemical structure. Now avermectins is received by chemical synthesis. Active application of these preparations has dictated necessity of studying in the possibility of formation of resistance to them.

Literary data about high resistance to these connections is not numerous. Collected naturally and subjected, laboratory room flies have developed very high resistance to abamectin during 7 generations (Scott et al., 1991). In the USA, resistance to abamectin populations of Colorado potato beetle have been received (ПР=15-30) by rigid selections of these insects (Argentine, Marshall, 1990), and one of them has been received from a laboratory sensitive strain, and another – from field population. In California and Florida, field populations of the ordinary web tick with resistant index to abamectin 7-690 are revealed (Campos et al., 1997). Caterpillars *P xylostella*, collected in the nature, contained in laboratory on processed abamectin leaves, have developed 30-135-кратную resistance to insecticide (Zhao et al., 2007).

There is much more data about low resistance to avermectins or about its absence. At selection, in vitro by avermectin of natural population Australian sheep blowfly, *Lucilia cuprina,* for 60 generations has developed insignificant resistance (RI <10) (Rugg et al., 1998). As resistance to avermectin in the horn fly slowly developed – at selection during 30 generations RI did not exceed value 3 (Byford et al., 1999). Resistance to abamectin is not found in head louses in the USA (Clark et al., 2002). Caterpillars *P. xylostella*, collected in different areas of China, were sensitive to abamectin, as well as to insecticides from other classes (Long et al., 2005). House flies, selected by avermectines A_1 and A_2 during 18 and 20 generations respectively, also have not generated resistance to these substances (Alexeev, Roslavtseva, 2006). For protection of hothouse roses against resistant populations of phytophagous mites *Tetranychoidea* the preparation fytoverm which possesses the prolonged action is recommended and renders ovicide action (Meshkov et al., 2007).

Last decade the wide circulation in practice of protection of plants from pest kinds of insects was received by preparations of a new class - neonicotinoids. Low norms of the expense, high system and translaminar action in plants, moderate firmness in objects of environment are peculiar to them high biological activity against a wide spectrum of wreckers of agricultural crops. In spite of the fact that preparations of this class have started to be applied not so long ago, in the literature there was already data about formation of resistance to them. At selection of the Green peach aphid *Myzus persicae* in vitro it was possible to receive strains with 14-fold (Chen et al., 2005) and 18-fold (Foster et al., 2003) resistance to neonicotinoids in comparison with a sensitive strain. In Great Britain in rare instances small level of resistance to these preparations meets at these wreckers and naturally (Foster et al., 2003). As a result of laboratory selection resistance of a strain of cotton aphid *Aphis gussypii* to imidacloprid managed to be raised in 20 times. Field researches have shown that resistance to this insecticide strongly varies depending on a population geographical position. Believe that in field conditions the cotton aphid is capable to develop, at

least, moderate level of resistance to imidacloprid (Li, Han, 2007). In the USA Colorado potato beetle imago and larvae collected in Lond Island, have shown, accordingly, 100 and 13-fold resistance to imidacloprid after two years of processing by this insecticide (Zhao et al., 2000).

Thus, insects can generate resistance to these insecticides. But while it basically is shown at tolerance level, application of this class of insecticides is effectively enough. For example, in delta of Volga (Russia) application confidor for processing of tomatoes from Colorado potato beetle has led to decrease in its number of 90% (Bajrambekov et al., 2004). In Belarus actara has shown almost 100% insecticide activity in relation to cereal thrips on winter тритикале and a rye (Bojko, Slobozhankina, 2004). Mospilan and actara have shown high efficiency in the Volga region against bug *Eurygaster integriceps* (Silaev et al., 2004). Neonicotinoids are effective against a pea aphid, *Sitona* species, Phytonomus rumicis (Karjakina, 2004) Now preparations of last two classes show high efficiency in relation to a wide spectrum of agricultural wreckers, their assortment that allows recommendation of their inclusion in schemes of rotation of preparations for the purpose of decreasing the speed of formation of resistance to intensively applied insecticides and acaricides extends.

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Comparative efficacy of single (*Cry1*Ac) and stacked Bt genes (*Cry1*Ac+*Cry2*Ab) in interspecific Bt cotton hybrids against tobacco caterpillar, *Spodoptera litura* (Fab.)

ABSTRACT

Bt cotton cultivars generally referred to as Bollgard (BG) contain a single gene (Cry1Ac) that offers protection against bollworms *viz., Helicoverpa armigera* (Hubner), *Erias vittella* (Boisdual) and *Pectinophora gossypiella* (Saunders). Although the Cry1Ac is toxic to the bollworms, voracious foliage feeder, *Spodoptera litura* (Fabricius) is less sensitive to Cry1Ac protein and is predicted to be a major pest in the emerging scenario. As an attempt to have broader spectra of activity within the lepidopterans, which were previously controlled effectively by single gene constructs and improved efficacy against bollworms, stacked or duel gene Bt cottons popularly known as Bollgard-II (BG-II) were approved in both USA and Australia since 2002 and in India since 2006 as the most convenient tool for resistance management. The dual-gene cotton hybrids produce approximately the same level of the Cry1Ac protein as that of single-gene Bollgard cultivars, but are further protection extended by Cry2Ab protein. Keeping in view of this fact, the present investigation was undertaken during 2009-10 season at ARS, Dharwad to study comparative bioefficacy of single and stacked Bt genes containing hybrids against tobacco caterpillar under laboratory condition.

The investigation on bioefficacy of Cry1Ac in MRC-6918 BG-I and Cry1Ac + Cry2Ab in MRC-7918 BG-II interspecific Bt hybrids against *S. litura* was carried out from 60 to 140 DAS at 20 days interval by leaf feeding bioassay method. About ten larvae were in each treatment were replicated four times. Two days old neonates were used for bio-assay. The larvae were released @ one/well on leaf disc of 2.0 cm diameter and closed tightly with serene wrap and lid. The leaf discs were changed with fresh ones every day. The leaf discs were placed on semi-wet filter paper disc of similar size to avoid drying of test material. Rearing trays of 25 wells were used for bioassay. The mortality of the larvae at 24 hours interval till seven days was recorded and converted as percent mortality and later corrected using mortality in the control treatment (DCH-2 non Bt) and only corrected mortality for each treatment was considered. Mean mortality during the respective days of interval was subjected to student "t" test for comparison.

Table 1: Comparative Bioefficacy of single (*Cry*1Ac) and stacked genes (*Cry*1Ac + *Cry*2Ab) against tobacco caterpillar, *Spodoptera litura* (Fab.)

Gene	Neonate mortality (%) of tobacco caterpillar					
	60 DAS	80 DAS	100 DAS	120 DAS	140 DAS	Mean
*Cry*1Ac (MRC-6918 BG-I)	35.00 (36.20)	23.06 (28.61)	12.50 (20.46)	10.28 (18.69)	5.00 (9.21)	17.17 (22.63)
*Cry*1Ac+ *Cry*2Ab (MRC-7918 BG-II)	100.00 (89.96)	97.22 (85.10)	92.50 (76.14)	81.94 (65.05)	62.50 (52.25)	86.83 (73.70)
"t" value	30.94	11.04	11.05	20.99	7.78	6.20
Percent increase over *Cry*1Ac	65.00	76.28	86.48	87.45	92.00	80.22

Table t value at 6 *df* : 2.44
Table t value at 8 *df* : 2.30
Figures in parenthesis are arcsine transformed values

The results revealed that, at 60 DAS, the mortality of *S. litura* was 100 per cent and proved the efficacy of stacked genes and was significantly superior to the leaves expressing single gene (35.00%). The mortality was gradually reduced to 23.06 per cent at 80 DAS and reached down to 5.00 per cent at 140 DAS. Whereas, mortality on leaves expressing stacked gene remained significantly high and ranged from 97.22 to 62.50 per cent from 80 to 140 DAS respectively. The mean mortality of larvae over the season clearly indicated that the enhanced efficacy of stacked genes was confirmed by rendering significantly higher mortality of *S. litura* (86.83%) compared to the mortality caused by single gene (17.17%). On the contrary extent of increase in mortality of *S. litura* on leaves expressing stacked genes was 80.22 per cent over leaves containing single gene. It can be concluded that, Cry1Ac was found to have no significant effect on *S. litura* with 35.00 per cent mortality at 60 DAS itself and per cent mortality was significantly reduced to 5.00 per cent at 140 DAS. The additional advantage of stacked genes in Bt cotton hybrid with expanded horizon of bioefficacy against *S. litura* was evident by

rendering higher percentage of mortality (100.00 to 62.50%) compared to the mortality on leaves expressing single gene.

Key words: Single gene (Cry1Ac), Stacked gene (Cry1Ac+Cry2Ab), Bioefficacy, Bt Cotton

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Pest Management and Contemporary Entomology (review of ECE 2010)

The IXth European Congress of Entomology was held in Budapest, beautiful capital of Hungary during 22-27 August 2010. According to concluding remarks of Dr. Tamas Vasarhelyi, Chairman of the Organizing Committee ECE 2010, Honorary Presidium Member, there were near 600 participants (532 registered participants, and some short time guests). More than 300 oral and more than 300 poster as well as 7 plenary presentations gave the scientific content of the Congress. Participants celebrated the 100th anniversary of the Hungarian Entomological Society (fig. 1) in the exhibition Hexapod Empire of the Hungarian Natural History Museum.

ECE was of an interdisciplinary character involving a wide range of research directions. ECE attracted taxonomists, systematics, ecologists, physiologists, toxicologists, biochemists, ethologists, experts of biocontrol. Plenary speakers were invited by the NOC (National Organizing Committee). ECE 2010 featured 7 plenary talks and 37 symposia, held in 3-5 parallel sessions, together with an Exhibition of 4 world's foremost scientific publishers (Brill, Elsevier, Wiley-Blackwell, Pensoft Publishers) and Noldus Information Technology.

Update problems and aims of pest management investigations have been elucidated in presentations and posters at the set of symposia: "Biocontrol in crops and storage"; "Biorational control of arthropod pests: mechanism and application"; "IPM challenges and prospects in annual and perennial crops"; "Role of biodiversity in pest management"; "Semiochemicals in agroecosystems"; "Xenobiotic effects and side-effects on arthropods". Symposia and poster sessions "Invasive species" and "Genetically modified plants – effects on insects" also were dedicated mainly to the pest management. 20 oral presentations and 35 posters were presented at the symposium "Biorational control of arthropod pests: mechanism and application", alike the 14 oral presentations and 30 posters presented at the symposium "IPM challenges and prospects in annual and perennial crops", the most plentiful symposia of the Congress.

In the first plenary talk of ECE (Sylvia Dorn, Switzerland, Elected Presidium Member and Deputy Chair) ecological consequences and implications of orchard pests adaptations for integrated pest management have been discussed. A. R. Horowitz, P. Ellsworth and Isaac Ishaaya briefly summarized various new environmentally friendly approaches for pest management in the presentation "Biorational control of arthropod pests: an overview". One such approach is based on disrupting the activity of specific hormones and other signaling molecules acting on specific insect receptors. Another approach is the potential use of natural products of plant origin for pest control. Novel biotechnology control strategies ("the genetic approach") were discussed. New term "Biorational control" has been proposed to replace the term "Integrated pest management". Dr. Isaak Ishaaya

presented new chemical tools of pest management in his talk. Dirk Babendreier on behalf of group of Switzerland researchers presented "Framework for sustainable use of pesticides across the EU Member States: challenges and opportunities for implement IPM", about new Directive 2009/128/EC of the European Parliament. Member States must develop a National Action Plan, including targets, measures and timetables to reduce pesticide risks and hazards, as well as dependence on pesticides. An important but challenging task is the harmonization of IPM across Europe, while ensuring the environmental and economic sustainability of food production in each MemberState. An example of pesticide-free pest control has been presented by Hungarian specialists, A. Pinter and F. Toth.

An important sign of ECE 2010 was the comprehension of pest resistance-susceptibility monitoring necessity. Attempts of entomopathogenic fungi, bacteria and viruses use for pest control were presented in 12 submitted abstracts. For the most part investigations carried out in Turkey, Iran and India. Primary set of entomopathogenic fungi was little: there were *Lecanicilliummuscarium*, *L. lecanii*, *Paecilomyces lilacinus*, *Beauveria bassiana* and *Metharizium anisopliae*. Their strains can infect the wide spectrum of insect species. Some results were dedicated to the prospective investigations of synergistic effects of different origin pathogens, including joint effect of *Bacillus thuringiensis* and *Metharizium anisopliae*.

In 26 posters and presentations application for pest control of pest's parasitoids and predators examined. Various ways of biological pest control were presented in several sections: "Role of biodiversity in pest management", "Biocontrol in crops and storage", "Landscape ecology and management", "IPM challenges and prospects in annual and perennial crops, Carabid ecology". There were reports in the sections of "Role of biodiversity in pest management" and "Landscape ecology and management" which described the effect of biodiversity on pests and presented researches of methods to increase biodiversity, investigations including the impact of fertilizers, plant communities near the fields and modern agricultural technologies. Negative influence of intraguild relations is proved for small and large predators. Significant differences of species composition and number of pest's predators in plant communities of different ages, positive relationship of biodiversity and the number of flowering plants has been showed. Also results presented of a studies showing influence of the type and quantity of fertilizers on the number of entomophagous species. Increase of the number is proved for using of organic fertilizers. Laboratory researches of dependence of *Grafosoma* fecundity from application of fertilizers are presented.
Classical works on studying of biology and phenology of kinds of agents of the biocontrol: predatory beetles, parasitoids of eggs and larvae, and pathogenic nematodes have been presented in the section "Biocontrol in crops and storage". Investigations of the behavioral characteristics of parasitoids are considered. There were works generalizing experience on introduction of methods of the biocontrol in production with reception of positive results at commercial operation. Successful examples of biological control based on integrated application of entomophagous: predators and parasitoids or pathogens. For example, at cultivation of tomatoes on the closed ground high efficiency of simultaneous use predatory ground beetles and parasitoids eggs is shown. Also the problem of the most effective entering pathogenic nematodes in soil for decrease in number of *Diabrotica* has been studied.

The problems of attracting entomophagous on the field and influence of the insecticides for the ratio of pests and predators are discussed in the section IPM challenges and prospects in annual and perennial crops.

Problem of availability of pest for mass predators was announced in the section "Carabid ecology". It is a new problem and its resolve may be very important for improve methods of the biocontrol.

Although wide coverage of biological control and a large number of reports were presented at the conference, but it did not work, there were not any investigations using the methods of detection of new significant predators. Studies showing the influence of individual species on pest populations in natural conditions aren't presented at the conference.

It remains open questions about the commercial importance of biocontrol methods, reducing costs and developing new approaches.

Remarkable that in IXth European Congress of Entomology it was presented many reports based on such molecular methods, as polymerase chain reaction (PCR) and sequencing.

Some results for several insects' species was shown in separate symposia "RNA interference, a novel tool in analyzing hormone function". So, this methods was used for research in insect phylogeny, phylogeography, population polymorphism, divergence of close related species, in study of insect vector-disease transmission, insecticide resistance researches, create and investigations of transgenic plants and insects.

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Research Progress in Understanding Insecticide Resistance in Oriental Migratory Locust, *Locusta migratoria manilensis*

The migratory locust, *Locusta migratoria* Linnaeus (Orthoptera: Acrididae), is a serious agricultural pest in many regions of the world, including sub-Saharan Africa, the Arabian and Indo-Pakistan Peninsulas, Europe and Mediterranean coastal areas, eastern Asia, and Australia. *L. migratoria* primarily feeds on bulrush and gramineous plants, which makes it a major agricultural pest due to its huge consumption of crops, and more importantly its periodic massive outbreaks. In China, the control of the oriental migratory locust, *L. migratoria manilensis*, used to rely on organophosphate insecticides (OPs). However, extensive applications of OPs have inevitably resulted in the development of insecticide resistance in some natural populations of the locust (Ma et al., 2004; He et al., 2004; Yang et al., 2008, 2009).

To elucidate the mechanism of insecticide resistance in the oriental migratory locust, we initially compared general esterases from two populations of the oriental migratory locust, collected from Huanghua and Pingshan Counties, Hebei Province, China. General esterase activities in the Huanghua population were 1.8-fold higher than those in the Pingshan population. Increased esterase activity in the Huanghua population appeared to be mainly due to several additional esterase bands detected on non-denaturing polyacrylamide gel electrophoresis (PAGE). Inhibition studies of general esterases using four inhibitors, including paraoxon, malaoxon, eserine and carbaryl, indicated that most general esterases in the two populations were B-type. The increased esterase activity in the Huanghua population appeared to be associated with a 1.8-fold decreased susceptibility to malathion. Such differences may attribute to the difference in control practices for the locust between Huanghua and Pingshan Counties (He et al., 2004). Because we only compared the susceptibilities to malathion between the two field populations and did not include a known susceptible population for comparisons at that time, we did not know the exact levels of malathion resistance in the field populations.

We further evaluated a possible involvement of insensitive acetylcholinesterase (AChE) in reduced susceptibility of the Huanghua population. We first purified AChE by affinity chromatography from two populations of the locust. The purification factors and yields were 1661-fold and 19.3%, respectively, for the Huanghua population, and 3897-fold and 39.6% for the Pingshan population. Both the purification factor and yield were significantly lower in the Huanghua population than in the Pingshan population. AChE activity was almost completely inhibited by 10^{-6} M eserine and BW284C51, but $\leq$ 5.8% of AChE activity was inhibited by ethopropazine at the same concentration, suggesting that purified AChE from either population was a typical insect AChE. However, AChE purified from the Huanghua population was 62-, 2.0-, and 1.6-fold less sensitive to inhibition by the three organophosphate compounds, chlorpyrifos oxon, demeton-*S*-methyl, and paraoxon, respectively, than that from the Pingshan population. Significantly lower purification factor and low yield associated with reduced sensitivity of AChE to inhibition by the organophosphates indicated that AChE purified from the Huanghua population was biochemically and pharmacologically different from that of the Pingshan population. Reduced sensitivity of AChE appeared to contribute to organophosphate resistance in the locust from Huanghua County, where insecticides have commonly been used to manage outbreaks of the locust (Ma et al., 2004).

We also compared the susceptibility to malathion, and the activity and sensitivity of AChE between other two field populations of the locust collected from Wudi County of Shandong Province in East China and Huangliu County of Hainan Province in South China. Huangliu population showed 8.5-fold resistance to malathion compared with Wudi population. AChE from Huangliu population showed 4.8-fold higher activity than that from Wudi population toward the model substrate acetylthiocholine (ATC). Kinetic studies indicated that AChE from Huangliu population had 2.6-fold lower affinity, but 5.0-fold higher catalytic activity toward ATC than AChE from Wudi population. Significantly increased activity of AChE in Huangliu population was also confirmed by non-denaturing PAGE. Inhibition kinetics revealed that

AChE from Huangliu population was 9.8-, 2.4-, 8.0- and 7.7-fold less sensitive to inhibition by paraoxon, malaoxon, chlopyrifos oxon, demeton-*S*-methyl, respectively, than those from Wudi population. This study revealed that a mild resistance to malathion in Huangliu population was associated with reduced sensitivity and increased catalytic activity of AChE. Our results suggested that alterations of AChE might play an important role conferring or contribute to Malathion resistance in Huangliu population of the locust (Yang et al., 2008).

In a separate study, we compared the susceptibilities to three OPs (malathion, chlorpyrifos, and phoxim), responses to three metabolic synergists [triphenyl phosphate (TPP), piperonyl butoxide (PBO), and diethyl maleate (DEM)], activities of major detoxification enzymes [general esterases, glutathione S-transferases (GSTs), cytochrome P450 monooxygenases (P450s)], and sensitivity of AChE between a laboratory susceptible (LS) colony and a field-derived resistant (FR) colony of the locust. The FR was significantly resistant to Malathion (57.5-fold), but marginally resistant to chlorpyrifos (5.4) and phoxim (2.9). The Malathion resistance of the FR colony was significantly diminished by TPP (synergism ratio: 16.2) and DEM (3.3), but was unchanged by PBO. In contrast, none of these synergists significantly affected the toxicity of Malathion in the LS colony. Biochemical studies indicated that general esterase and GST activities in the FR colony were 2.1- to 3.2-fold and 1.2- to 2.0-fold, respectively, higher than those in the LS colony, but there was no significant difference in P450 activity between the LS and FR colony. Furthermore, AChE from the FR colony showed 4.0-fold higher activity but was 3.2-, 2.2-, and 1.1-fold less sensitive to inhibition by malaoxon, chlorpyrifos-oxon, and phoxim, respectively, than that of the LS colony. All these results clearly indicated that the observed Malathion resistance in the FR colony was conferred by multiple mechanisms, including increased detoxification by esterases and GSTs, and increased activity and reduced sensitivity of AChE to OP inhibition (Yang et al., 2009).

All these studies clearly indicated that increased esterase activity was a major mechanism of malathionresistance in field populations of the locust in China. To further study the OP resistance at the molecular level, we screened carboxylesterase (CarE)-like genes from a large locust expressed sequence tag (EST) database and assessed their potential roles in malathion resistance. Twenty-five ESTs derived from different CarE-like genes in the locust EST database were identified by bioinformatics methods. Among these CarE-like genes, we found a total of 12 candidate genes showing significantly increased transcript levels ranging from 2.6- to 11.6-fold in the FR colony compared with the LS colony as evaluated by real-time quantitative PCR. To evaluate the potential role of the two CarE genes, *LmCarE9* and *LmCarE25*, in conferring malathion resistance in the FR colony, we used RNAi techniques to silence these two genes individually or both at the same time followed by malathion bioassays. Preliminary experiments showed that the expression of *LmCarE9* and *LmCarE25* can be repressed 12 h after the injection of their dsRNA into the second instar nymphs and the silence can reach its maximum level 24 h after the dsRNA injection. The nymph mortalities increased from 34.3 to 65.2 and 54.2% respectively after *LmCarE9* and *LmcarE25* were silenced. Compared with the control locusts which were injected with the same volume of deionized distilled water, the susceptibility of locust to malathion was significantly increased after injection of dsRNA of *LmCarE9* and *LmCarE25*. However, silencing both *LmCarE9* and *LmCarE25* did not show significant synergism when exposed to malathion. These results supported our notion that at least some of the 12 CarE-like genes might play an important role in conferring malathion resistance in the FR colony (Zhang et al., 2011).

Because our previous studies also indicated that increased GST activity contribute to malathion resistance in the locust, we carried out a relatively detailed analysis of the GST genes based on the locust EST database. We identified a total of 10 GST-like genes, characterized their molecular properties, determined their developmental stage- and tissue-specific expression patterns and assessed the effect of deltamethrin on theirexpressions. Phylogenetic analysis revealed nine GSTs in three different classes, including seven in sigma, one in delta and one in theta. The remaining GST (*LmGSTu1*) was unclassified. Real-time quantitative PCR analysis showed that deltamethrin at 0.08 and/or 0.12 μg/mL induced the expression of almost all 10 GSTgenes in third-instar nymph locusts. However, deltamethrin at 0.16 and/or 0.2 μg/mL decreased the expressions of *LmGSTd1*, *LmGSTs1*, *LmGSTs5* and *LmGSTs6*. This study provided the first insight into molecular characteristics of *GSTs* and their transcriptional response to deltamethrin exposures in the oriental migratory locust(Qin et al., 2011). Further studies using RNAi will help us identify key GST genes responsible for insecticide resistance in this important insect pest.

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