Resistant Pest Management Newsletter

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

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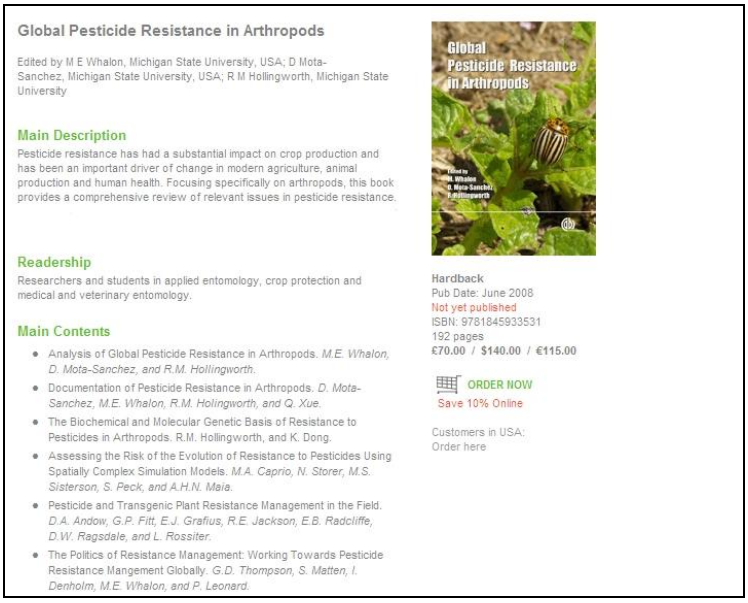
Letter from the Editors

Dear Newsletter Subscribers,

We would like to take this opportunity to let you all know about a new book that will be coming out in June of 2008 that you may be interested in hearing about. The book is called ***Global Pesticide Resistance in Arthropods***. It was written by many authors in the field of pesticide resistance and is edited by M.E. Whalon, D. Mota-Sanchez, and R.M. Hollingworth, who are, as you may know, the editors of this newsletter. This books focus is on the state of pesticide resistance today, the policies that are in place to delay resistance to pesticides, and the methods used to predict and control resistance. Please review this book when it becomes available in June of 2008 from CABI Publishers.

Sincerely,
Abbra Puvalowski
RPMNews Coordinator



Global Pesticide Resistance in Arthropods

Edited by M E Whalon, Michigan State University, USA; D Mota-Sanchez, Michigan State University, USA; R M Hollingworth, Michigan State University

Main Description

Pesticide resistance has had a substantial impact on crop production and has been an important driver of change in modern agriculture, animal production and human health. Focusing specifically on arthropods, this book provides a comprehensive review of relevant issues in pesticide resistance.

Readership

Researchers and students in applied entomology, crop protection and medical and veterinary entomology.

Main Contents

- Analysis of Global Pesticide Resistance in Arthropods. *M.E. Whalon, D. Mota-Sanchez, and R.M. Hollingworth.*
- Documentation of Pesticide Resistance in Arthropods. *D. Mota-Sanchez, M.E. Whalon, R.M. Holingworth, and Q. Xue.*
- The Biochemical and Molecular Genetic Basis of Resistance to Pesticides in Arthropods. R.M. Hollingworth, and K. Dong.
- Assessing the Risk of the Evolution of Resistance to Pesticides Using Spatially Complex Simulation Models. *M.A. Caprio, N. Storer, M.S. Sisterson, S. Peck, and A.H.N. Maia.*
- Pesticide and Transgenic Plant Resistance Management in the Field. *D.A. Andow, G.P. Fitt, E.J. Grafius, R.E. Jackson, E.B. Radcliffe, D.W. Ragsdale, and L. Rossiter.*
- The Politics of Resistance Management: Working Towards Pesticide Resistance Mangement Globally. *G.D. Thompson, S. Matten, I. Denholm, M.E. Whalon, and P. Leonard.*

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Resistance Management Reviews

Pest Resistance to Insect Resistant Cotton and Corn Plants Delayed by Refuge Strategy, according to researchers at the University of Arizona who have reported finding incidences of Bt-resistant bollworm

The researchers analyzed " ... published data from monitoring studies of six major caterpillar pests of Bt crops in Australia, China, Spain and the U.S. ... most caterpillar pests of cotton and corn remained susceptible to Bt crops. 'The resistance occurred in one particular pest in one part of the U.S. ... 'The other major pests attacking Bt crops have not evolved resistance. And even most bollworm populations have not evolved resistance' ... The field outcomes refute some experts' worst-case scenarios that predicted pests would become resistant to Bt crops in as few as three years ... Bt cotton reduces use of broad-spectrum insecticides and increases yield..."

Document Title: The title of the February 7, 2008 University of Arizona News Release is "First documented case of pest resistance to biotech cotton"

The title of the research report published online on February 7, 2008 in the journal, Nature Biotechnology, is ""Insect resistance to Bt crops: evidence versus theory"

Summary. The following information is taken from the February 7, 2008 University of Arizona News Release

A pest insect known as bollworm is the first to evolve resistance in the field to plants modified to produce an insecticide called Bt, according to a new research report.

Bt-resistant populations of bollworm, *Helicoverpa zea*, were found in more than a dozen crop fields in Mississippi and Arkansas between 2003 and 2006. "What we're seeing is evolution in action," said lead researcher Bruce Tabashnik. "This is the first documented case of field-evolved resistance to a Bt crop."

Bt crops are so named because they have been genetically altered to produce Bt toxins, which kill some insects. The toxins are produced in nature by the widespread bacterium *Bacillus thuringiensis*, hence the abbreviation Bt.

The bollworm resistance to Bt cotton was discovered when a team of University of Arizona entomologists analyzed published data from monitoring studies of six major caterpillar pests of Bt crops in Australia, China, Spain and the U.S. The data documenting bollworm resistance were first collected seven years after Bt cotton was introduced in 1996.

"Resistance is a decrease in pest susceptibility that can be measured over human experience," said Tabashnik, professor and head of UA's entomology department and an expert in insect resistance to insecticides. "When you use an insecticide to control a pest, some populations eventually evolves resistance."

The researchers write in their report that Bt cotton and Bt corn have been grown on more than 162 million

hectares (400 million acres) worldwide since 1996, “generating one of the largest selections for insect resistance ever known."

Even so, the researchers found that most caterpillar pests of cotton and corn remained susceptible to Bt crops.

"The resistance occurred in one particular pest in one part of the U.S.," Tabashnik said. "The other major pests attacking Bt crops have not evolved resistance. And even most bollworm populations have not evolved resistance."

The field outcomes refute some experts' worst-case scenarios that predicted pests would become resistant to Bt crops in as few as three years, he said.

“The only other case of field-evolved resistance to Bt toxins involves resistance to Bt sprays," Tabashnik said. He added that such sprays have been used for decades, but now represent a small proportion of the Bt used against crop pests.

The bollworm is a major cotton pest in the southeastern U.S. and Texas, but not in Arizona. The major caterpillar pest of cotton in Arizona is a different species known as pink bollworm, *Pectinophora gossypiella*, which has remained susceptible to the Bt toxin in biotech cotton.

Tabashnik and his colleagues' article, "Insect resistance to Bt crops: evidence versus theory," will be published in the February issue of Nature Biotechnology. His co-authors are Aaron J. Gassmann, a former UA postdoctoral fellow now an assistant professor at Iowa State University; David W. Crowder, a UA doctoral student; and Yves Carriére, a UA professor of entomology. Tabashnik and Carriére are members of UA's BIO5 Institute.

The U.S. Department of Agriculture funded the research.

"Our research shows that in Arizona, Bt cotton reduces use of broad-spectrum insecticides and increases yield," said Carriére. Such insecticides kill both pest insects and beneficial insects.

To delay resistance, non-Bt crops are planted near Bt crops to provide "refuges" for susceptible pests. Because resistant insects are rare, the only mates they are likely to encounter would be susceptible insects from the refuges. The hybrid offspring of such a mating generally would be susceptible to the toxin. In most pests, offspring are resistant to Bt toxins only if both parents are resistant.

In bollworm, however, hybrid offspring produced by matings between susceptible and resistant moths are resistant. Such a dominant inheritance of resistance was predicted to make resistance evolve faster.

The UA researchers found that bollworm resistance evolved fastest in the states with the lowest abundance of refuges.

The field outcomes documented by the global monitoring data fit the predictions of the theory underlying the refuge strategy, Tabashnik said.

Although first-generation biotech cotton contained only one Bt toxin called Cry1Ac, a new variety contains both Cry1Ac and a second Bt toxin, Cry2Ab. The combination overcomes pests that are resistant to just one toxin.

The next steps, Tabashnik said, include conducting research to understand inheritance of resistance to Cry2Ab and developing designer toxins to kill pests resistant to Cry1Ac.

Bruce E Tabashnik[1], Aaron J Gassmann[1,2], David W Crowder1 & Yves Carriére[1]
[1]Department of Entomology, University of Arizona, Tucson, Arizona 85721, USA.
[2]Current address: Department of Entomology, Iowa State University, Ames, Iowa 50011, USA.

Resistance Management from around the Globe

Sensitivity of the cucurbit powdery mildew fungus, *Podosphaera xanthii*, to registered fungicides in 2007 in New York and implication for control

ABSTRACT
Seedling bioassays were used to monitor sensitivity of the powdery mildew fungus to currently registered, fungicides at-risk for resistance in commercial and research plantings of zucchini, summer squash and pumpkin during the 2007 growing season in Long Island, NY. Resistance to QoI fungicides (FRAC Group 11) was common based on results with trifloxystrobin (formulated as Flint). Fungicide sensitivity of pathogen populations in spring zucchini at conventionally managed farms was similar to that early in powdery mildew development in Halloween pumpkin, which was almost one month later. This indicates that bioassays conducted in spring crops provide a good indication of what the fungicide sensitivity will be for

the pathogen population in main season crops. Strains tolerating high concentrations of boscalid (175 µg/ml formulated as Endura)(Group 7) and myclobutanil (120 µg/ml formulated as Nova)(Group 3) were present at powdery mildew onset in spring-planted zucchini. These were the highest concentrations tested. Fungicide sensitivity was similar for pathogen populations in fields with the main season crop of Halloween (decorative) pumpkin. The pathogen is very sensitive to the new fungicide quinoxyfen (Quintec)(Group 13), which was registered for use on melons in 2007. Strains tolerating a low concentration (5 µg/ml) were not detected in zucchini but were at very low frequencies in pumpkin. Tolerance of the DMI fungicides myclobutanil and triflumizole (Procure) was similar. Fungicide sensitivity changed little during the growing season. Strains tolerating 200 µg/ml boscalid, 150 µg/ml myclobutanil, and 10 µg/ml quinoxyfen were detected during the growing season. Strains tolerating DMI fungicides at 100-120 µg/ml were less common at the end of the growing season (September – October).

INTRODUCTION

Fungicides continue to be an important tool for managing powdery mildew, which is the most common disease occurring on cucurbits throughout the US. Although there are varieties with genetic resistance to this disease, an integrated program is recommended to reduce selection pressure for pathogen strains able to overcome genetic resistance in the plant, thus use of fungicides will continue to be the principal practice for managing powdery mildew in cucurbit crops. Mobile fungicides are needed because their systemic, translaminar or volatile activity enables them to be re-distributed to the lower surface of leaves where powdery mildew develops best and where direct fungicide spray deposition is limited. Unfortunately mobile fungicides are at-risk for resistance development due to their single-site mode of action and the cucurbit powdery mildew pathogen has demonstrated ability to develop resistance.

Procure, Pristine, and Quintec are presently the mobile fungicides most commonly recommended for cucurbit powdery mildew. Procure is a DMI (demethylation inhibiting) fungicide (FRAC Group 3) registered in the US for this use in 2002. Procure is recommended over Nova, another DMI, because it can be used at twice the rate of active ingredient and Nova was ineffective at full label rate in NY in 2006 (McGrath and Davey 2007a). Procure has provided excellent but inconsistent control (McGrath and Davey 2006 and 2007a). DMI resistance is quantitative. Pristine contains a carboxamide (boscalid)(FRAC Group 7) and a QoI (pyraclostrobin)(FRAC Group 11). It has provided good to excellent control in recent fungicide efficacy experiments in NY. Pristine has provided control similar to Endura (boscalid) when Cabrio (pyraclostrobin) was ineffective, documenting no synergistic activity between these compounds. Endura is not registered for use on cucurbits. Pristine was registered in the US for powdery mildew in 2003. QoI resistance, which is qualitative, was detected in the US in 2002. Quintec (quinoxyfen)(FRAC Group 13) was registered in 2007 for melons.

The primary objective of this study was to monitor fungicide sensitivity of the cucurbit powdery mildew fungus on Long Island, NY, during the 2007 growing season in commercial and research plantings. Fungicide sensitivity was also examined in Pennsylvania early in powdery mildew development. This monitoring project was started in 2003.

MATERIALS AND METHODS

An in-field seedling bioassay was used to assess fungicide sensitivity of powdery mildew fungal pathogen populations. Pumpkin seedlings were started in a growth chamber, then transplanted to pots and grown in a greenhouse until about the 4th true leaf stage. Their growing point and unexpanded leaves were removed just before treatment. Seedlings were treated with various doses of fungicides using a back-pack sprayer equipped with a single nozzle boom operated at 40 psi. Treated seedlings were left overnight to dry. Then they were placed in fields amongst cucurbit plants with powdery mildew symptoms. Each group of seedlings had 1 treated seedling for each fungicide dose tested plus two non-treated seedlings. They were left for about 4 hours during the middle of the day to be exposed to the wind-dispersed spores of the powdery mildew pathogen in the fields. Afterwards the seedling were kept in a greenhouse until symptoms of powdery mildew were clearly visible, which took at least 10 days. Then severity (percent tissue with symptoms) was visually estimated for each leaf. Frequency of pathogen strains in a field able to tolerate each fungicide dose was estimated by calculating the ratio of severity on fungicide-treated plants relative to non-treated plants for each leaf in each group, then determining the field average. The bioassay was conducted on 7 dates from 28 Jun through 2 Oct 2007 in commercial and research fields.

The fungicides tested were: myclobutanil (formulated as Nova®) at 20, 40, 80, 100, 120 and 150 ppm (µg/ml); triflumizole (Procure®) at 100, 120 and 150 ppm; boscalid (Endura®) at 125, 150, 175 and 200 ppm; quinoxyfen (Quintec®) at 1, 5, 10 and 20 ppm; and trifloxystrobin (Flint®) at 50 or 150 ppm. Endura was used rather than the fungicide with boscalid registered for this use, Pristine, because it also contains pyraclostrobin. Since QoI resistance is qualitative only one concentration is needed for its detection. Myclobutanil and trifloxystrobin have been used as representatives for FRAC Group 3 and 11 fungicides, respectively, in previous experiments. Nova was also tested with two adjuvants (Activator 90 and LI700) to determine if efficacy could be improved.

Some powdery mildew developed on seedlings while in the greenhouse before they were treated for bioassays conducted in August and September, probably resulting from spores entering

through ceiling vents. These spots were marked with a Sharpie pen so that they would not be included in severity assessments. The ink appeared to kill fungal tissue on the leaf surface. Additionally, one set of treated seedlings were left in the greenhouse while the others were in production fields to determine if the contamination had any additional impact on the bioassay.

RESULTS and DISCUSSION

Information on fungicide sensitivity early in powdery mildew development during the 2007 production season and before extensive use of fungicides for powdery mildew was obtained by conducting bioassays in commercial spring plantings of zucchini in Suffolk County on Long Island, NY, and in eastern PA. Powdery mildew develops in spring plantings of summer squash before main season crops like pumpkin. Crops grown during the summer are more severely affected by powdery mildew than those started in spring, thus mobile fungicides are used more in main season crops. Information on fungicide sensitivity from spring plantings can be useful for guiding fungicide recommendations for summer crops.

The first two bioassays were conducted in commercial spring plantings of zucchini in NY on 29 Jun in one field and on 13 Jul after mildew was found in four more fields (Table 1). Resistance to QoI fungicides (FRAC Group 11) was found in only 3 of the 5 fields, but at these sites it was at a very high level (89-100%). Considering this type of resistance is qualitative, thus resistant pathogen strains are completely uninhibited, and the pathogen spores can be dispersed widely by wind, these fungicides were not recommended for controlling powdery mildew in 2007. Sensitivity to DMI (Group 3) fungicides varied among fields. In 4 of the 5 fields strains were detected tolerating 100 ppm myclobutanil. This is considered a moderately high level of insensitivity that could affect control with DMI fungicides, especially with the lowest label rate. This level of insensitivity may be the reason Nova at the highest rate was ineffective when tested at LIHREC in 2006. A very low frequency of tolerance of 120 ppm was found in 1 field. A low frequency of isolates tolerating 175 ppm boscalid (key ingredient in Pristine) were detected in all fields. A very low frequency of isolates tolerating 5 ppm of quinoxyfen was found in 4 fields. The most sensitive pathogen population was in Farm D, which is certified organic. This result was surprising because mobile fungicides had not been applied to the conventionally-managed crops and the primary inoculum each year in northern states is thought to be wind-dispersed asexual spores (conidia) from southern production areas rather than ascospores surviving over winter in production fields. Additionally, the bioassay conducted on 26 Jul 2006 did not reveal a difference in fungicide sensitivity of the pathogen population at this farm compared to other farms (McGrath and Davey 2007b).

Table 1. Percentage of isolates estimated tolerant to different concentrations (ppm) of fungicides in commercial spring plantings of zucchini in NY and PA.

Assay		Quinoxyfen			Boscalid			Myclobutanil				Trifloxystrobin	
date	Location	5	10	20	100	150	175	20	80	100	120	50	150
6/29	Farm A	5	1	0	27	16	-	1	0	0	1	26	-
7/13	Farm A	0	0	0	15	11	2	13	13	9	2	-	89
7/13	Farm B	1	0	0	38	38	22	100	100	26	0	-	0
7/13	Farm C	1	0	0	46	38	20	100	70	100	91	-	91
7/13	Farm D; organic	0	0	0	17	9	1	0	0	0	0	-	1
7/13	Farm E	1	0	0	45	18	12	100	100	39	7	-	100
7/24	Farm L; PA	0	0	0	1	2	1	60	15	3	7	30	-
7/25	Farm M; PA	2	0	0	6	1	2	95	16	16	32	16	-

'-' indicates dose not tested.

The third bioassay was conducted in two commercial spring plantings of zucchini in Lancaster and Berks counties in PA (Table 1). Results suggest that these pathogen populations were more sensitive to fungicides, in particular boscalid, then the populations in the NY zucchini plantings examined in the previous bioassay and the populations in the three adjacent experiments examined at the same time. The population at Farm L, which is managed organically, did not differ substantially from the population at Farm M, managed conventionally.

Based on these results from the spring plantings, Quintec was predicted to be the most effective fungicide. When these fungicides are applied at the highest label rate, the rate of active ingredient is 1.36 oz/A quinoxyfen for Quintec applied at 6 oz/A, 4 oz/A triflumizole for Procure at 8 oz/A, and 4.66 oz/A boscalid for Pristine at 18.5 oz/A. Concentration in a spray solution varies greatly with the gallonage used to make the application. When applied at 50 gpa, the concentration of these active ingredients is 212, 599, and 698 ppm for Quintec, Procure, and Pristine, respectively, which is 42, 4, and 3.5 times higher than the highest concentrations tolerated by powdery mildew strains detected in commercial fields in 2007 (5, 150, and 200 ppm, respectively). Higher concentrations of triflumizole and boscalid were not tested; it is possible that strains with even higher tolerance were present. Resistance to FRAC Group 7 and 13 fungicides (boscalid and quinoxyfen) is thought to be quantitative as it is for Group 3 (DMI) fungicides. QoI fungicides (Group 11) were not recommended in 2007 because resistant strains were detected in both NY and PA.

Fungicide sensitivity of the powdery mildew fungal pathogen populations in spring zucchini at conventionally-managed farms was similar to that early in powdery mildew development in Halloween (decorative) pumpkin, which was almost one month later (Table 1 13 Jul and Table 2 10 Aug). This indicates that bioassays conducted in spring crops provide a good indication of what the fungicide sensitivity will be for the pathogen population in main season crops, confirming previous results. Fungicide sensitivity of the pathogen population in the organic

pumpkin planting was very similar to that of the spring squash planting at this farm (D), which was found to be relatively sensitive when examined almost two months earlier (Table 2 7 Sep).

Table 2. Percentage of isolates estimated tolerant to different concentrations (ppm) of fungicides in commercial pumpkin plantings on LI, NY.

Assay		Quinoxyfen			Boscalid				Myclobutanil			Triflumizole		
date	Location	1	5	10	125	150	175	200	100	120	150	100	120	150
8/10	Farm E; Field 2	66	3	-	29	-	20	-	22	9	0	22	19	4
8/10	Farm F; Field 1	9	0	-	17	-	9	-	26	6	0	64	32	0
8/10	Farm G	9	3	-	13	-	38	-	13	6	0	100	100	0
8/10	Farm H; Field 1	11	0	-	35	-	0	-	11	4	0	74	4	0
8/10	Farm I	97	0	-	12	-	14	-	26	10	0	14	2	0
8/23	Farm E; Field 2	45	3	-	69	21	14	-	100	21	0	28	7	0
8/23	Farm F; Field 2	5	3	-	31	23	15	-	15	3	0	15	2	0
8/23	Farm H; Field 1	20	2	-	45	40	17	-	30	25	0	17	12	10
8/23	Farm H; Field 2	3	1	-	35	13	10	-	8	1	3	1	1	1
8/23	Farm J (contacts) *	5	1	-	35	25	13	-	1	0	3	10	0	0
9/7	Farm D; organic	1	1	-	16	6	6	-	1	0	0	0	0	0
9/7	Farm H; Field 2	2	2	-	2	1	1	-	1	0	0	0	0	0
9/7	Farm I	6	4	-	44	28	20	-	6	0	0	0	0	0
9/7	Farm J (contacts)	7	1	-	10	5	3	-	19	0	0	3	0	0
9/7	Farm K (contacts)	24	1	-	100	100	100	-	56	18	1	0	0	0
10/2	Farm H; Field 1	-	5	2	20	-	10	10	0	0	0	0	0	0
10/2	Farm H; Field 2	-	0	9	19	-	16	13	6	0	0	0	0	0
10/2	Farm J (contacts)	-	1	0	6	-	6	9	13	0	3	0	0	0

* Only fungicides with contact activity (e.g. copper, chlorothalonil) were applied in these fields.
Pristine and Procure were applied to pumpkin crops in other fields, with the exception of Farm D.

Impact of fungicide use on fungicide sensitivity of powdery mildew fungal pathogen populations was examined by conducting four bioassays in commercial fields with Halloween pumpkin on LI, NY, during the growing season (Table 2). Most crops were treated with Pristine and Procure applied on a weekly schedule beginning very early in powdery mildew development. At two farms only fungicides with contact activity (e.g. copper, chlorothalonil) were applied. Sensitivity of the pathogen population in these fields did not differ greatly or consistently from fields where mobile fungicides were applied (Table 2). No fungicides were used at the organic farm. Pathogen sensitivity was low but not lower than all other fields.

Table 3. Percentage of isolates estimated tolerant to different concentrations (ppm) of fungicides in research plantings of summer squash and pumpkin at LIHREC.

Assay	Experiment	Quinoxyfen				Boscalid					Myclobutanil					Triflumizole			Trifloxy-
date	(treatment) *	1	5	10	20	100	125	150	175	200	20	80	100	120	150	100	120	150	strobin 50
7/24	Exp 1; variety trial	-	1	0	0	35	-	20	8	-	65	39	44	25	-	-	-	-	78
7/24	Exp 2; variety trial	-	0	0	0	25	-	1	2	-	82	53	59	35	-	-	-	-	100
7/24	Exp 3; variety trial	-	4	0	0	43	-	21	1	-	29	86	57	86	-	-	-	-	100
8/10	Exp 4 (Program)	83	13	-	-	-	17	-	26	-	-	-	53	12	12	39	39	6	-
8/10	Exp 5 (nontreated)	71	18	-	-	-	46	-	41	-	-	-	52	24	10	36	58	18	-
8/23	Exp 4 (Program)	71	2	-	-	-	36	27	10	-	-	-	9	0	0	18	4	4	-
8/23	Exp 4 (Program)	-	-	-	-	-	24	13	12	-	-	-	1	2	1	12	0	0	-
9/7	Exp 5 (nontreated)	1	1	-	-	-	25	19	1	-	-	-	1	0	0	0	0	0	-
9/7	Exp 5 (Quintec)	6	2	-	-	-	6	4	1	-	-	-	0	0	1	2	0	0	-
9/7	Exp 5 (Procure)	5	2	-	-	-	15	15	1	-	-	-	0	0	0	0	0	0	-
9/7	Exp 5 (Endura)	0	1	-	-	-	100	53	0	-	-	-	0	0	0	0	0	0	-
9/7	Exp 5 (Program)	1	2	-	-	-	20	4	2	-	-	-	32	25	0	0	2	0	-
10/2	Exp 5 (Quintec)	-	68	27	-	-	100	-	95	27	-	-	20	14	0	20	7	0	-
10/2	Exp 5 (Procure)	-	5	8	-	-	84	-	74	0	-	-	0	0	0	0	0	0	-
10/2	Exp 5 (Endura)	-	0	0	-	-	100	-	45	25	-	-	10	30	0	10	0	0	-
10/2	Exp 5 (Program)	-	3	2	-	-	40	-	60	35	-	-	50	0	0	0	0	0	-

Impact of fungicide use was also examined by conducting five bioassays in research plantings at LIHREC during the growing season (Table 3). Experiments 1-3 were early-planted variety trials with powdery mildew resistant varieties that were not treated with mobile fungicides for powdery mildew. Seedlings were placed next to susceptible varieties. Experiments 4 and 5 were fungicide evaluations. Treatments were individual fungicides applied weekly or a Program with Quintec, Procure, and Pristine applied in alternation and tank-mixed with a protectant fungicide. Seedlings were placed in the center of plots. However, it is still possible spores deposited on seedlings included some from other plots due to the ease with which powdery mildew spores are wind dispersed. A higher frequency of strains tolerating high concentrations of boscalid were detected on 7 Sep where only Endura had been applied. Strains tolerating high concentrations of quinoxyfen were detected on 2 Oct where only Quintec had been applied. This apparent evidence of selection of less tolerant strains under sole use of a fungicide was not evident with other fungicides or at other dates. Best control of powdery mildew in this experiment was obtained with Quintec used alone or in a fungicide program for resistance management (alternation among Quintec, Procure, and Pristine; all tank-mixed with the protectant fungicide Microthiol Disperss) and with an experimental fungicide (93-98% control on upper leaf surfaces and 74-81% control on lower surfaces based on AUDPC values)(McGrath and Fox, 2008). Procure and Pristine were not as effective, but they did provide adequate control (90-91% control on upper leaf surfaces, respectively, with the highest label rate). Procure controlled powdery mildew on lower leaf surfaces more effectively than Pristine (78% vs 58% control), which was similar to results obtained in 2005 but opposite results obtained in 2006 when another DMI fungicide, Nova, was ineffective.

Pathogen sensitivity to the two DMI fungicides, myclobutanil and triflumizole, was fairly similar. The pathogen was not consistently more sensitive to either one.

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Monica M. Miazzi[1] and Margaret T. McGrath[2]
[1]Dipartimento di Protezione delle Piante e Microbiologia applicata
Via Amendola 15/9, 70126 Bari, Italy
monica.miazzi@libero.it
[2]Department of Plant Pathology and Plant-Microbe Biology, Cornell University
Long Island Horticultural Research and Extension Center
3059 Sound Avenue, Riverhead, New York 11901-1098
mtm3@cornell.edu

Distribution and Impact of Glyphosate-Resistant Palmer amaranth (*Amaranthus palmeri*) in the Southern United States (U.S.)

Glyphosate (N-phosphonomethyl glycine)-resistance in Palmer amaranth has emerged as an imminent threat to economical weed management in cotton (*Gossypium hirsutum*) and soybean (*Glycine max*.) in the southeastern Coastal Plain of North Carolina, South Carolina, and Georgia and in Arkansas and Tennessee in the North Delta (see below). This article provides up-to-date information on the distribution of glyphosate-resistant Palmer amaranth populations and alerts the international weed science community to a significant resistance occurrence that poses a serious challenge to weed management in agronomic and other crops in the U.S.

Distribution of Confirmed Glyphosate-Resistant Palmer amaranth Populations

State	Counties with Resistant Populations	Estimated Hectares Infested
Arkansas	14	304,000
Georgia	18	112,000
North Carolina	11	2,200
South Carolina	3	4,500
Tennessee	3	1,400

Palmer amaranth is a dioecious summer annual. In temperate latitudes, it produces one generation per year. If continuous greenhouse screening could be done, bioassays of two and possibly three generations per year might be possible. However, not all weed scientists have the facilities to screen continuously. Moreover, the putatively resistant plants must be allowed to mature seed. Field failures are generally reported in June or July, and mature seed may not be collected until September or later. Testing of the F1 progeny is an absolute requirement for confirmation of resistance and many weed scientists exercise caution and reconfirm resistance in F2 progenies before reporting. Accordingly, determination and reporting of glyphosate resistance in Palmer amaranth populations has typically taken 18 months or more from the time an initial field failure is referred for evaluation. Conversely, the weed's mode of propagation, by obligate out-crossing through wind-transported pollen, appears rapid and possibly long-range as well (Sosnoskie et al., 2007). Movement of glyphosate-resistant Palmer amaranth pollen is the subject of ongoing research in Georgia and North Carolina.

The number of newly-confirmed populations in 2007 in Arkansas and Georgia suggests that the progression of the problem in the field is outpacing the capacity of local researchers to test and officially report populations that are at the forefront of an expanding infestation. Official reporting of resistance cannot be better than retrospective; however, it is reasonable to anticipate that the resistance is spreading and growers in counties that are likely to be impacted in the coming year should be alerted. In North Carolina, such a number of widely scattered populations have been confirmed that the monitoring program may be terminated after 2008. For the purpose of making practical management recommendations, the principal row-crop producing areas of the entire states of Arkansas, Georgia, and North Carolina may be considered infested within a few years.

In newly-reported populations, glyphosate-resistance in Palmer amaranth appears to be caused in two different ways: 1) by local transport through movement of seed and/or pollen, and 2) by spontaneous occurrence of newly-resistant populations. In Tennessee, shikimate accumulated in both the susceptible and the resistant biotypes, indicating that EPSPS (5-enolpyruvylshikimate-3-phosphate synthase), the enzyme whose inhibition is considered responsible for the herbicidal effects of glyphosate on susceptible plants, was inhibited (Steckel et al., 2008). In contrast, a glyphosate-resistant Palmer amaranth population in Georgia did not accumulate shikimate (Culpepper et al., 2006). Evidence of more than one resistance mechanism strongly suggests the occurrence of multiple mutations leading to expression of resistance.

Anticipated Impacts

Palmer amaranth is a robust annual that frequently grows to 2 or more meters in height. Seed production is prolific and estimated to be as great as 600,000 seed from a large, healthy plant (Keeley et al., 1987). Continuous emergence from May through September has been observed in Missouri (Kendig and Nichols, 2005). Palmer amaranth is highly competitive with corn, soybean, and cotton (Klingaman and Oliver,1994; Massinga and Currie, 2002; Morgan et al., 2001), and is already considered one of the most troublesome weeds in agronomic crops in the Southern Region (Webster, 2005).

Glyphosate, used in conjunction with transgenic, glyphosate-resistant cultivars, is the principal herbicide in the dominant weed management system in soybean and cotton in the U.S. (http://www.ers.usda.gov/data/BiotechCrops/).
Minimally, glyphosate resistance in such a common and troublesome weed species would force changes in the weed management practices of many growers. In fact, no combination of products or practices have been found that would serve as reliably or economically as

has the present program that now includes the use of glyphosate. Estimates of direct financial impact are $56-$78/hectare for additional herbicides alone in cotton (Bryant, 2007), with the additional expectation of as yet unquantified crop yield losses.

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R. L. Nichols[1], M. G. Burton[2], A. S. Culpepper[3], C. L. Main[4], M. W. Marshall[5], T. C. Mueller[4], J. K. Norsworthy[6], R. C. Scott[6], K. L. Smith[6], L. E. Steckel[4], and A. C. York[2]

[1]Cotton Incorporated, [2]North Carolina State University, [3]University of Georgia, [4]University of Tennessee, [5]Clemson University, [6]University of Arkansas

INSECTICIDE PERSISTENCE AND RESIDUAL TOXICITY MONITORING IN TEA MOSQUITO BUG, *HELOPELTIS THEIVORA* WATERHOUSE (HETEROPTERA: HEMIPTERA: MIRIDAE) IN DOOARS, WEST BENGAL

ABSTRACT. Persistence (PT values) and residual toxicity **(**LT_{50} values) of imidacloprid 17.5 SL, thiomethoxam 25 WG, deltamethrin 2.8 EC, alphamethrin 10 EC, cypermethrin 25 EC, λ-cyhalothrin 5 EC, fenpropathrin 30 EC, monocrotophos 37 SL, oxydemeton methyl 25 EC, quinalphos 25 EC and endosulfan 35 EC against *Helopeltis theivora* Waterhouse were studied by exposing field collected tea mosquito bug adults for 24 hours to TV1 tea leaves treated with three concentrations, (0.05, 0.10 and 0.25 percent) for a period of 4-28 days by following probit analysis and product (PT) of average residual toxicity methods.

Persistence of neonicotinoids (thiomethoxam and imidacloprid), synthetic pyrethroids (alphamethrin, deltamethrin, cypermethrin, λ-cyhalothrin, fenpropathrin) and monocrotophos (organophosphate) last for a longer duration (18 - 28 days) with increased PT values (938.25 – 1423.13) at 0.25% concentration whereas, oxydemetonmethyl, endosulfan, and quinalphos persisted for a relatively short duration (7-11 days) with lesser PT values (307.00 – 513.87). But at a lower concentration (0.05%), recommended dose by TRA, the thiomethoxam, imidacloprid, λ-cyhalothrin, fenpropathrin and monocrotophos exhibited persistence toxicityfor 14-16 days along with higher PT values (689.50 – 806.00) while persistence was 7-11 days with 124.00 – 573.37 PT values in alphamethrin, deltamethrin, cypermethrin, oxydemetonmethyl, endosulfan, and quinalphos respectively.

Higher LT_{50} values of 10.59 – 11.27 days were observed at 0.25% concentration of thiomethoxam, λ-cyhalothrin, imidacloprid and fenpropathrin followed by 8.29 – 9.04 days in deltametrhrin, alphamethrin and monocrotophos, moderately by 4.11 – 6.99 days in cypermethrin and oxydemetonmethyl and least by 2.92 – 3.02 days in endosulfan and quinalphos. Nevertheless, lesser LT_{50} values (1.11 – 4.98 days) were noted in the recommended dose of λ-cyhalothrin, imidacloprid, alphamethrin, deltamethrin, cypermethrin, oxydemetonmethyl, endosulfan, and quinalphos and those insecticides are commonly used in tea. Thus, for combating and delaying the problem of resistance either the TRA must reassess the dose or the planters must change over their strategies in the light of above findings.

Key words: Insecticide, $LT_{50,}$ Persistence, Residual toxicity, *Helopeltis theivora*

INTRODUCTION. Tea mosquito bug (*Helopeltis theivora* Waterhouse) is considered as one of the major pests of tea in Assam, Dooars, Terai and Darjeeling because it attacks only to the young shoots that is the actual crop of tea. Many Tocklai released clones, garden released clones; and seed jats are susceptible to *H. theivora* attack at varying degrees. 80% of the tea plantations are being affected by this pest alone which in turn is reducing the productivity 10-50% (Gurusubramanian and Bora, 2007). The nymphs and adults of *H. theivora* suck the sap of the young leaves, buds and tender stems, and while doing so it injects toxic saliva which causes the breakdown of tissues surrounding the puncture, which becomes dark brown

shrunken spots after 24 hours. The badly affected leaves became deformed and even curl-up. In severe attacks, bushes virtually cease to form shoots, and the affected area may not flush for weeks together. In addition, due to oviposition, the tender stems develop cracks and over-callousing which led to blockage of vascular bundles thereby affecting the physiology causing stunted growth and sometimes die-back of the stems (Rahman *et al.*, 2005).

In North East India, Tocklai Experimental Station, Tea Research Association (TRA), Jorhat, Assam, India is the premier institute to test and certify the plant protection chemicals for use in tea plantations. Earlier TRA recommended different pesticides [endosulfan, quinalphos, phosphomidon, phosalone, acephate, dimethoate, chlorpyrifos, monocrotophos, oxydemetonmethyl, λ-cyhalothrin, β-cyfluthrin, ethofenprox, cartap hydrochloride, alphamethrin, cypermethrin, deltamethrin, profenophos, thiomethoxam, imidacloprid, dicofol, ethion, propargite, fenazaquin, sulfur and neem formulations] for controlling tea pests (Anonymous, 1993, 1999). During the last several decades, the control of pests, diseases and weeds in tea fields is predominantly by the use of synthetic chemicals. From the recent survey in tea gardens of Dooars, it was observed that synthetic pesticides constituted 85% of the total pesticides used, wherein, acaricides and insecticides accounted for 25% (3.60 l/ha) and 60% (8.46 l/ha) respectively. Within the synthetic insecticides, organophosphate compounds (64% - 5 rounds per year) were most preferred followed by organochlorine (26% - 2 rounds/year) and synthetic pyrethroids (9% - 7 rounds per year) (Sannigrahi and Talukdar, 2003). The latest surveillance report of the European Community (EC) indicated that the presence of residues in Indian tea is a cause of great concern. Assam and Darjeeling teas continue to record high numbers of positive values for organochlorine and synthetic pyrethroids pesticide residues, very few of which exceeded the EU maximum residue level. Thus, use of DDT (10.4 to 47.1%), endosulfan (41.1 to 98.0%), dicofol (0.0 - 82.4%) and cypermethrin (6.0 - 45.1) remain comparatively high during 2001 to 2004 in different tea growing areas of the North-East states of India (Anonymous, 2001, 2002, 2003 and 2004). Due to regular application of insecticides, *H. theivora* has been realized as a menace year round and had a noticeable decrease in susceptibility to different classes of insecticides (Sarker and Mukhopadhyay, 2003, 2006, 2006a; Rahman *et al*., 2006 and 2007; Sarmah *et al*., 2006; Bora *et al*., 2007, 2007a and 2008; Gurusubramanian *et al*., 2008).

Thus, the tea mosquito bug constituted a major constraint in obtaining maximum tea yield. It was, therefore, considered imperative to assess residual toxicity of some commonly used insecticides at variable concentrations for their efficacy against *H. theivora* for economic and effective management.

MATERIALS AND METHODS. The TV 1 clones were sprayed with imidacloprid 17.5 SL, thiomethoxam 25 WG, deltamethrin 2.8 EC, alphamethrin 10 EC, cypermethrin 25EC, lamda-cyhalothrin 5 EC, fenpropathrin 30 EC, monocrotophos 37SL, endosulfan 35 EC, quinalphos 25 EC and oxydemeton methyl 25 EC at three different concentrations (0.05, 0.1 and 0.25 percent) for evaluating the persistence of residual toxicity against *H. theivora*. Each treatment contained 150 bushes with three replications. One plot was not treated and used as an untreated control. Two and a bud from five tea bushes were selected randomly after one hour of spray from each treated and untreated plots for "0" day observation and collected in marked paper bags separately. Bags with shoots were brought to the laboratory. Five tea shoots were kept in a glass tube containing water and wrapped with cotton. Glass tubes containing tea shoots were placed in the glass chimneys. The muslin cloth was tied with the help of rubber bands on top of the glass chimneys, and the tubes were kept at 27 ± 2°C in a culture room. Ten field collected and preconditioned adults of *H. theivora* were released in each glass chimney containing tea shoots collected from the respective plots. Observations were recorded 24 hours after the release of *H. theivora* adults. Moribund insects were counted as dead. The same procedure was repeated every day until insect mortality declined to ten percent (4-28 days) in all the three observations (Sarup *et al*., 1969; Rahman *et al*., 2007).

The relative efficacy of each treatment was determined by a criterion developed by Saini (1959), which used the product (PT) of average residual toxicity (T) and the period in days (P) for which the toxicity persisted. The average residual toxicity was calculated by first adding the values of corrected percent mortality caused by the insecticidal residues on the tea plant at various intervals and then divided by the total number of observations.

$$T = \frac{\text{Sum of percent mortality of tea mosquito bug on different days}}{\text{No. of observations}}$$

The LT_{50} values for different concentrations of insecticides were calculated by probit analysis (Busvine, 1971; Finney 1973) for all of the replications of the experiment. The t- test was employed for comparing the log LT_{50} values of different insecticides used in the present investigation (Singh *et al*., 1998 a and b). For example, the "t" value for testing the difference between the log LT_{50} values of endosulfan 0.05% and 0.25% was calculated as follows.

$$\text{Sum SEM} = \sqrt{(\text{SEM of endosulfan 0.25 \%})^2 + (\text{SEM of endosulfan 0.05 \%})^2}$$

$$\text{“t” value} = \frac{\log LT_{50} \text{ value of endosulfan } 0.25\% \; - \; \log LT_{50} \text{ value of endosulfan } 0.05\%}{\text{Sum SEM}}$$

The table value of “t” is 2.0369 and 2.7385 at 5 and 1 percent level respectively.

Table 1. PT values and order of relative efficacy of different insecticides at variable concentration against adults of *Helopeltis theivora* Waterhouse.

Insecticide	Concentration (%)	Period (P) (Days)	Percent average residual toxicity (T)	PT value	Order of relative efficacy (ORE)
Endosulfan 35 EC	0.05	4	31.000	124.00	33
	0.10	6	45.800	274.80	29
	0.25	10	43.500	435.00	25
Quinalphos 25 EC	0.05	5	36.750	183.75	32
	0.10	6	39.333	236.00	30
	0.25	7	43.857	307.00	27
Monocrotophos 37 SL	0.05	14	49.25	689.50	20
	0.10	15	51.500	772.50	17
	0.25	18	52.125	938.25	11
Oxydemeton methyl 25 EC	0.05	6	38.00	228.00	31
	0.10	7	39.333	275.33	28
	0.25	11	46.714	513.87	24
Deltamethrin 2.8 EC	0.05	11	50.500	555.50	23
	0.10	17	51.500	875.50	12
	0.25	23	57.375	1319.63	4
Cypermethrin 25EC	0.05	8	49.142	393.14	26
	0.10	14	49.142	688.00	21
	0.25	18	56.000	1008.00	9
Alphamethrin 10EC	0.05	11	52.125	573.37	22
	0.10	14	62.000	868.00	13
	0.25	20	56.000	1120.00	10
Fenpropathrin 30 EC	0.05	16	49.625	794.00	16
	0.10	19	61.375	1166.13	8
	0.25	28	49.875	1396.50	3
λ- cyhalothrin 5 EC	0.05	14	49.625	694.75	19
	0.10	16	51.125	818.00	14
	0.25	21	61.125	1283.63	5
Imidacloprid 17.5 SL	0.05	15	48.750	731.25	18
	0.10	20	58.375	1167.50	7
	0.25	24	59.250	1422.00	2
Thiomethoxam 25 WG	0.05	16	50.735	806.00	15
	0.10	21	57.000	1197.00	6
	0.25	23	61.175	1423.13	1

Mean of three observation

RESULTS AND DISCUSSION. The duration of effectiveness (persistent toxicity) and residual toxicity of eleven commonly used insecticides under four different classes (organochlorine (endosulfan), organophosphates (quinalphos, monocrotophos and oxydemetonmethyl), synthetic pyrethroids (deltamethrin, cypermethrin, alphamethrin, fenpropathrin and λ-cyhalothrin) and neonicotinoids (imidacloprid and thiomethoxam)) were evaluated on the basis of PT (persistence) (Table 1) and LT_{50} (residual toxicity) (Table 2) values respectively against adults of *H. theivora* when exposed to tea leaves (TV1) treated with three different concentrations (0.05, 0.10 and 0.25%) as foliar sprays. It was evident from Table 1 that the higher concentration (0.25%) of fenpropathrin (28 days), imidacloprid (24 days), thiomethoxam (23 days), deltamethrin (23 days), λ-cyhalothrin (21 days), alphamethrin (20 days), cypermethrin (18 days) and monocrotophos (18 days) persisted for a longer duration (18 - 28 days) against *H. theivora*. Imidacloprid and thiomethoxam at 0.10% concentration caused tea mosquito bug mortality for 20 and 21 days respectively. Oxydemetonmethyl, endosulfan, and quinalphos at 0.25% persisted for a relatively short duration, 11, 10 and 7 days respectively (Table 1). Lower concentration of all the chosen insecticides (0.05%) exhibited persistence toxicity for4-8 days in endosufan, quinalphos, oxydemetonmethyl and cypermethrin and 11-15 days in alphamethrin, deltamethrin, λ-cyhalothrin, monocrotophos, fenpropathrin, thiomethoxam and imidacloprid. As a whole, it was observed that the toxicity of organochlorine, organophosphates, synthetic pyrethroids and neonicotinoids persisted for 4-10 days, 5-18 days, 8-28 days and 15-24 days respectively (Table 1). Among the synthetic pyrethroids, shorter persistence duration (8 - 11 days) was recorded in the lower concentration (0.05%) of cypermethrin, alphamethrin and deltamethrin.

Table 2. Relative efficacy of different insecticides against Tea mosquito bug, *Helopeltis theivora* Waterhouse.

Insecticide	Concentration (%)	X^2	Regression equation	LT_{50} (Days)	Fiducial limit (Days)	Relative residual toxicity	ORE
Endosulfan 35 EC	0.05	1.68	y = 12.505 - 2.187 x	1.115	1.531 - 0.813	1.00	33
	0.10	4.07	y = 12.505 - 2.187 x	2.705	3.407 - 2.148	2.42	28
	0.25	3.02	y = 14.627 - 2.766 x	3.027	3.617 - 2.533	2.71	26
Quinalphos 25 EC	0.05	1.50	y = 12.117 - 2.205 x	1.689	2.198 - 1.298	1.51	32
	0.10	1.69	y = 13.056 - 2.403 x	2.248	2.724 - 1.856	2.01	30
	0.25	3.47	y = 16.217 - 3.252 x	2.925	3.365 - 2.544	2.62	27
Monocrotophos 37 SL	0.05	3.94	y = 13.424 - 2.252 x	5.502	6.625 - 4.569	4.93	19
	0.10	5.04	y = 12.893 - 2.071 x	6.468	7.936 - 5.302	5.80	16
	0.25	4.69	y = 15.566 - 2.696 x	8.292	9.729 - 7.067	7.43	10
Oxydemeton methyl 25 EC	0.05	0.09	y = 13.444 - 2.567 x	1.946	2.376 - 1.595	1.74	31
	0.10	1.05	y = 12.826 - 2.332 x	2.269	2.766 - 1.862	2.03	29
	0.25	4.55	y = 14.294 - 2.569 x	4.112	4.908 - 3.449	3.69	24
Deltamethrin 2.8 EC	0.05	0.22	y = 15.662 - 2.930 x	4.348	5.160 - 3.663	3.90	23
	0.10	0.85	y = 13.942 - 2.382 x	5.667	6.427 - 4.745	5.08	17
	0.25	2.15	y = 14.761 - 2.467 x	9.046	10.811 - 7.570	8.11	7
Cypermethrin 25 EC	0.05	3.53	y = 13.253 - 2.325 x	3.532	4.242 - 2.940	3.17	25
	0.10	4.85	y = 11.612 - 1.762 x	5.633	7.169 - 4.426	5.05	18
	0.25	4.84	y = 13.766 - 2.283 x	6.916	8.314 - 5.745	6.20	15
Alphamethrin 10 EC	0.05	1.97	y = 14.769 - 2.674 x	4.564	5.329 - 3.908	4.09	22
	0.10	6.59	y = 15.084 - 2.595 x	7.686	9.091 - 6.498	6.89	11
	0.25	4.50	y = 13.714 - 2.207 x	8.881	10.714 - 7.361	7.96	8
Fenpropathrin 30 EC	0.05	3.56	y = 15.110 - 2.362 x	6.979	8.195 - 5.944	6.26	14
	0.10	4.63	y = 18.808 - 3.524 x	8.287	9.436 - 7.257	7.43	9
	0.25	2.10	y = 14.707 - 2.412 x	10.599	12.654 - 8.878	9.50	4
λ- Cyhalothrin 5 EC	0.05	2.86	y = 14.494 - 2.568 x	4.982	5.872 - 4.226	4.47	20
	0.10	3.94	y = 14.908 - 2.566 x	7.263	8.553 - 6.168	6.51	12
	0.25	4.71	y = 10.762 - 2.571 x	10.762	12.728 - 9.099	9.65	2
Imidacloprid 17.5 SL	0.05	5.86	y = 11.054 - 1.648 x	4.723	5.999 - 3.719	4.23	21
	0.10	6.70	y = 11.479 - 1.628 x	9.521	12.213 - 7.422	8.53	5
	0.25	5.15	y = 12.433 - 1.846 x	10.608	13.281 - 8.473	9.51	3
Thiomethoxam 25 WG	0.05	2.73	y = 14.609 - 2.495 x	7.106	8.397 - 6.013	6.37	13
	0.10	3.30	y = 13.523 - 2.146 x	9.371	11.381 - 7.716	8.40	6
	0.25	6.19	y = 14.231 - 2.278 x	11.272	13.597 - 9.344	10.10	1

Mean of three observations; Y = Probit kill; ORE = Order of relative efficacy; X = log (timex1000)

Higher percent average residual toxicity (T) was observed in synthetic pyrethroids (49.14 – 62.00%) followed by neonicotinoids (48.75 – 61.17%), organophosphates (36.75 – 52.12%) and finally by organochlorine (31.0 - 45.8%). Average residual toxicity was noted between 49.14% and 62.0% in monocrotophos, cypermethrin, deltamethrin, alphamethrin, fenpropathrin, λ-cyhalothrin, imidacloprid and thiomethoxam whereas in endosulfan, quinalphos and oxydemetonmethyl, it was between 31.0% and 46.71% (Table 1).

PT values (persistence of insecticides) ranged between 124 and 435, 183.75 and 938.25, 393.14 and 1396.50 and 731.25 and 1423.13 for organochlorine, organophosphate, synthetic pyrethroids and neonicotinoids respectively. The highest PT was

observed in 0.25% thiomethoxam (1423.13) whereas endosulfan at 0.05% registered lowest PT value. On the basis of PT values the order of relative efficacy of the chosen eleven insecticides is shown in Table 1. Based on PT values, it was noticed that thiomethoxam, imidacloprid, fenpropathrin, deltamethrin, λ-cyhalothrin, alphamethrin, cypermethrin and monocrotophos persisted for a longer period and effectively check the tea mosquito population (PT value - 1008.00 – 1423.13) at 0.25% concentration, and oxydemetonmethyl, endosufan and quinalphos showed a shorter duration and lesser average residual toxicity (PT value - 307.00 - 513.87). However, at 0.05% concentration the order of persistence of insecticides (PT value: 124.00 – 806.00) was thiomethoxam, fenpropathrin, imidacloprid, λ-cyhalothrin, monocrotophos, alphamethrin, deltamethrin, cypermethrin, oxydemetonmethyl, quinalphos and endosulfan (Table 1).

The LT_{50} value increased with the concentration as well as varied with the class of insecticides that showed the relative efficacy of the selected eleven insecticides against tea mosquito bug. Neonicotinoids (4.72 – 11.27 days) and synthetic pyrethroids (3.53 – 10.76 days) registered relatively higher LT_{50} value than organophosphate (1.68 – 8.29) and organochlorine (1.11 – 3.02). Higher LT_{50} values of 10.59 – 11.27 days were observed in thiomethoxam, λ-cyhalothrin, imidacloprid and fenpropathrin followed by 8.29 – 9.04 days in deltamethrin, alphamethrin and monocrotophos, moderately by 4.11 – 6.99 days in cypermethrin and oxydemetonmethyl and least by 2.92 – 3.02 days in endosulfan and quinalphos (Table 2) at 0.25% concentration. Further, the order of LT_{50} values changed at the lower concentration (0.05%) of the insecticides. The relative residual toxicity of different insecticides was calculated by considering the LT_{50} value of these insecticides at three different concentrations and accordingly the order of relative efficacy was assigned accordingly. It may be highlighted here that the relative residual toxicity of monocrotophos(0.25%), thiomethoxam (0.05%), fenpropathrin (0.05%), λ-cyhalothrin (0.05%), imidacloprid (0.05%), alphamethrin (0.05%), deltamethrin (0.05%), oxydemetonmethyl (0.25%), cypermethrin (0.05%), endosulfan (0.25%) and quinalphos(0.25%) at the recommended concentrations by TRA was 7.43,6.37, 6.26, 4.47, 4.23, 4.09, 3.90, 3.69, 3.17, 2.71 and 2.62 times higher than the residual toxicity of endosulfan at 0.05% against *H. theivora* (Table 2).

Based on "t" values calculated for testing the difference between log_{50} values of different concentrations of eleven insecticides against *H. theivora* (Table 3), thiomethoxam, imidacloprid, fenpropathrin and λ-cyhalothrin were significantly superior in imparting higher residual toxicity *vis-à-vis* other insecticides used in this study. Among the three concentrations, 0.25% was significantly in high order. Thiomethoxam proved to be highly toxic to *H. theivora* as evidenced by significant difference in "t" values with respect to organophosphates and organochlorine. Toxicity of imidacloprid, λ-cyhalothrin, and fenpropathrin were at par. Endosulfan and quinalphos manifested low residual toxicity (Table 3).

Table: 3: 't' values of three concentrations of different insecticides assessed against *Helopeltis theivora* Waterhouse

Con. (%)	Log LT50 ± SEM	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
0.05	3.048 ± 0.0830	-																															
0.10	3.432 ± 0.0228	9.20	-																														
0.25	3.491 ± 0.0161	11.24	1.75	-																													
0.05	3.228 ± 0.0154	4.71	7.41	11.39	-																												
0.10	3.352 ± 0.0139	8.08	2.99	6.08	5.99	-																											
0.25	3.466 ± 0.0139	11.11	1.27	0.78	11.49	3.61	-																										
0.05	3.741 ± 0.0140	18.43	11.57	12.20	24.66	19.74	13.95	-																									
0.10	3.811 ± 0.0157	19.92	13.73	14.73	26.62	21.96	16.50	3.33	-																								
0.25	3.919 ± 0.0123	23.47	18.75	21.37	34.89	30.46	24.33	9.51	5.40	-																							
0.05	3.289 ± 0.0130	6.46	5.45	9.32	3.03	13.31	9.31	13.66	25.71	35.79	-																						
0.10	3.356 ± 0.0152	8.08	2.77	5.65	5.92	0.19	5.36	18.68	20.87	28.72	3.35	-																					
0.25	3.614 ± 0.0146	14.93	6.74	6.12	18.20	13.03	7.36	6.28	9.20	15.88	16.66	12.28	-																				
0.05	3.638 ± 0.0291	13.96	5.58	4.72	12.46	8.88	5.34	3.19	5.24	8.89	10.22	8.39	0.73	-																			
0.10	3.753 ± 0.0173	18.07	11.22	11.53	22.72	18.14	12.98	0.54	2.48	7.79	11.48	17.26	6.15	3.40	-																		
0.25	3.956 ± 0.0117	24.60	20.46	23.86	37.72	33.37	27.07	11.81	7.43	2.16	38.33	31.41	18.28	10.13	9.75	-																	
0.05	3.548 ± 0.0197	12.46	3.85	2.65	12.80	14.12	3.40	8.00	10.47	15.92	10.97	7.74	2.69	2.56	7.82	17.81	-																
0.10	3.751 ± 0.0178	17.93	11.03	11.22	12.00	17.72	12.66	0.44	2.23	7.74	11.00	16.88	5.95	3.31	0.08	9.62	7.66	-															
0.25	3.840 ± 0.0138	21.06	15.28	16.95	29.70	25.02	19.17	5.03	1.38	4.26	29.15	23.60	11.30	6.27	3.93	6.44	12.16	3.95	-														
0.05	3.659 ± 0.0187	15.42	7.72	7.28	17.80	13.17	8.28	5.51	6.22	11.60	16.29	12.62	1.89	0.60	3.70	13.30	4.09	5.36	7.80	-													
0.10	3.886 ± 0.0195	15.75	15.13	16.07	26.53	22.34	17.57	6.04	3.00	1.42	25.51	21.45	11.19	7.08	5.11	3.08	12.20	5.11	1.93	8.40	-												
0.25	3.948 ± 0.0176	23.01	17.91	19.62	30.90	26.46	21.51	9.24	5.82	1.34	30.22	25.51	14.64	9.11	7.92	0.37	15.15	7.88	4.84	11.28	2.36	-											
0.05	3.844 ± 0.0121	21.51	15.96	18.05	31.58	26.75	20.54	5.56	1.66	4.33	33.53	25.15	12.16	6.53	4.31	6.66	12.81	4.32	0.21	15.01	1.03	4.88	-										
0.10	3.918 ± 0.0131	23.32	18.54	21.11	34.15	29.65	23.66	9.26	5.24	0.05	34.18	28.10	15.51	8.77	7.60	2.17	15.67	7.55	4.10	11.35	1.36	1.36	4.15	-									
0.25	4.025 ± 0.0130	26.19	22.63	26.28	39.43	35.25	29.26	14.86	10.49	5.85	40.00	33.45	20.96	12.13	12.53	3.94	20.21	12.39	9.73	16.05	5.94	5.51	0.16	5.81	-								
0.05	3.697 ± 0.0128	17.44	10.15	10.53	25.43	18.35	12.28	2.32	5.64	12.47	22.41	17.22	4.27	1.86	2.60	14.97	6.56	2.46	7.60	1.68	8.11	8.96	8.35	12.07	18.02	-							
0.10	3.861 ± 0.0128	21.85	16.43	18.53	31.65	27.07	21.01	6.34	2.47	3.25	31.42	25.50	12.73	7.03	5.02	5.49	13.37	5.02	1.11	8.93	1.07	3.10	0.96	3.11	9.01	9.06	-						
0.25	4.032 ± 0.0147	25.96	22.14	25.27	37.92	33.66	28.01	14.33	10.27	5.88	37.90	32.03	20.19	12.08	12.29	4.06	19.75	12.21	9.55	15.75	5.98	5.66	0.88	5.81	0.35	7.26	0.81	-					
0.05	3.674 ± 0.0153	16.45	8.83	8.69	20.25	13.65	10.09	3.23	6.25	12.45	19.25	14.79	2.84	1.09	3.83	14.68	5.06	3.29	8.05	0.62	8.38	11.56	8.71	12.13	17.55	1.15	9.39	16.88	-				
0.10	3.979 ± 0.0201	26.52	18.05	19.37	29.68	25.69	21.02	9.75	6.38	2.54	28.87	24.72	14.71	9.66	8.52	0.99	15.33	8.30	5.72	11.67	3.32	1.16	5.76	2.55	1.92	1.84	4.95	2.12	12.10	-			
0.25	4.026 ± 0.0144	25.87	22.08	25.23	38.00	33.70	28.00	14.25	10.09	5.65	37.98	32.05	20.09	11.97	12.13	3.78	19.39	12.06	9.34	15.55	5.98	5.43	0.68	5.56	0.05	7.13	0.30	0.59	16.76	1.90	-		
0.05	3.852 ± 0.0125	21.67	16.15	18.05	31.51	26.98	20.75	5.93	2.05	3.80	33.27	25.30	12.39	6.77	4.64	6.08	13.04	4.65	0.64	15.61	1.47	4.46	0.46	3.64	9.61	8.70	0.50	9.27	9.03	5.38	9.15	-	
0.10	3.972 ± 0.0130	24.57	20.30	23.16	36.11	31.79	25.94	11.78	7.70	2.04	36.13	30.04	17.90	10.37	9.90	0.88	17.66	9.82	6.76	13.49	3.61	1.11	6.99	2.84	2.80	4.62	5.90	2.98	14.46	0.28	2.71	6.45	-
0.25	4.052 ± 0.0105	27.58	24.80	29.89	44.34	40.46	33.87	17.97	12.88	8.26	46.24	38.03	24.60	15.44	14.87	6.19	22.70	14.68	12.32	18.45	7.34	5.12	3.16	8.07	1.63	1.64	11.64	1.11	20.54	3.24	1.46	12.42	4.66

The value of "t" is above 2.0369 significant at 5.0 percent level and above 2.7385 significant at 1 percent level.

It has been estimated that tea industry in India harbour about 300 species of pests and therefore, extreme care must be exercised before a pesticide is introduced to tea for pest control (Gurusubramanian *et al*., 2005) to avoid residue build-up. Organophosphate, organochlorine, carbamate, and synthetic pyrethroid insecticides have been in use on tea in North-East India for the past 100 years. Much of the efficacy and sustainability of these groups of insecticides in tea mosquito bug management would depend on the susceptibility. Endosulfan, quinalphos, cypermethrin, alphamethrin, deltamethrin and oxydemetonmethyl at recommended dose persisted for shorter durations of 7-11 days in the Dooars area. In this situation, either the planters may reduce the frequency of the above mentioned chemicals or TRA may reassess the dose of cypermethrin, alphamethrin, and deltamethrin to 0.10%, which showed 14-17 days of persistence with increased PT values (688.0 – 875.0) (Table 1) and LT_{50} values (5.63 – 7.68 days) (Table 2), instead 0.05%. Earlier, Rahman *et al* (2005) observed the PT and LT_{50} values of different insecticides against tea mosquito bug in Jorhat, Assam and found the persistence of 10 – 23 days with 300 – 1771 PT values. Variation in relative toxicity was observed between male and female populations of Jorhat (Gurusubramanian and Bora, 2007) and Darjeeling (Bora *et al*., 2007) and among the populations of *H. theivora* collected from different sub-districts of Dooars (Rahman *et al*., 2005) due to selection pressure by insecticides. A comparison of expected effective doses of different classes of

insecticides against tea mosquito bug collected from Jorhat, Assam based on their LC_{50} values with recommended dose revealed a pronounced shift in the level of susceptibility of *H. theivora*. The recommended dose of synthetic pyrethroids (fenpropathrin, cypermethrin, λ-cyhalothrin, and deltamethrin), organophosphates (profenophos, dimethoate, oxydemetonmethyl, phosalone, and quinalphos) neonicotinoids (thiomethoxam and imidacloprid), and organochlorine (endosulfan), however, was practically ineffective against this pest (Bora *et al.,* 2008; Gurusubramanian *et al*., 2008). The presence of various oxido-reductase enzymes in the salivary and mid gut along with the basic hydrolyzing enzymes enable *H. theivora* to be one of the most destructive pests of tea by depredating the young leaves and growing shoots of tea (Sarker and Mukhopadhyay, 2006). In addition, qualitative and quantitative changes were recorded in the enzyme patterns of the tea mosquito bug indicating a higher tolerance/resistance status due to the formation of greater amounts of esterases (Sarker and Mukhopadhyay, 2003), glutathione S-transferase and acetylcholinesterase (Sarker and Mukhopadhyay, 2006a). One of the main reasons for higher tolerance or resistance by tea mosquito bug to different pesticides was due to mixing of incompatible insecticides with acaricides to combat mixed infestation of tea mosquito bug and red spider mite which, not only decreased the insecticide toxicity but also shifted the level of relative toxicity (Rahman *et al*., 2005).

Because of a lesser requirement (100 ml/ha) having knock down effect and cost effectiveness, the synthetic pyrethroids are being used widely in tea plantations, and their consumption is about 3-5 litres/ha (Gurusubramanian *et al.* 2005). Planters using insecticides as a prophylactic against the tea mosquito bug, due to it being the wet season pest and their peak season (May-July) coinciding with the rainy season (June-July), caused the consumption of pesticides to increase, with about 8-16 applications per year of synthetic pyrethroids on top of other chemical applications. Hence, irrespective of the group to which insecticides belong, evidence of the development of resistance to synthetic pyrethroids, organophosphates, organochlorines and neonicotinoids has been experimentally proved in the tea mosquito populations of Dooars, West Bengal and Jorhat, Assam (Rahman *et al*., 2005; Sarker and Mukhopadhyay, 2006a; Gurusubramanian and Bora, 2007; Bora *et al*., 2008; Gurusubramanian *et al*., 2008).

In the recent years, it has become a major concern to the tea industry as the importing countries are imposing stringent restrictions for acceptability of the "made" tea due to pesticide residues. Changes in pest management tactics are resulting from environmental and human safety concerns, development of insect pest susceptibility change against a few insecticides is now a reality, and increases in pesticide cost and availability. Thus, before spraying any chemicals, the tea planters must i)consider the impact of pesticides on non target organisms, human health, wild life habitat and environment and ii) adopt IPM strategies to reduce the pesticide load to produce residue free tea, increase the exports and meet out the consumers' demand. Potential cultural practices for conserving and enhancing the natural enemies need to be integrated with our current crop management strategies for developing sustainable tea crop protection. Therefore, the following integrated resistant management practices must be followed for combating and delaying the problem of resistance so that it does not assume unmanageable proportions:

1. After infestation remove all the infested shoots to rejuvenate the shoot growth as well as to remove the laden eggs before spraying.
2. In severe infestations, LOS (level of skiff) operations should be followed to minimize the infestation of the next generation.
3. Shade status neither should be overshaded nor unshaded.
4. Alternate hosts must be eliminated (Guava (*Psidium guajava),* oak (*Quercus* spp.), melastoma (*Melastoma* sp.), Thoroughwort (*Eupatorium* sp.), fragrant thoroughwort (*Eupatorium odoratum),*Dayflower (*Commelina* spp.), Sesbania (*Sesbania cannibina),* Jackfruit (*Artrocarpus heterophylla*), Bortengeshi (*Oxalis acetocello*), Ornamental jasmine (*Gardenia jesminoid*), Mulberry (*Morus alba*), Kadam (*Enthocephalus cadamba*), Jamun (*Eugenia jambolana*), Boal (*Ehretia acuminata*), Mikania (*Mikania micrantha*), *Acacia moniliformis* and *Premna latifolia).*
5. By following proper monitoring detect the *H. theivora* at an early stage and manually collect the adults (Morning – 06.30 h – 08.30 h; Evening – 16.00 h – 18.00 h) by use of a trained labour force.
6. Unpruned sections must be monitored regularly during December-February and proper care should be taken to kill the residual population.
7. Care needs to be adopted in skiffed and pruned sections during bud breaking (March – April) which are prone to *H. theivora* attack.
8. Underperformance of spraying equipments should be avoided.
9. Toxicity persistence of different insecticides at recommended dose falls between 7 – 16 days. Hence interval between two subsequent rounds must be 7-15 days.

10. Avoid spraying of endosulfan, quinalphos and cypermethrin in severely infested sections due to shorter persistence.
11. Selection and usage of chemicals, assurance of the quality, required spraying fluid, and trained man power for overall good coverage.
12. Under dense tea bush population care must be taken for good and uniform coverage of chemicals.
13. Incompatible chemicals must be avoided in tank-mix formulations in severely affected sections.
14. Recommended dose of chemicals should be followed and avoid sub- and supra- lethal doses to minimize the chances of susceptibility change in *H. theivora* populations.
15. Avoid spraying during hot sunny days which degrade the chemical activity, cause phytotoxicity and has no direct contact with insects. Hence, spray in the early morning and late afternoon.
16. With prior knowledge about *H. theivora* infestation patterns, mark the infested bushes in the early stages and go for spot application to check the pest as well as to reduce the chemical load instead of blanket application.
17. Conserve and preserve the natural enemies present in the natural tea ecosystem by minimizing the load of chemicals for their natural regulation.

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Somnath Roy[1], G.Gurusubramanian[2] and Ananda Mukhopadhyay [1]
[1]Entomology Research Unit, Department of Zoology, University of North Bengal, Darjeeling-734 430, West Bengal, India.
E-mail: entosomnath@yahoo.co.in; dr_amukherjee_nbu@rediffmail.com; Fax No.0353-2581546
[2]Department of Zoology, Mizoram Central University, Tanhril, Aizawl -796 009, Mizoram, India.
E-mail: gurus64@yahoo.com; Fax No.0389-2330642/644

Application of bi-PASA and development of PCR-REN for detection of point mutation 980A>G in *AChE* gene of Colorado Potato Beetle in South Ural's local population

Key words: Colorado potato beetle, resistance, acetylcholinesterase, mutation, PCR, South Urals.

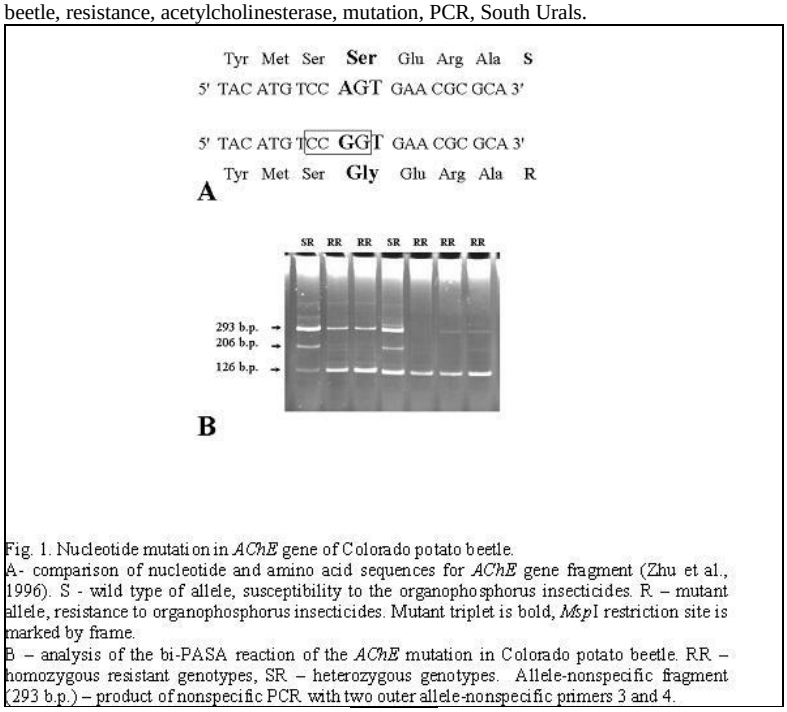

Fig. 1. Nucleotide mutation in *AChE* gene of Colorado potato beetle.
A- comparison of nucleotide and amino acid sequences for *AChE* gene fragment (Zhu et al., 1996). S - wild type of allele, susceptibility to the organophosphorus insecticides. R – mutant allele, resistance to organophosphorus insecticides. Mutant triplet is bold, *Msp*I restriction site is marked by frame.
B – analysis of the bi-PASA reaction of the *AChE* mutation in Colorado potato beetle. RR – homozygous resistant genotypes, SR – heterozygous genotypes. Allele-nonspecific fragment (293 b.p.) – product of nonspecific PCR with two outer allele-nonspecific primers 3 and 4.

Acetylcholinesterase *(AChE)* is the target site of inhibition by organophosphorus and carbamate insecticides in insects (Smallman and Mansing, 1969). In the Colorado potato beetle *AChE* insensitivity and resistance to azinphos-methyl is associated with a transition 980A>G and serine to glycine amino acid substitution (Fig. 1, A) in its active center and with the insensitivity of this *AChE* form to the organophosphorus insecticides action (Zhu, Clark, 1995).

For detection of this point mutation in *AChE* of Colorado potato beetles, we have used bi-directional PCR amplification of specific alleles (bi-PASA) (Clark et al., 2001). This method allows the separation of homozygous resistant RR or susceptible SS genotypes and heterozygous genotype SR. The Bi-PASA reaction contains four separate primers. The two inner primers (No 1 and 2 in Table 1) are allele-specific at the mutation site. The two outer primers (No 3 and 4) are allele-nonspecific. Under the following PCR and electrophoresis, susceptible homozygotes (wild type, SS) are determining by the presence of 206 b.p. fragment, resistant homozygotes (RR genotype) - by the presence of 126 b.p. fragment, and heterozygous genotype (SR) by the presence of both 206- and 126 b.p. fragments (Figure. 1b). Primers for bi-PASA realization were synthesized in Sintol (Russia, Moscow) by (Clark et al., 2001).

The PCR was run for 35 cycles, each consisting of denaturation at 94°C for 30 seconds, annealing at 62°C for 30 seconds, and extension at 72°C for 1 minute after initial denaturation of the DNA template at 94°C for 1 minute. A final extension at 72°C lasts for 5 minutes (Clark et al., 2001). Following PCR, the bi-PASA product were resolved by size using 5% PAAG electrophoresis in lxTAE buffer and visualized by ethidium bromide staining.

Table 1. Primer sequences used in bi-PASA (Clark et al., 2001)

Name	Sequences, 5` to 3`
1) Resistant allele-specific forward primer	CGT GGA GCT ACA TGT CCG
2) Susceptible allele-specific reverse primer	TTG TTC TGC GCG TTC ACT
3) Allele-nonspecific forward primer	GCT ATA CGT TGG ATC AAA GAC A
4) Allele-nonspecific reverse primer	ACT GCT CTC ATA CAG TCC ATC A

In the model local population of Colorado Potato Beetle (Ufimsky region, South Urals, Bashkortostan) with the high level of resistance to organophosphorus insecticides, the DNA analysis of imago (N=98) was carried out looking for the presence of the point mutation 980A>G in the gene of *AChE*. We identified resistant homozygous RR (frequency of occurrence 0.7) and heterozygous SR (frequency of occurrence 0.3) genotypes (Fig. 1, B).

Colorado potato beetle in the local populations of Ufimsky region is characterized by maximum resistance to pirimifos-methyl and malathion (mortality in one of the local populations under the application of diagnostic concentrations was about 0%) (Udalov, 2006).

We suppose that long-term treatments with organophosphorus insecticides at the initial stage of the Colorado potato beetle expanding on the territory of Bashkortostan eliminated individuals with the sensitive genotypes (homozygous genotypes SS were not identified).

For the accessory confirmation of 980A>G mutation identified, we had applied the PCR-REN (Restriction ENdonuclease) method. The given mutation availability led to the arrival of the restriction site for *Msp*I restrictase (Fig 1, A). After the PCR with pare of allele-nonspecific primers, the 293 b.p. fragment obtained was treated by restrictase. In the case of mutation absence, the restriction should not to occur and electrophoresis should follow by the obtaining of a single fragment of 293 b.p. In the case of homozygous mutant genotype (G/G) two fragments 187 and 106 b.p. should be obtained, and in the case of heterozygote we shall obtain 3 fragments at a rate of 293, 187 and 106 b.p.

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Maksim B. Udalov and Galina V. Benkovskaya
Institute of Biochemistry and Genetics, Ufa Scientific Centre,
Russian Academy of Sciences,
Ufa, Russia, prosp. Oktyabrya, 71. E-mail: udalov-m@yandex.ru

Research in Resistance Management

Joint action of *Beauveria bassiana* (Bals.) Vuill. with Certain Synthetic Insecticides and Neem Formulations used for the Management of Rice Leaffolder, *Cnaphalocrocis medinalis* Guenee

ABSTRACT: Twelve commonly used synthetic insecticides and three neem formulations in rice pest management were evaluated at three different concentrations *viz.*, 10X, 1X and 0.1X (where X=recommended field rate) with the entomopathogenic *Beauveria bassiana* (Bals) Vuill isolate: BbCm KKL 1100 under *in vitro* using the agar plate poisoned food technique for predicting their combined performance against rice leaf folder, *Cnaphalocrocis medinalis* in a field situation. The sensitivity of *B. bassiana* in terms of radial growth extension was quantified during the whole test period of 14 days at 26±2°C under laboratory condition. Generally, all of the insecticides/neem formulations tested had adverse effects on the mycelial growth either partially or completely. At the 10X rate, all the insecticides pronounced fungicidal effect on *B. bassiana* exhibiting total (100%) inhibition of mycelial growth, while the neem formulations strongly inhibited the fungus growth impairing 70-86% biomass production. At the 1X rate, carbaryl and carbofuron showed total (100%) inhibition, but all other insecticides and neem formulations showed a fungistatic effect with 41.48–75.5% mycelia inhibition observed. The 0.1X rate of insecticides showed a varying degree of fungistatic effects, but chlorpyriphos (27.33%) and NSKE (22.2%) exhibited slightly harmful effects and are probably compatible with *B. bassiana* in the field. This is not the case with rest of the insecticide formulations, which are moderate to strongly antagonistic to the fungus.

KEY WORDS: Joint action, insecticides, *Beauveria bassiana*, rice agro-ecosystem

INTRODUCTION

The fungal entomopathogen, *Beauveria bassiana* (Bals.) Vuill. is being investigated for use as a biological control agent against diverse insect-pests in agriculture. In India, the fungus has established natural epizootics on different species of rice insects (Hazarika and Puzari, 1990; Padmanaban *et al.* 1990; Ambethgar, 1996), and extensively on the rice leaf folder, *Cnaphalocrocis medinalis* Guenee (Ambethgar, 2002). Insecticides used for control of rice insects may have a

synergistic or antagonistic effect on the potentiality of *B. bassiana* in fields (Aguda *et al.*, 1984). Regular use of a wide spectrum of insecticides can result in their accumulation in the field environment and this may impede natural epizootics (Moorhouse *et al.* 1992). Under such natural epizootic conditions, it is expected to enhance effectiveness through joint action of prevalent fungal entomopathogens and compatible insecticides (Jacobson *et al.*, 2001), which would reduce not only the cost of protection, but also reduce the gravity of resistance to insecticide. This knowledge of compatibility between *B. bassiana* and all commonly used insecticides is crucial, so that growers may select appropriate compounds to schedule treatments accordingly in order to minimize any deleterious effects on biocontrol efficiency.

In this context, if *B. bassiana* is to be incorporated into the integrated pest management programme of rice, it is necessary to determine the effects of commonly used insecticides on it. Till now, information on the joint action of insecticides *vis-à-vis* fungal entomopathogens in rice ecosystem is scanty. The present study aims to examine the joint action of some selected insecticides and neem products on the growth of a pre-selected virulent *B. bassiana* isolate under *in vitro* condition for predicting their combined performance against *C. medinalis* in a field situation.

MATERIALS AND METHODS

Fungal isolate used: The *Beauveria bassiana* isolate BbCm KKL 1100 used in this study was retrieved from original collections made of mycosed larvae of rice leaf folder, *Cnaphalocrocis medinalis* from a rice leaffolder endemic area of Karaikal Enclave, Pondicherry Union Territory, India (Ambethgar, 1996). The fungus was initially isolated on slants of Sabouraud dextrose agar fortified with 1% yeast extract (SDAY) medium and incubated for 2-3 weeks at 26±2^{o}C. Inocula of the fungus were produced on SDAY in Petri plates, which were sealed and incubated at 26±2^{o}C in complete darkness in a B.O.D incubator for 14 days to allow maximum mycelial growth.

Synthetic insecticides and neem formulations: The insecticides and neem formulations used in this study were selected among those commonly used in rice pest management programme (Table 1). The effect of these insecticides on the radial growth of *B. bassiana* was evaluated under laboratory conditions. The pesticide dose was calculated by the field application rate based on 500 litre of spray fluid per hectare (Crop Production Guide, 2000). Fifteen insecticides each at three concentrations (the field application rate (I.0 X), a 10 time lower rate (0.1 X) and a 10 time higher rate (10.0 X)) were tested by poisoned food technique (Moorhouse *et al.*, 1992) in SDAY medium.

In vitro inoculation procedure: Twenty ml of SDAY medium was sterilized in individual boiling tubes. The sterile agar was cooled in a water bath to approximately luke warm temperature of 55^{0}C and the insecticide emulsions of required concentration were incorporated into the melted sterile SDAY aseptically, thoroughly mixed, poured into 9 cm diameter sterile Petri dishes and allowed to solidify and set overnight under laminar flow cabinet. An agar disc along with mycelial mat of *B. bassiana* was cored from the periphery of a 10-day-old colony by 10 mm diameter cork borer and transferred-inverted onto the center of a SDAY plate amended with differential insecticide concentrations. Growth medium (SDAY) without insecticide but inoculated with mycelial disc served as control. The plates were sealed and incubated at 26±2^{o}C in complete darkness in a B.O.D incubator for 14 days to allow maximum growth. Each insecticide-concentration-fungus combination and corresponding control was replicated three times. The diameter of growing fungus culture in excess of the plugs in each Petri dish was measured on 14 days after inoculation using a millimeter ruler along the same pre-marked radial line on the four cardinal points from the disc. The replicated values recorded at each reading were averaged, and the data was expressed as percentage of growth inhibition in production of fungal colony on pesticide treated SDAY in comparison to corresponding control using the following formula (Hassan, 1989).

$$X = \frac{Y - Z}{Y} \times 100$$

Where: X, Y, Z stand for percentage of growth inhibition, radial growth of fungus in control and radial growth of fungus in poisoned medium respectively. The pesticides were further classified in evaluation categories of 1-4 scoring: 1 = harmless (< 20% reduction in beneficial capacity), 2 = slightly harmful (20-35 %), 3 = moderately harmful (35-50%), and 4 = harmful (>50 %) in toxicity tests according to Hassan's classification scheme (Hassan, 1989).

RESULTS AND DISCUSSION

In the integrated pest management system, it is essential to know the influence of pesticides frequently used in crop protection on the introduced entomopathogens. A number of recent studies have suggested pesticides may adversely affect insect pathogenic fungi under field conditions. Selective pesticides that can be used to control pests without any adversely effect on introduced entomopathogens are needed. In the present study, all the tested insecticides and neem products lowered the mycelial growth of *B. bassiana* in SDAY medium either partially or completely at all the three concentrations (the recommended field application rate (1.0X), 10 times

lower rate (0.1X) and 10 times higher rate (10.0X)). The mean radial extension of *B. bassiana* was significantly reduced by the recommended rate of all insecticides examined. The data in Table 1 shows that there were significant differences among the insecticides in inhibition of fungus growth. The results indicate that carbofuran and carbaryl completely inhibited fungus growth at all three concentrations. Radial extension of the fungus was also prevented by 10 times higher concentration of all the insecticides tested. Three out of twelve insecticides tested (quinalphos (41.48%), monocrotophos (47.41%), chlorpyriphos (48.33%) and phosphamidon (48.88%)) showed a moderate fungistatic effect towards the mycelial growth of *B. bassiana* at field recommended rates, while all other insecticides caused a strong fungistatic effect with more than 50% inhibition of mycelial growth. At 10 times reduced concentration (0.1X), only chlorpyriphos (27.33% inhibition) and dimethoate (32.6%) were slightly harmful to the development of *B. bassiana*, while all other insecticides posed strong inhibition to the fungus. This observation conceived the idea that mixing the least harmful insecticide like chlorpyriphos at a sub-lethal dose with fungal formulation spray of *B.bassiana* may be helpful to induce mycosis on the field population of *C. medinalis* larvae by weakening host insect physiology following the combination sprays. In this direction, Ribba *et al.* (1983) advocated half of the recommended dose of chlorpyriphos in combination with *B. bassiana* for the control of *Ostriana nubilalis* Hubner.

In general, increasing the concentration of the chemicals from 0.1 to 10 times the recommended rate caused a strong inhibition or fungicidal effect on the fungus. At 1 time the rate of concentration no insecticide was compatible with the fungus and showed fungistatic reactions. Published literature on the influence of insecticides on entomopathogenic fungi is contradictory. However, varying the levels of reduction in the mean radial extension of *B. bassiana* was dependent on insecticide concentration. Urs *et al.* (1967) reported insecticides such as methyl parathion, malathion, endrin, DDT and HCH were inhibitory on the development of *B. bassiana* in the order mentioned. Cadatal and Gabriel (1970) have reported that carbaryl, endosulfan and endrin exhibited partial to complete inhibition of mycelial growth and sporulation in *B. bassiana*, while insecticides such as fenitrothion and chlorfenvinphos were innocuous. Clark *et al.* (1982) reported that insecticides such as azinphos methyl and carbofuron showed moderate inhibition of *B. bassiana*, reducing mycelial growth by about 50 per cent, while permethrin did not inhibit the fungus at all, and its growth was very similar to that of the untreated control. Gardner *et al.* (1979) reported that carbaryl and methyl parathion at 0.04% partly impaired the growth of *B. bassiana*. But in the present study, carbaryl and carbofuran pronounced fungicidal effect to *B. bassiana* in all the three concentrations tested. The inhibitory effects of insecticides on the processes of germination, radial growth and sporulation vary depending on the fungal species and strain (Anderson *et al.*, 1989). Anderson *et al.* (1989) further reported that insecticides such as abamectin, triflumuron and thuringiensin were compatible with *B. bassiana* even at higher rates. Carbaryl, fenvelerate, and thuringiensin combinations with *B. bassiana* produced decreased mycoses compared with *B. bassiana* alone for the control of Colorado potato beetle (Anderson *et al.*, 1989).

Table 1. Joint action of certain insecticides on the growth of *B. bassiana* in SDAY

Insecticides		Lower dose (0.1 X)		Normal dose (1 X)		Higher dose (10 X)		Scoring at 0.1 X
		MRG	PIC	MCD	PIC	MCD	PIC	
Acephate	75 SP	31.33	65.18	22.00	75.55	0.0	100.00	4
Carbaryl	50 WP	0.0	100.00	0.0	100.00	0.0	100.00	4
Carbofuran	3 G	0.0	100.00	0.0	100.00	0.0	100.00	4
Chlorpyriphos	20 EC	69.63	27.33	46.50	48.33	0.0	100.00	2
Dichlorvas	76 WSC	50.66	43.71	35.33	60.74	0.0	100.00	3
Dimethoate	30 EC	60.66	32.60	41.00	54.44	0.0	100.00	2
Endosulfan	35 EC	51.33	42.96	36.66	59.66	0.0	100.00	3
Fenthion	100 EC	40.66	54.82	26.66	70.33	0.0	100.00	4
Monocrotophos	36 WSC	59.33	34.07	47.33	47.41	0.0	100.00	2
Phosalone	35 EC	54.00	40.00	36.66	59.66	0.0	100.00	3
Phosphamidon	85WSC	56.00	37.77	46.00	48.88	0.0	100.00	3
Quinalphos	25 EC	39.00	56.66	52.66	41.48	0.0	100.00	4
Azadirachtin	0.03 EC	57.66	35.93	44.00	51.11	18.3	80.00	3
Neem Oil	3%	57.33	36.30	40.33	55.18	12.33	86.30	3
NSKE	5%	70.00	22.22	46.33	48.52	20.66	70.04	2
Control	-	90.00	0.00	90.00	0.00	90.00	0.00	-

MRG=Mean radial growth (in mm), PIC= Per cent inhibition over control

1 = Harmless (<20% inhibition) 3 = Moderately harmful (36-50% inhibition)
2 = Slightly harmful (20-35% inhibition) 4 = Harmful (>50% inhibition)

In the present study, chlorpyriphos was found to be slightly harmful to *B. bassiana* at 0.1 X dose (Table 1). Contrary to this finding, chlorpyriphos had a strong inhibition on the growth and sporulation of *B. bassiana* in a dose-dependent manner even at concentrations lower than recommended rates of field use (Rama Mohan Rao, 1989). Although the different insecticides tested in the present investigations inhibited the growth of *B. bassiana* in the poisoned agar media *in vitro*, the combined use of entomopathogenic fungus and insecticides need not be totally ruled out. Certain insecticides at sub-lethal doses have been combined with entomofungi for obtaining better control of some insect pests. For instance, increased mortality due to mycosis of *Beauveria tenella* Sacc. (Mc.Leod) in *Melolontha melolontha* L. was noted by the addition of reduced doses of insecticides (Olmert and Kenneth, 1974; Aguda *et al.*, 1984). The commercial

mycoinsecticide 'Boverin' based on *B. bassiana* with reduced doses of trichlorophon have been used to suppress the second-generation outbreaks of *Cydia pomonella* L in orchard ecosystems (Olmert and Kenneth, 1974).

A study on the joint action of certain neem products such as azadirachtin, neem oil and NSKE at three levels of concentrations showed growth inhibition of *B. bassiana* at varying degree similar to synthetic insecticides. However, only NSKE at 10 times reduced concentration (0.1X) showed slight inhibitory effect of 22.2% as graded as slightly harmful to development of *B. bassiana*. Gupta *et al.* (2002) reported on the joint action of certain commercial neem formulations like Achook, Field marshal, Margocide and Nimbecidine in 10,000, 1000, and 100 ppm to *B. bassiana* under *in vitro* conditions. This may be due to the chemical nature of active ingredients and toxic principles present in the formulations.

In conclusion, it is worth exploring the effects of chlorpyriphos, NSKE and some other newer insecticidal compounds at sub-lethal doses with *B. bassiana* as two-in-one tank mix strategy to realize multiple mortality factors for field control of insect pests in rice ecosystem. While doing so, adequate care should also be taken because these insecticidal compounds at sub-lethal doses may end up with complications like resurgence of sucking insects in rice ecosystems. The present studies also opened a new vista on the combined use of fungal entomopathogens together with compatible insecticides as multiple mortality factors against target pests, which would also help in delay and expression of resistance to newer insecticides.

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V. AMBETHGAR[1], M. SWAMIAPPAN[2], R. RABINDRAN[2] AND R. J. RABINDRA[3]

1 Regional Research Station, TNAU, Vridhachalam-606 001, Tamil Nadu (India)

2 Centre for Plant Protection Studies, TNAU, Coimbatore-641 003, Tamil Nadu (India)

3 Project Directorate of Biological Control, ICAR, Bangalore-560 024, Karnataka (India)

Corresponding Author E-mail: drva_1965@yahoo.co.in

Susceptibility of the Bagworm *Auchmophila kordofensis* Rebel (Lepidoptera: Psychidae) to the three different insecticides groups Spionsad, Chlorpyrifos and Cypermethrin

ABSTRACT

The insect pest (*Auchmophila kordofensis*) (Lepidoptera:Psychidae), locally known as umcigara is widely spread in Kordofan States, Sudan. The objective of the study was to investigate the susceptibility, mortality rates (LD_{50} and LD_{90}), and the homogeneity/heterogeneity of *Auchmophila kordofensis* (Rebel) when treated with one of three selected insecticides. The pest, fourth larval instar (L4), was obtained from Kordofan State (ElObeid region), and collected from *Acacia* Spp (*A. nubica*, *A. tortilis*, *A. nilotica*, *A. Seyal*) and *Prosopis julifora* trees. The study was conducted in the Biology Laboratory, Faculty of Agricultural Sciences, University of Gezira. Spinosad (Tracer® 240 SC), chlorpyrifos (Drusban® 48% EC) and cypermethrin (Devicyprin® 25%EC) were tested (bioassayed) at seven different concentration, topically (thoracic segments) and by the dipping method. Results were taken after 24 hours, viz. mortality percentages, LD_{50}, LD_{90} and log-dose probability lines slopes (Ld-p lines) for each insecticide and route of administration. The results obtained showed that dipping method for spinosad showed a LD_{50} value of 8.87±1.38ppm and a LD_{90} value of 48.73±8.5ppm; and slope = 1.71 while the topical method resulted in a LD_{50} value of 17.37+2.97ppm and a LD_{90} 34.67±1.00ppm and slope = 4.4 Chlorpyrifos displayed an LD_{50} value of 7.38±0.85ppm and a LD_{90} value of 25.57±0.55ppm for dipping method; and slope = 2.32, whereas, those for the topical were LD_{50} 15.13±3.62ppm and LD_{90} 28.84+0.39ppm, respectively and slope = 4.53 The dipping and topical methods for cypermethrin showed LD_{50} values of 7.04±1.25ppm, 15.45±2.68ppm, and LD_{90} values of 34.50±4.88ppm and 28.18±1.66ppm; Slope = 1.85 and 5.02 respectively. The study showed that cypermethrin has an excellent performance on *A. kordofensis*, followed by chlorpyrifos and spinosad, taking into consideration that the last chemical is environmentally friendly and a safe product. Recommendations: Further elaborate and detailed field and laboratory investigations about this pest are urgently needed for more understanding, (*i.e.* biological, ecological, physiological, biochemical and behavioural aspects). Such information would help in preparation and implementation of effective control strategies.

Introduction

Among the biological constraints to the *Acacia* spp the acacia bagworm, *Auchmophila kordofensis* (Rebel) (Lepidoptera: Psychidae), locally known as umcigara, is considered as one of the most destructive pests of trees in the forests. It is a caterpillar that lives in a silken cocoon resembling a bag, by which the caterpillar is attached to the branch of the host tree. The damage encountered due to bagworm attack is defoliation, death of branches and/or death of the whole tree may occur.

Psychidae larvae construct portable bags, or cases, of bits of leaves and twigs, and eventually pupate in this bag. Wingless females lay their eggs in the bag, and usually never leave it until the eggs are laid (Borror and White, 1970).

The acacia bagworm, *Auchmophila kordofensis* (Rebel), is known locally as "umcigara", the name derived from the status of the larvae, which is found living within the bag permanently, (Elbadwi, 1995). *A. kordofensis* was first classified by Rebel (1906) (Rebel and Zeray, 1914) in the order Lepidoptera, super family Zygenoidae and family Psychidae. In the Sudan, *A. kordofensis* was reported in Kordofan in 1914 on *Acacia Senegal* and was shown to be on other *Acacia* species in Darfur (western Sudan), Kassala (eastern Sudan), Blue Nile (Southeastern Sudan) and around Khartoum Central Sudan (Rebel and Zeray, 1914).

Many species of bagworms show polyphagous feeding habits and have been recorded from many different host plants (Dennis, 1983). Rebel and Zeray (1914) were the first to classify the Acacia bagworm according to specimen collected from Kordofan State from *Acacia nubica* and, hence, the name of the species *kordofensis* was offered. Andrews (1948) list the hosts of the Acacia bagworm as follows: *Acacia nubica*, *A. tortilis*, *A. nilotica*, *A. seyal*, *A. Senegal*, and Mesquite, *Prosopis Julifora* (Swartz. DC). The over-wintering eggs begin hatching in late spring, about the first week of June in Kordofan State, when Mock Orange (*Philadelphus* spp.) are in full bloom and when the first red fruits are on Honeysuckle (Horre and Sons 2002). The larvae immediately begin feeding and constructing their protective houses out of silk and bits and pieces of their host plant. They prefer arborvitae and juniper, but are not picky nor are they nimble enough to move much, and will devour just about any tree or shrub, evergreen or deciduous.

The bagworm has become a serious pest and is known to do severe damage by defoliating plantation trees (Peak, 1956). According to Dennis (1983), bagworms generally defoliate the host by eating the leaves and the plant becomes generally festooned with hanging bags (Appe. E3). Basri and Kevan (1995) described the damage caused by the oil palm bagworm, *Metise plana*, as removal of most leaf tissues from the oil palm foliage to be used for body growth and bag construction.

Persistent droughts and repeated heavy infestations by the Acacia bagworm in the Sudan have adversely affected the contribution of these trees to ecological stability (Ahmed *et al*., 2005, and Mahmoud, 2005). In addition, there is a growing concern that the insect might endanger *A. senegal*, the producer of gum Arabic. These real and potential threats have triggered interest in the Acacia bagworm and elevated it to the status of a national issue that requires intensive investigations (Mahmoud, 2005).

Efficient control of bagworms is a matter of timing. Spraying should be applied while the bagworms are small, because late sprays are much less effective (Horre and Sons, 2002). The larger the worms, the harder they are to kill with chemicals. Also, Hamman (2002) reported that the best time to apply insecticides for bagworm control is soon after eggs have been hatched, or while the larvae are small and actively feeding. The control becomes difficult and not effective as the larvae mature and close their bags. Davidson and Lyon (1979) stated that pesticides are more effective, if applied soon after eggs hatch. One should select the pesticide that does least damage to the natural enemies.

Peak (1956) reported that the bagworms attacking *Cypress* trees in South Africa, are controlled successfully by applying Cryolite® at the rate of 20-30 (a.i) lb/acre (*.i.e* 8.88 – 13.33 kg/acre), but later it was found that it was better to apply 10% toxaphene- Diesel oil, as an atomized spray, at the rate of three to four gallons/acre. Dennis (1983) reported that trichlorphen and DDT insecticides have both been used successfully in the past against oil palm bagworms.

The environmental friendly insecticides, like the chitin synthesis inhibitor diflubenzuron, were found to be ideal for forest pest management, when used against the chir pine bagworm, *Eumeta crameri* (Waker) (David *et al.,* 1992). Klass (1986) stated that control of bagworms can be obtained by using some insecticides, i.e. carbaryl (Sevin®), chlorpyrifos (Dursban®), trichlorfen (Dylox®), diazinon or acephate and orthen. The insecticides, which are also recommended for bagworm control, included malathion, bendiocarb (Dycarb®, Ficam®), permethrin (Astor®) and trichlorfen (Dylox®, Proxol®)(Klass, 1986). Adam and Elamin (2006) also recommended Tracer 240SC at 0.188 %, Sumigold 20% at 0.56% and D-C-Tron plus at 1.25% product (v/v) for bagworm control.

Conventional insecticides are among the most popular chemical control agents because they are readily available, rapid acting, and highly reliable. A single application may control several different pest species and usually forms a persistent residue that continues to kill insects for hours or even days after application. Because of their convenience and effectiveness, insecticides quickly became standard practice for pest control during the 1960's and 1970's. Overuse, misuse, and abuse of these chemicals have led to widespread criticism of chemical control and, in a few cases, resulted in long-term environmental consequences. Resistance arises through the over-use or mis-use of an insecticide against a pest species and results in the selection of resistant forms of the pest and the consequent evolution of populations that are resistant to that insecticide.

In Sudan, the bagworms are only exposed to chemicals during the campaign of tree locust control; usually the control is done by spraying the acacia trees, particularly the *Acacia Senegal*, to protect the trees. The campaign is done annually for the production of the gum (gum Arabic) which contributes to the economy of the country. The continuous exposure of the bagworm causes the resurgence of the bagworm as the future pest of the *Acacia senegal* .

The scope of the study is to determine the susceptibility of the three insecticides (spinosad, chlorpyrifos and cypermethrin) from different groups of chemicals with different mode of action through the lethal doses (viz. LD_{50} and LD_{90}), the slopes of the log-dose probability (Ld-p) lines, and the LD_{90}/LD_{50} ratios (RR' ratio) for each chemical using two route of administration.

Materials and Methods

Insects

The fourth instar larvae (L4) of *A. kordofensis* were collected from Elobeid district, found associated with *Acacia* spp. especially in *A. nubica*. These larvae were kept in cages and brought to Wad Medni, Biology Laboratory, Faculty of Agricultural Sciences (FAS), U. of G.

The larvae were kept under laboratory conditions and supplied with green leaves of *A. nubica*, as feed. This population was used for the bioassay as L4.

Insecticides tested

Three insecticides from different chemical groups were used:

1- Spinosad (Naturalytes), as Tracer® 240 SC.

2- Chlorpyrifos (Organophosphate), as Dursban® 48 EC.

3- cypermethrin (Pyrethroid), as Devicyprin® 25 EC .

The 4^{th} instar larvae were subjected to insecticide treatments. Exploratory studies were performed for each insecticide to determine the suitable range of concentrations using the topical application (thoracic segments) and the dipping method.

For each insecticide, seven concentrations (5, 10, 20, 30, 40, 50, and 60 ppm) were tested to obtain the regression line. Each concentration was replicated five times (5 larvae). The test was repeated three times for reproducibility.

The larvae were distributed in glass Petri - dishes (9 cm in dia.) with one insect per dish. The dishes were arranged in CRD with 3 replicates of the 4 treatments, and the control dish was treated with absolute water.

Routes of exposure tested

Dipping method

Each larva was picked by forceps and immersed into the freshly prepared solution of the specified/designated concentration for ten seconds only, transferred to the designated Petri-dish and provided with *A. nubica* leaves. After 24 hours, the percent mortality was calculated taking into the consideration the natural mortality (control).

Topical application method

Each larva treated using a micropipette by one of the concentrations (10 µl) in third thoracic segment. The rest of the steps were as above.

Data collection and statistical analysis

The experiment data and observations were recorded after 24 hours for each treatment and each route of administration. The mortality was corrected by Abbott's formula (Abbott, 1925 and Finney, 1952). Results were analyzed by probit analysis to determine the critical lethal dose, which kill 50% and 90% of the treated population, *i.e.* LD_{50} and LD_{90}. Whenever the

control mortality exceeded 20%, the test was repeated (Change, *et. al.*, 1980 and Busvine, 1980).

Data was presented in tables and figures.

Results and Discussion

The bioassay results of the field-collected L4 population of *A. kordofensis* using spinosad, chlorpyrifos and cypermethrin with seven concentrations and comparing two different bioassay (routes of administration) methods are presented below. The results will discussed by comparing the methods, the LD_{50s}, the LD_{90s} and the slopes of the Ld-p lines.

Table 1. Acute toxicity results for spinosad using the 4th larval instar of the *A. kordofensis* adopting topical application and dipping methods.

Treatments	LD_{50} ppm	LD_{90} ppm	Slope	RR'
Dipping	8.87 ± 1.38	48.73 ± 8.56	1.71	5.49
Topical	17.37 ± 2.97	34.67.2 ± 1.00	4.40	1.91

Dipping: LC_{50} = 18.82 LC_{90} = 102.33

Topical: LC_{50} = 33.88 LC_{90} = 67.61

RR'=LD_{90}/LD_{50}; L4 average weight = 2.1mg.

Spinosad

Dipping treatment

The results are illustrated in Table 1. Figure 1 shows that when the larvae were dipped in the tested spinosad suspension concentrations (*i.e.* 5, 10, 20, 30, 40, 50 and 60 ppm). None of the tested concentrations gave 100% mortality. The probit analysis indicated that the LD_{50} and the LD_{90} were 8.87 and 48.73 ppm, respectively, and hence the LD_{90}/LD_{50} (*i.e.* RR') is 5.49 (Table 1; Figure 1). The most surprising finding suggested that spinosad could be used to suppress bagworm using reasonable concentrations within 24 hours. The population was shown to be relatively heterogeneous to this insecticide by dipping method (slope =1.71; Figure 1), when compared to the other two tested insecticides and the topical application method. The difference between the LD_{90} and LD_{50} values was large, and hence the RR' ratio was high (RR'= 5.49). These results might suggest that the doses required for field application, which are supposed or expected to inflict excellent control, must be much higher than one would expect when comparing the LD_{50}s of comparable or recommended insecticides. This emphasizes again that the LD_{50} of any toxicant, including insecticides, might be misleading. One must consider the LD_{90} to complete the picture. However, the 24 hour results revealed that spinosad can be considered as one of the promising chemical control agent for this pest.

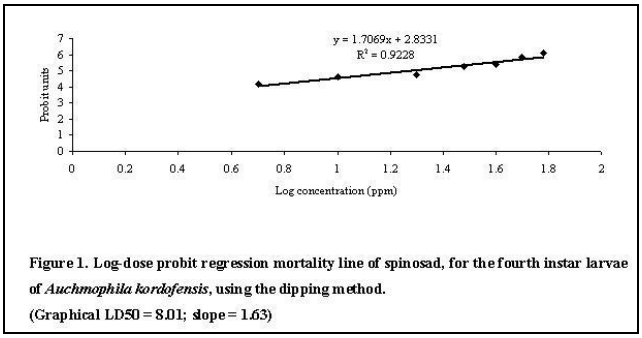

Figure 1. Log-dose probit regression mortality line of spinosad, for the fourth instar larvae of *Auchmophila kordofensis*, using the dipping method. (Graphical LD50 = 8.01; slope = 1.63)

Topical application

The results of topical treatment of the same above-mentioned concentrations using the same instar showed in Table 1 and Figure 2. Topical treatment by spinosad, showed that a higher dose (almost double) was required for an LD_{50} (17.37 ppm), compared to the LD_{50} of 8.87 ppm required by dipping treatment. On the contrary, lower dose was required for an LD_{90} (34.67ppm) compared to LD_{90} (48.73 ppm) recorded for the dipping treatment (Table 1; Figures 1 and 2). Spinosad has been shown to cause rapid feeding cessation in lepidopterous insects. The slope of the Ld-P line for the method was 4.4. This shows that the population is more homogeneous to topical treatment than to the dipping method (slope =1.71). The difference between the LD_{90} and LD_{50} values was smaller than that when the chemical was tested by the dipping method. This was reflected on the LD_{90}/LD_{50} ratio (RR' = 1.91), compared to 5.49.

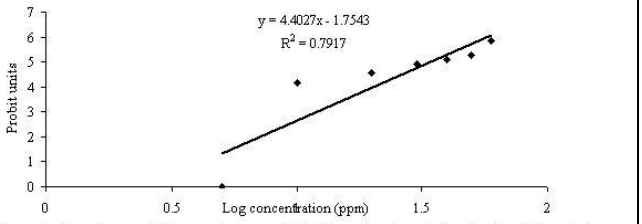

Figure 2. Log-dose probit regression mortality line of spinosad, for the fourth instar larvae of *Auchmophila kordofensis*, using the topical application method. (Graphical LD50 = 17.44; slope = 4.0).

Comparing efficiency of the two bioassay methods

The susceptibility of the L4 of the bagworm varied significantly in the two application methods. The findings indicated that the dipping method is significantly better than the topical methods (the LD_{50} 8.87 and 17.37 ppm, respectively), but the LD_{90} value in topical method was smaller than that of the dipping methods (LD_{90}= 48.73 ppm and 34.67 ppm, respectively; Table 1 and Figure 3) indicated that C.V.% was high (17.56), which means that the population was more homogeneous to the topical method.

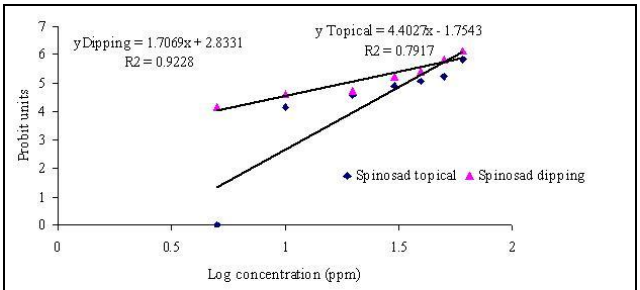

Figure 3. Log-dose probit regression mortality line of spinosad for both the dipping and topical application methods using the fourth instar larvae of *Auchmophila kordofensis* .

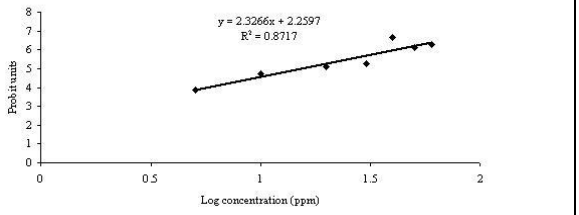

Figure 4. Log-dose probit regression mortality line of chlorpyrifos for the dipping method using the fourth instar larvae of *Auchmophila kordofensis* .
(Graphical LD50 = 7.57; slope = 2.24).

Adam and Elamin (2006) used Tracer 240Sc (at 0.188%,0.25%and 0.313%) and the effect on larval population of the Aacacia bagworm using the first post –spray count indicated that all concentrations of tracer dropped the population of the acacia bagworm to the zero level seven days after application (i.e. all concentration s of tracer achieved 100% mortality). Only one spray was needed from tracer for the whole season to keep the populations of Acacia bagworm between 0.0 and less than 1.0 larvae per twig. Spraying Tracer at 0.188% on pre-spray counts of 5.7 drop post-spray counts to 0.9 at 3 days, 0.0 at 7 days and 0.7 at 14 days. Spraying Tracer at 0.25% on pre-spray counts of 5.6 drop post-spray counts to 0.1 at 3 days, 0.0 at 7 days and 0.5 at 14 days. Finally, Spraying Tracer at 0.313% on pre-spray counts of 4.9 drop post-spray counts to 0.1 at 3 days, 0.0 at 7 days and 0.0 at 14 days.

Table 2. Acute toxicity results for chlorpyrifos using the 4th larval instar of the *A. kordofensis* adopting the topical application and dipping methods.

Treatments	LD_{50}	LD_{90}	Slope	RR'
Dipping	7.38 ± 0.85	25.57 ± 0.55	2.32	3.46
Topical	15.13 ± 3.62	28.84 ± 0.39	4.53	1.91
	Dipping: LC_{50} = 15.49		LC_{90} = 53.70	
	Topical: LC_{50} =29.51		LC_{90} = 56.23	

Chlorpyrifos

Dipping treatment

Results of the susceptibility are summarized in Table 2 and Figure 4 The results presented showed that the L4 of field populations of *A. kordofensis*, dipped in chlorpyrifos at the test concentrations resulted in a LD_{50} value of 7.38 ppm, and LD_{90} value of 25.57 ppm (RR' = 3.46; Table 2). The population was shown to be heterogeneous to this insecticide (slope=2.32).The LD_{90} values for field populations ranged from 25.02 to 26.12 ppm. These results indicate that the population was heterogeneous in their susceptibility to chlorpyrifos using the dipping treatment (Table 2; Figure 4). These differences were highly significant, no mortality was observed among the larvae in the controls.

Topical application

Topical treatment of the test concentrations specified showed that the LD_{50} value was 15.13ppm compared to 7.38ppm for the dipping treatment, whereas the LD_{90} was 28.84ppm compared to 25.57ppm dipping treatment and the slope was (4.53) for the topical treatment (Table 2, Figure 5). These results indicate that the population was homogeneous in their susceptibility to chlorpyrifos using topical treatment (Figure 5). The difference between the LD_{50} and LD_{90} values (RR') was 1.91. These results reflect that the chlorpyrifos exerted its effect by contact action but needs complete coverage.

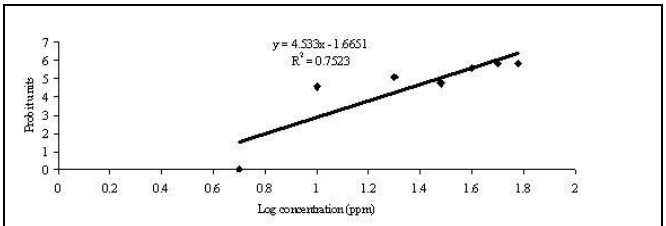

Figure 5. Log-dose probit regression mortality line of chlorpyrifos, for the fourth instar larvae of *Auchmophila kordofensis*, using the topical method.
(Graphical LD50 = 15.38; slope = 4.46).

Comparing efficiency of the two bioassays methods

The susceptibility of bagworm varied significantly in the two adopted methods of application. The findings indicated that dipping method is significantly more effective than the topical methods. The LD_{50} value (7.38ppm) of the dipping was less than the LD_{50} (15.13ppm) of the topical treatment. However, the LD_{90} (28.84ppm) of the topical was slightly higher than the LD_{90} (25.57ppm) of the dipping method. The results indicated that the population was more homogeneous to topical treatment than to the dipping treatments (Table 2 and Figure 6).

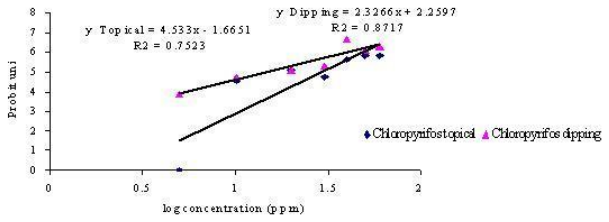

Figure 6. Log-dose probit regression mortality line of chlorpyrifos, for both the dipping and topical application methods, using the fourth instar larvae of on *Auchmophila kordofensis*.

Cypermethrin

Table 3. Acute toxicity results for cypermethrin using the 4th larval instar of the *A. kordofensis* adopting topical application and dipping methods.

Treatment	LD_{50}	LD_{90}	Slope	RR'
Dipping	7.04 ± 1.25	34.50 ± 4.88	1.85	4.90
Topical	15.45 ± 2.68	28.18 ± 1.66	5.02	1.82
	Dipping: LC_{50} = 14.79,		LC_{90} = 72.44	
	Topical: LC_{50} = 30.12,		LC_{90} = 54.995	

Dipping treatment

The results presented in Table 3 and Figure 7 from when the larvae were dipped in the cypermethrin tested concentrations showed that the LD_{50} was 7.04ppm, whereas the LD_{90} was 34.5ppm (RR' = 4.90; Table 3). The population was also homogeneous to this insecticide (slope=1.85; Table 3; Figure 7).The difference between the LD_{90} and LD_{50} values was large and was reflected on the RR' value which was calculated as 4.90.

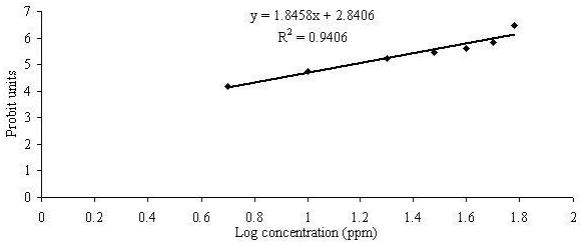

Figure 7. Log-dose probit regression mortality line of cypermethrin, for fourth instar larvae of on *Auchmophila kordofensis*, using the dipping method.
(Graphical LD50 = 7.14; slope = 1.83)

Topical application

Topical treatment by cypermethrin, showed that the LD_{50} was 15.45ppm, compared to 7.04ppm for the dipping method. However, the LD_{90} was 28.18ppm for the topical application compared to 34.5ppm for the dipping counter part (Table 3). The slope was high (5.02) indicating that this population is strongly homogeneous to this insecticide (Figure 7). The difference between the LD_{90} and LD_{50} values was large, and was reflected on the RR' value of 1.82.

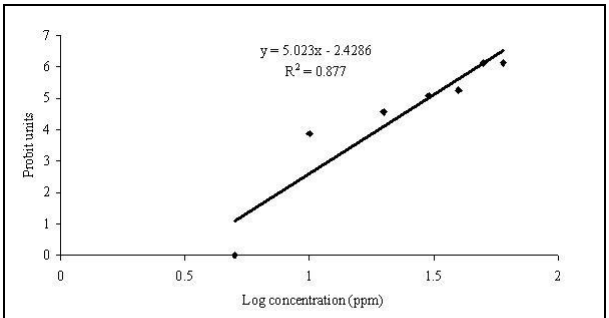

Figure 8. Log-dose probit regression mortality line of cypermethrin for the topical method using the fourth instar larvae on *Auchmophila kordofensis* .
(Graphical LD50 = 15.81; slope = 5.4).

Comparing efficiency of the two bioassays techniques:

The susceptibility of bagworm varied significantly between the two adopted methods (dipping and topical). The findings indicated that the dipping method is significantly better than the topical methods in term of LD_{50} (LD_{50} 7.07 and 15.45ppm, respectively), although the population was more homogeneous to cypermethrin when applied topically.

When comparing the two bioassay methods in terms of the LD_{90} for cypermethrin, the obtained values were 34.50 and 28.18ppm, respectively (Table 3) indicating that C.V.% was high (14.14), When the larvae were treated topically with the cypermethrin series of concentrations, the results indicated that the dose range is narrow. The RR' value was 1.82, and the LD-p line slope was large (5.02), compared to the dipping method in which the RR' was large (4.90), and the Ld-p line slope was much smaller (1.85; Table 3; Figure 9). These results indicated that, in this field population, the bagworm was homogeneously responding to cypermethrin and exhibited a high level of homogeneity to the topical application.

Peak (1956) reported that the bagworms attacking *Cypress* trees in South Africa are controlled successfully by applying Cryolite product (v/v) at the rate of 20-30 (a.i) lb/acre (*.i.e* 8.88 – 13.33 kg/acre), but later it was found that it was better to apply 10% toxaphene- Diesel oil, as an atomized spray, at the rate of three to four gallons/acre.

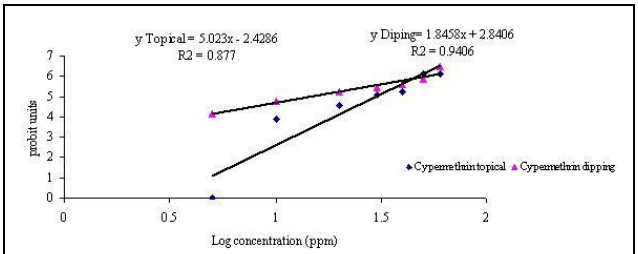

Figure 9. Log-dose probit regression mortality line of cypermethrin for both the dipping and topical application methods using the fourth instar larvae on *Auchmophila kordofensis* .

Comparing the insecticides and The Routes of administration

In the present investigation three insecticides belonging to different classes with different modes of action (*viz*. spinosad, chlorpyrifos, and cypermethrin) were tested against the L4 of the bagworm. The results showed that the LD_{50}s varied between the tested insecticides and among the adopted bioassay methods. The results also indicated that these larvae are very susceptible to all tested insecticides measured in terms of the LD_{50}s and LD_{90}s. The calculated LD_{50} of the tested insecticides by using the dipping method ranged from 8.87 to 7.04ppm, whereas that of the graphical ranged from 8.01 to 7.14 (Table 4). The differences were not significant.

Table 4. Acute Toxicity parameters for the three bioassayed insecticides (chlorpyrifos, cypermethrin and spinosad) to the 4th larval instar of the *A. kordofensis* when adopting topical application and dipping methods.

Treatments	Methods	LD_{90}/LD_{50} (RR)[3]	LD_{50}(±S.E.)(ppm)		LD_{90}(±S.E.)(ppm)		Slope		C.V.% LD_{50}	C.V.% LD_{90}
			Cal.	Graph.	Cal.	Graph.	Cal.	Graph.		
1. Spinosad	Dipping	5.49	8.87±1.38	8.01	48.73± 8.5	48.01	1.71	1.63	15.56	17.56
	Topical	1.91	17.37± 2.97	17.44	34.67± 1.00	34.87	4.40	4.0	17.91	2.88
2. Chlorpyrifos	Dipping	3.46	7.38 ± 0.85	7.57	25.57±0.55	25.71	2.32	2.24	11.52	2.15
	Topical	1.91	15.13± 3.62	15.38	28.84 ± 0.39	28.72	4.53	4.46	23.93	1.35
3.Cypermethrin	Dipping	4.90	7.04 ±1.25	7.14	34.50 ±4.88	34.76	1.85	1.83	17.76	14.14
	Topical	1.82	15.45±2.68	15.81	28.18,±1.66	28.21	5.02	5.40	17.35	5.89

The slope of the Ld-P lines obtained by all insecticides tested using the dipping treatment was low and the range was from 1.72 to 2.32. The population was more homogeneous towards chlorpyrifos, followed by cypermethrin, then spinosad (1.72; Table 4). Generally, the population responded homogeneously to all tested chemicals. This is logical since this pest was not previously exposed the test chemicals.

When these insecticides were applied topically, this population proved to be even more homogeneous to the treatments, and exihibited Ld-P line slopes as follows: 4.40 (spinosad), 4.53 (chlorpyrifos) and 5.02 (cypermethrin; Fig 10). This indicated that the pest was more homogeneous to cypermethrin treatments than the other two. However, the overall picture is that the pest is still susceptible and homogeneous to the tested chemicals and treatments, which are good indicators that might assist in establishing IPM programs for such a pest.

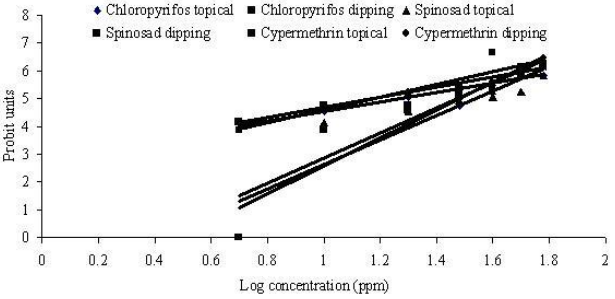

Figure 10. Log-dose probit regression mortality lines for chloropyrifos, spinosad, cypermethrin on *Auchmophila kordofensis* larvae showing the results of both routes of administration.

When the insecticides were applied topically, the LD_{50} values were 17.37ppm (spinosad), 15.13ppm (chlorpyrifos) and 15.45ppm (cypermethrin; Table 3). Again, the values did not seem to be significantly different between insecticides. However, when comparing the differences between the LD_{50} values regarding the route of administration, it is quite obvious that the dipping method was more effective, and that the dose almost doubled when the insecticide was tested topically.

The LD_{90} results for spinosad, regarding the dipping route of administration, was 48.73ppm, whereas that of the topical application was less (34.67ppm). This is contradicting the LD_{50} results of the same insecticide where the topical dose was almost double that of the dipping route of administration. The LD_{90} for chlorpyrifos was 25.57ppm for the dipping and 28.84ppm for the topical application. The picture in the LD_{50} values was as in spinosad (doubling). However, cypermethrin behavior was closer to that of spinosad; the dipping (34.5ppm) LD_{90} was higher than that of the topical application (28.1ppm). Such results can be explained as follows: the doses that are required to kill 50% of the treated population, using any of the tested chemicals, can be easily obtained from either route of administration (penetrate or distribute), reach to the site of action in a suitable dose and time before being metabolized. However, the dose that kills 90% must be higher and needs more time within the same 24 hours to penetrate, be distributed, be metabolized (degraded or activated), and attack the target (site of action). What is to be said here is that the LD_{50} must be accompanied by the LD_{90} to give the full toxicologic story of a chemical on a specific pest or pest stage. Any of these insecticides can be successfully used in controlling this pest. However, one must consider the full toxicologic information/profile, especially the resistance development, the effect on natural enemies, persistence, price, *etc*.

Results of the present study suggest that there is an urgent need to initiate country-wide programs to study the biology, ecology, life tables, physiology and behavior of the bagworms before becoming a serious

problem to the forests, especially the gum Arabic plantations, and their control. It is also important to assess pest status of various bagworm species in the Sudan.

Conclusion and Recommendations

The results of the study showed the following:

1. Cypermethrin was the most effective of the three insecticides (LD_{50} 7.04ppm & LD_{90} 34.76ppm in dipping method, and LD_{50} 15.45ppm & LD_{90} 28.18ppm in the topical method) in laboratory tests against this instar of the bagworm. The slopes ranged from 1.85 to 5.02 in the dipping and topical method respectively, compared to topical method where the population was more homogenous (Slope = 5.02).
2. Chlorpyrifos was highly effective with dipping method. The population was more homogeneous to this insecticide than to spinosad (slope 2.32). It was even more homogeneous when topically applied (slope was 4.53).
3. Spinosad showed a good performance against the fourth instar larvae; the population was heterogeneous responding to it with an Ld-P line slope of 1.71 by the dipping method and 4.40 for the topical application method.
4. Further elaborated and detailed field and laboratory investigations about this pest are urgently needed for more understanding (*i.e*. biological, ecological, physiological, biochemical, and behavioral aspects). Such information would help in the preparation and implementation of effective control strategies.

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Kowkab F.E. Babiker[1], Yousif O. H. Assad[2] and Nabil H. H. Bashir[2]

1 Chememport company, P.O.Box 825 Khartoum, Sudan. Email:kowkab75@live.com Fax:+249183770139

2 Department of Pesticides and Toxicology, Faculty of Agricultural Sciences, University of Gezira, P.O.Box 20, Wad Medani, Sudan. Email: bashirnabil@hotmail.com and yousifassad@maktoob.com

ACUTE TOXICITY OF ABAMECTIN TO EARTHWORMS, *EUDRILUS EUGENIAE* (KINBERG) AND *PERIONYX EXCAVATUS* (PERRIER)

ABSTRACT

Earthworms, 'intestines of the earth,' are very important in breaking down organic matter. Laboratory studies conducted to determine the acute toxicity of abamectin, a broad spectrum pesticide to earthworms, *Eudrilus eugeniae* (Kinberg) and *Perionyx excavatus* (Perrier) revealed that the earthworms are highly susceptible to abamectin. The LC_{50} values of abamectin to *E. eugeniae* by artificial soil test bioassay method were 33.2, 17.4 and 4.6 mg kg^{-1} of soil at 2, 7 and 14 days, respectively, while that of *P. excavatus* were 37.1, 21.1 and 6.7mg kg^{-1} of soil, respectively. The LC_{50} values of abamectin to *E. eugeniae* at 2, 7 and 14 days by dung test bioassay method were 177.1, 53.3, 35.9 mg kg^{-1} of dung, respectively, whereas to *P. excavatus* the values were 172.6, 47.2 and 30.5 mg kg^{-1} of dung, respectively. The median lethal time (LT_{50}) was 14.8, 11.2, 6.8 and 3.1 days for abamectin at 3.8, 9.5, 19.0 and 28.5 ppm to *E. eugeniae* in soil and 16.1, 9.0, 6.7 and 2.7 days for abamectin at 19.0, 38.0, 76.0 and 114.0 ppm to *E. eugeniae* in dung, respectively. Likewise for *P. excavatus*, the median lethal time (LT_{50}) was 17.3, 10.1 and 5.0 days for abamectin at 9.5, 19.0 and 28.5ppm in soil and 19.8, 11.5, 5.7 and 2.7 days for abamectin at 19.0, 38.0, 76.0 and 114.0ppm in dung, respectively.

INTRODUCTION

The break down of organic matter in soil and the recycling of nutrients that it contains are key processes in maintaining soil fertility. Earthworms, 'intestines of the earth,' are most important in breaking down organic matter, incorporating it into soil and releasing the mineral nutrients that it contains. Indiscriminate and scrupulous use of broad spectrum synthetic chemicals results in the destruction of non-target organisms where they are deposited or get transported. Most of the synthetic insecticides have a long life period, which has resulted in the process of bioaccumulation and biomagnification in the environment and in living organisms (Sahai, 1992). The wide spread usage of synthetic insecticides ultimately pollute the lithosphere environment thereby affecting the soil fauna (mainly earthworms) which are known to improve the quality of the soil continuously, often referred as 'farmers friends'. It is observed that the population, development, and cocoon production would be adversely affected if the soil where their development takes place were contaminated even at sublethal concentrations. Knowledge of the effects of pesticides on these earthworms is important to avoid unnecessary loss of fertility. In this context, the present study was taken up to determine the acute toxicity of abamectin to *Eudrilus eugeniae* (Kinberg) and *Perionyx excavatus* (Perrier)

MATERIALS AND METHODS

Mass multiplication of earthworms

The nucleus culture of *E. eugeniae* was obtained from Regional Research Station Paiyur. Earthworms were mass multiplied in buckets of 20 L capacity. Soil (~ 20 kg) collected at TNAU Farm, cleaned for pebbles, plant debris and other extraneous materials, was amended with 10 percent decomposed farmyard manure. The buckets were maintained in a cool and dark place and moisture was maintained at 40 percent level by periodic sprinkling of water. After six months, medium sized earthworms with well-developed clitellum were selected for the bioassays.

The nucleus culture of *P. excavatus* was obtained from the Department of Environmental Science, Tamil Nadu Agricultural University, Coimbatore. Earthworms were mass multiplied in loose compost heaps with a high nitrogen level. A mixture of dung material (cow, sheep and horse) with kitchen wastes was used as feed material for *Perionyx*. Propagation was done under shady places with a sufficient level of moisture. After six months, medium sized earthworms with well developed clitellum were selected for the bioassays.

Experimental conditions

The effect of abamectin on earthworm, *E. eugeniae*, population was tested by following the artificial soil test method proposed by Biollogische Bundesanstalt fur Land-und Forst Wirsts chaft, Braunschweig (BBA) and modified by Ganeshkumar (2000).

The test substrate was prepared by mixing fine quartz sand 83.5 percent (particle size between 0.06 and 0.2mm), bentonite 5 percent, finely ground and dried sphagnum peat 10 percent (pH 2.6+0.5), pulverized calcium carbonate (1%) and ground dried cow dung (0.5%). The pH was adjusted to 7±0.5 and sufficient water was added to bring moisture content to 40 percent of the dry weight of the substrate. The complete mixture was moist enough, but not so wet that water appeared when the artificial soil was compressed.

After range finding tests, one kg of conditioned soil in tubular pots (18x6 cm) was treated with abamectin 1.9 EC at 0.2, 0.5, 1.0, 1.5 and 2.0 ml for *E. eugeniae* and *P. excavatus*. Fifteen earthworms washed in water were placed on the top of the substrate. The tubular pots were covered with perforated polythene cover to prevent the worms from crawling out and to avoid evaporation. The set up was kept under shade. After 7 days, 5 g of finely ground dried cow dung was mixed inside the container and water lost by evaporation was replaced. The number of live earthworms was counted based on mechanical stimulus, and earthworms were considered dead if they did not respond to a gentle mechanical stimulus (Edwards and Bohlen, 1992). Sufficient replicates were used to construct a reliable regression line known as log concentration probit mortality (lcpm)

line/ curve. The mortality data obtained was corrected for the mortality in the control by Abbott's formula (Abbott, 1925) and the data was subjected to probit analysis (Finney, 1971) and confirmed by EPA probit analysis program used for calculating LC/EC values Version 1.5.

Acute toxicity of abamectin to earth worms in dung

Five hundred grams of conditioned dung in tubular pots (18x 6 cm) was treated with abamectin 1.9 EC of 0.5, 1.0, 2.0 and 3.0 ml for *E. eugeniae* and *P. excavatus*. After that the procedure was followed as per the method dealt for soil.

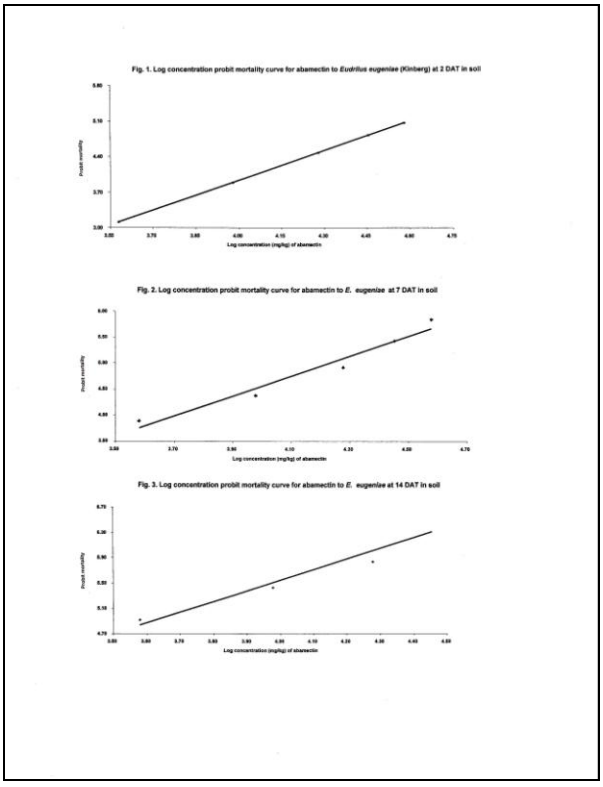
RESULTS AND DISCUSSION

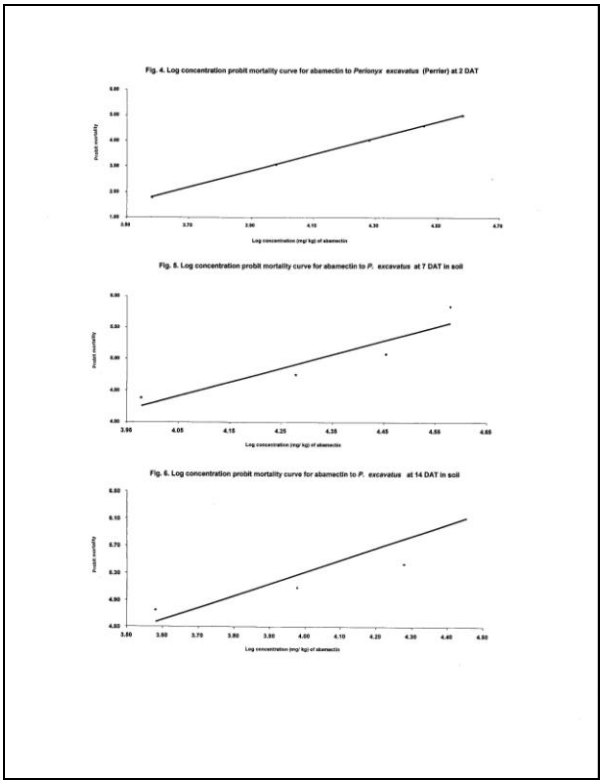
The LC_{50} values of abamectin to *E. eugeniae* by the artificial soil test bioassay method were 33.2, 17.4 and 4.6 mg kg^{-1} of soil at 2, 7 and 14 days, respectively, while that of *P. excavatus* were 37.1, 21.1 and 6.7mg kg^{-1} of soil, respectively. In the dung test bioassay method, the LC_{50} values of abamectin to *E. eugeniae* at 2, 7 and 14 days were 177.1, 53.3, 35.9 mg kg^{-1} of dung, respectively, to *P. excavatus* the values were 172.6, 47.2 and 30.5 mg kg^{-1} of dung, respectively (Table 1). (Figures 1-12).

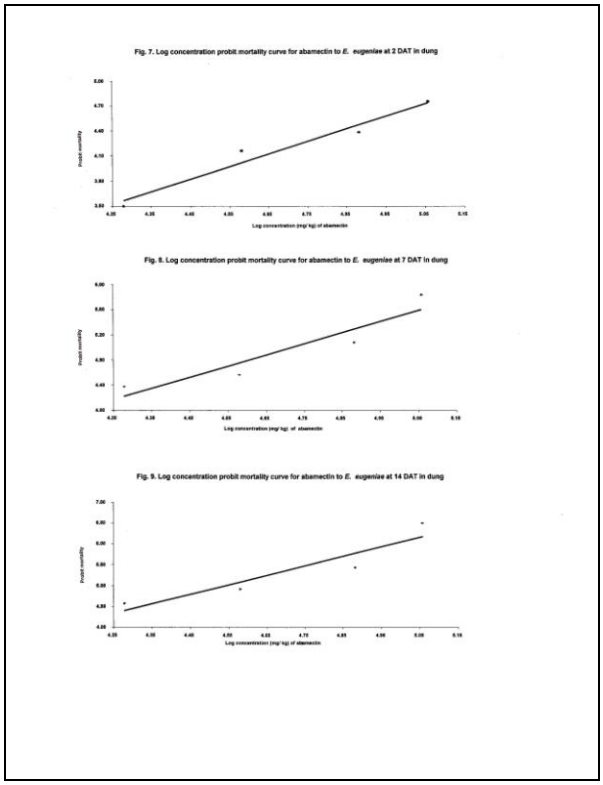
Table 1. Concentration - mortality response of abamectin 1.9 EC to earth worms

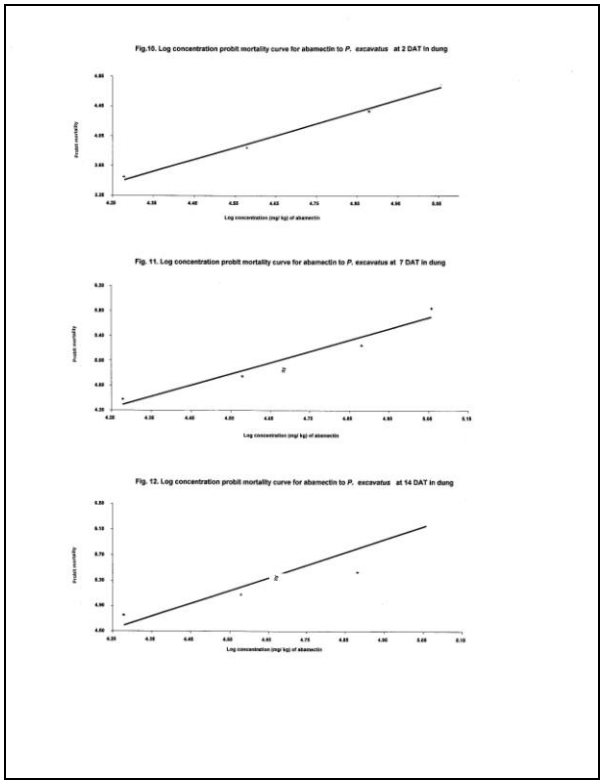
Organisms	Test Matrix	Days	LC_{50} (mg/kg)	95 per cent fiducial limit		Regression equation	χ^2 at P = 0.05
				LL	UL		
E. eugeniae	Soil	2	33.2	25.4	58.9	Y = 0.332 + 3.069 ±0.904 x	0.290 ^{NS}
		7	17.4	11.6	26.7	Y = 2.580 + 1.952 ±0.489 x	0.774 ^{NS}
		14	4.6	0.7	7.9	Y = 3.932 + 1.602 ±0.552 x	0.436 ^{NS}
	Dung	2	177.1	89.3	141802.3	Y = 1.736 + 1.452 ± 0.675 x	0.232 ^{NS}
		7	53.3	30.7	97.4	Y = 1.947 + 1.768 ±0.592 x	1.321 ^{NS}
		14	35.9	18.9	52.7	Y = 1.744 + 2.094 ±0.611 x	1.735 ^{NS}
P. excavatus	Soil	2	37.1	30.0	70.8	Y = -1.814+ 4.340 ± 1.477 x	0.364 ^{NS}
		7	21.1	12.8	33.8	Y = 2.103+ 2.186 ± 0.776 x	1.337 ^{NS}
		14	6.7	1.9	11.1	Y = 3.597 + 1.574 ± 0.531 x	2.137 ^{NS}
	Dung	2	172.6	92.1	85449.4	Y =1.358 + 1.628 ± 0.710 x	0.054 ^{NS}
		7	47.2	25.8	79.3	Y = 1.985+ 1.801 ± 0.591 x	0.431 ^{NS}
		14	30.5	10.8	47.0	Y = 2.310 + 1.813 ± 0.604 x	1.810 ^{NS}

number of worms used (15) NS = Not significant









The median lethal time (LT_{50}) was 14.8, 11.2, 6.8 and 3.1 days for abamectin at 3.8, 9.5, 19.0 and 28.5 ppm to *E. eugeniae* in soil and 16.1, 9.0, 6.7 and 2.7 days for abamectin at 19.0, 38.0, 76.0 and 114.0 ppm to *E. eugeniae* in dung, respectively. Likewise for *P. excavatus*, the median lethal time (LT_{50}) was 17.3, 10.1 and 5.0 days for abamectin at 9.5, 19.0 and 28.5 ppm in soil and 19.8, 11.5, 5.7 and 2.7 days for abamectin at 19.0, 38.0, 76.0 and 114.0 ppm in dung, respectively (Table 2).

Table 2. Time - mortality response of abamectin 1.9 EC to earthworms

Organisms	Test Material	Concentration (ppm)	LT_{50} (days)	95 per cent fiducial limit		Regression equation	χ^2 at P = 0.05
				LL	UL		
E. eugeniae	soil	3.8	14.8	11.3	123.4	Y = 0.855 + 3.544± 1.537X	0.029 ^{NS}
		9.5	11.2	7.8	24.2	Y = 2.628 +2.259 ± 0.737X	0.952 ^{NS}
		19.0	6.8	3.7	11.7	Y = 3.532 +1.761 ±0.574 X	1.200 ^{NS}
		28.5	3.1	0.9	4.9	Y = 4.135 + 1.774 ±0.555 X	0.895 ^{NS}
	dung	19.0	16.1	9.5	914.8	Y = 2.948 + 1.701± 0.730 X	0.130 ^{NS}
		38.0*	9.0	-	-	Y = 3.871 + 0.883 ±0.557 X	0.035 ^{NS}
		76.0	6.7	1.5	26.0	Y = 4.027 +1.174 ±0.534 X	0.202 ^{NS}
		114.0	2.7	0.9	4.2	Y = 4.134 + 2.021 ±0.580 X	0.022 ^{NS}
P. excavatus	soil	9.5	17.3	12.2	164.5	Y = 1.877 + 2.522 ± 1.019X	0.429 ^{NS}
		19.0	10.1	6.2	30.5	Y = 3.331 +1.658 ± 0.606 X	0.923 ^{NS}
		28.5	5.0	2.5	7.7	Y = 3.667 +1.898 ± 0.564 X	3.119 ^{NS}
	dung	19.0	19.8	10.8	8072.6	Y = 3.089 + 1.472 ± 0.676 X	0.029 ^{NS}
		38.0	11.5	6.6	105.9	Y = 3.492 +1.421 ± 0.588 X	0.046 ^{NS}
		76.0	5.7	1.2	12.8	Y = 4.048 +1.255 ± 0.534 X	0.265 ^{NS}
		114.0	2.7	0.9	4.2	Y = 4.134 +2.021 ± 0.580 X	0.022 ^{NS}

number of worms used (15) NS = Not significant
*- Slope not significantly different from zero , hence LC fiducial limits cannot be computed

In the present study, it was concluded that abamectin was more toxic to earthworms in soil compared to dung. The growth of the worms was also inhibited when concentrations increased. These findings are in agreement with the report of Halley *et al*. (1993) that avermectins were unlikely to be toxic to earthworms with LC_{50} values of 315 ppm and 28 ppm in soil, for ivermectin and abamectin, respectively. According to Edwards *et al*. (2001), the earthworms were not affected by the ivermectin in the dung pats. However, earthworms treated with ivermectin ranging from 2, 4, 8, 12, 16, 20 and 60 mg kg^{-1} lost weight compared to the untreated earthworms.

A high concentration of the chemical in the dung (20 mg kg^{-1} dry weight soil) inhibited earthworms from entering or remaining in contaminated soil. Madsen *et al*. (1990) investigated the effects of ivermectin in dung from heifers treated with 200 mg/kg of the pesticide on eight species of lumbricid earthworms. Although there was some depression in different species from 1 to 20 days after treatment, the differences were not statistically significant.

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R. Sheeba Jasmine[1], S. Kuttalam[2] and J. Stanley[3]
1- *Regional Plant Quarantine Station, Chennai.*
2- *Department of Agricultural Entomology, TNAU, Coimbatore*
shepris2000@yahoo.com

Susceptibility of different instars of *Helicoverpa armigera* to *Bacillus thuringiensis* insecticidal proteins

ABSTRACT

A study on the susceptibility of different instars of *Helicoverpa armigera* (Hubner) to *Bacillus thuringiensis* (*Bt*) insecticidal proteins (ICP) was conducted. The LC_{50} of Cry 1Aa, Cry 1Ab, Cry 1Ac, Cry 1C and Cry 1E were 108.54, 97.04, 66.62, 1240.83 and 1335.60 ng/cm^2, respectively to *H. armigera* neonates. The order of toxicity of different ICPs to second and third instar was Cry 1Ac > Cry 1Ab > Cry 1Aa. Overlap 95 percent fiducial limits at LC_{50} values of Cry 1Aa and Cry 1Ab showed similar toxicity of these toxins to second and third instars of *Helicoverpa armigera*. The possibility of these toxins and toxicity parameters in resistance management is discussed.

Key words: *Bacillus thuringiensis, Helicoverpa armigera, toxicity*

INTRODUCTION

Helicoverpa armigera (Hubner) is a polyphagous pest of world wide occurrence damaging a number of agricultural crops particularly pulses, cotton, horticultural crops, some millets and many weeds (Singh and Bains, 1986; Saini and Mehla, 1991). Due to indiscriminate use of insecticides in various crops, the pest has developed manifold resistance to many insecticides (Bhatia, 1986; Dhingra *et al.*, 1988; Jayaraj, 1988). Besides others problems like environmental pollution, pesticide residues, and secondary pest outbreaks have also been encountered.

Bacillus thuringiensis Berliner (Bt) based biopesticides are now being increasingly used in India in many crops as one of the alternatives to chemical insecticides. Bt produces crystalline inclusions during sporulation composed of one or several proteins known as insecticidal crystal (Cry) proteins (ICPs) or delta-endotoxins. These toxins kill the insects by binding to and creating pores in the midgut membranes (Schnepf *et al*., 1998). The Cry proteins are classified into four main groups, of which Cry1 and Cry2 proteins are mainly active against caterpillars (Crickmore *et al*., 1998).

Bt toxins are non-toxic to most non-target organisms including arthropod natural enemies (Entwistle *et al*., 1993). Transgenic cotton, engineered to continuously express delta- endotoxin from the Bt gene, hold great promise for controlling the boll worm complex (Barwale *et al*., 2004). The transgenic cotton expressing Bt protein(s) could reduce the impact of chemical insecticides and create an ecologically sound environment without reducing crop production as a part of an IPM strategy (Lutterell and Herog, 1994). Determining the base line susceptibility of key pest species to different Bt toxins is essential for monitoring the changes in their susceptibility over a period of time. Several common insect pest species have been selected for resistance to *B. thuringiensis* in the laboratory indicating that biological pesticides could suffer the same fate as chemical pesticides (Tabashnik *et al*., 1990; Tabashnik, 1994). Hence studies on the susceptibility of *Helicoverpa armigera* to the Bt ICPs will be helpful in devising appropriate resistance management strategies for Bt transgenics.

MATERIAL AND METHODS

Insects

Helicoverpa armigera larvae were collected from the chickpea and cotton fields in the Hisar district of Haryana. The field collected population was reared in the laboratory on a semi synthetic diet (Gupta *et al*., 1999). They were reared in the laboratory for four generations to develop diet-adapted laboratory population for bioassays.

Bt proteins

The Bt proteins were Cry1Aa, Cry1Ab, Cry1Ac, Cry 1C and Cry 1E.The multiplication and isolation of proteins from *E. coli* strains was carried out in the laboratory of the Department of Biotechnology and Molecular Biology, CCS HAU, Hisar, Haryana.

Multiplication and Isolation of Bt proteins from *E. coli* cultures

E. coli was inoculated in 50 ml Luria broth (LB) medium with 50µl/ml ampicillin and incubated at 37°C and 150 rpm overnight. From the seed culture, 1% was inoculated to a fresh 400 ml LB medium which should become thicker in consistency along with opacity, when it is ready for protein isolation.

The procedure adopted for purification of the protein from the incubated medium is given below:

1. To the medium 400µl isopropyl thio glycol (IPTG) was added and incubated for 2 hours.

Then centrifugation was carried out at 5000 rpm at 4°C for 10 minutes.

2. The pellet was resuspended in 20ml of lysis buffer (50Mm Tris Ph 8.0, 50m MEDTA and 18% sucrose), and to this 1 mg/ml lysozyme was added and incubated for 2 hours with slow shaking. Then centrifugation was carried out a 500 rpm, 4°C for 10 minutes.
3. Then the pellet was resuspended in crystal wash I (0.5M NaCl, 2%Triton-X) along with protease inhibitor trypsin.
4. The sample was sonicated for 5 minutes (2 pulse). To this 10 ml crystal wash I was added and then centrifuged at 500 rpm, 4°C for 10 minutes.
5. The pellet was washed three times with crystal wash I and crystal wash II (0.5 M Na Cl), and finally with sterilized distilled water. Each time the supernatant was discarded.
6. The obtained pellet was resuspended in 10 ml stabilizing buffer and incubated at 37°C for 3-4 hours.
7. Then the sample was centrifuged at 10000 rpm, 4°C for 10 minutes. The supernatant was treated as crystal protein. The toxins were stored at – 20°C for bioassays.

Bioassay of ICPs against *H. armigera*

The bioassays of ICPs were conducted by surface diet contamination (Liao *et al*., 2002) using cryo vials. The concentrations of Cry 1Aa, Cry 1Ab, and Cry 1Ac used in the bioassays ranged from 50-300, 50-400 and 50-175 ng/cm^2, respectively. Similarly a control was also used. The experiments were conducted when the insect stock culture was in F_4-F_6 generation. All the components of the semi-synthetic diet, except formalin were used for making the bioassay diet. Approximately 1 ml of the diet was suspended into cryo vials (without touching the sides of the vial) and allowed to settle. After the diet solidified, the Cry proteins were layered on the diet surface at 10µl per vial using a sterile glass rod. The vials were allowed to dry for 30 minutes, and to each vial, a larva was released using a soft hairbrush. Each treatment was replicated four times with 10 larvae as an experimental unit. The larval mortality was recorded every 24 hours, consecutively for seven days. Moribund larvae were also considered dead when the observations were terminated. The experiments were carried out at 27±2°C, 70±5 percent RH and natural day and light periods. The mortality data were subjected to probit analysis (Finney, 1971) to calculate median lethal concentration (LC_{50}), LC_{95} and Fiducial limits. The probit analysis was carried out using the statistical package for social sciences (SPSS) version 10.0 SPSS Inc., USA.

RESULT AND DISCUSSION

The neonates were the most susceptible to Bt ICPs than other instars studied. Among the ICPs, Cry 1Ac was the most toxic against all instars of *H.armigera*.

Table1. Relative toxicity of *B. thuringiensis* insecticidal crystal proteins against *Helicoverpa armigera* neonates

Toxin	LC_{50} (ng/ cm^2)	95% fiducial limits		LC_{95} (ng/ cm^2)	95% fiducial limits		'b'(±SE)	Chi Square (n-2)
		Lower	Upper		Lower	Upper		
Cryl Aa	113.03	100.01	121.39	358.51	289.71	289.71	3.35±0.40	3.63
Cryl Ab	101.73	90.32	111.10	326.57	237.31	237.31	3.30±0.44	4.03
Cryl Ac	69.41	55.31	89.63	348.63	203.01	203.01	3.15±0.39	3.43
Cry 1C	1244.01	1157.03	1335.71	2863.02	238.03	2389.03	4.65±0.65	2.15
Cry 1E	1340.42	1339.71	1535.12	3128.71	2648.01	2648.01	4.60±0.57	2.67

*In each case, the Chi square value from the goodness of fit test was less than tabular value (p =0.05), indicating that the data fit the probit model

The LC_{50} and LC_{95} of Cry 1 Ac against *H. armigera* neonates were 69.41 and 348.63 ng / cm^2 , respectively (Table 1).The order of toxicity of different ICPs to *H. armigera* neonates was Cry 1 Ac > Cry 1Ab > Cry 1 Aa > Cry 1 Ac > Cry 1E (66.41>101.73<113.03>1244.01>1340.42 ng/cm^2) (Table 1). As the Cry 1C and Cry 1E were relatively less toxic to *H. armigera* neonates, they were not evaluated against the late instars. The overlapping fiducial limits at LC_{50} for Cry 1 Aa and Cry 1Ab revealed that they were almost similar in toxicity to *H. armigera* neonates. The order of toxicity against second instar larvae was Cry 1 Ac > Cry 1Aa > Cry 1 Ab (112.16>166.32>197.13 ng/cm^2) (Table 2), wheras the order of toxicity against third instar larvae was Cry 1 Ac > Cry 1Ab > Cry 1 Aa (174.07>202.61>218.32 ng / cm^2) (Table 3). Even though variation occurred in the toxicity of Cry 1 Aa and Cry 1Ab, the overlapping fiducial limits at LC_{50} indicated no significant difference in their toxicity to *H. armigera* (Table 2 and 3). The less toxic nature of Cry 1C and Cry 1E has been recorded earlier (Liao *et al.,* 2002; Chakrabarti *et al.,* 1998).

Table2. Relative toxicity of *B. thuringiensis* insecticidal crystal proteins against second instar *Helicoverpa armigera* neonates

Toxin	LC_{50} (ng/ cm^2)	95% fiducial limits		LC_{95} (ng/ cm^2)	95% fiducial limits		'b'(±SE)	Chi Square (n-2)
		Lower	Upper		Lower	Upper		
Cryl Aa	166.32	156.10	177.01	359.32	312.12	445.32	4.88±0.57	2.93
Cryl Ab	197.13	176.31	221.36	385.21	308.02	682.17	4.62±0.59	2.57
Cryl Ac	112.16	102.37	123.56	363.31	292.05	507.32	3.20±0.40	4.47

*In each case, the Chi square value from the goodness of fit test was less than tabular value (p =0.05), indicating that the data fit the probit model

Susceptibility to bioinsecticides typically declines as the larvae develop (Hornby and Gardner, 1987). The late instars of *Helicoverpa zea* and *Plodia interpunctella* have been reported to be less susceptibile to *B. thuringiensis* than their early instars (Ali and Young, 1996).

The more toxic nature of Cry 1Ac to lepidopterans was already recorded by Kranthi *et al.* (1999a and 2000) and Jalali *et al*. (2004). Mahapatro *et al.* (20010 recorded the LC_{50} of Cry 1Aa, Cry 1Ab and Cry 1Ac to *E. Vitella* third instar was 224.5, 300.5 and 141.2 ng/ cm^2, respectively. Kranthi *et al.* (1999b) reported 4-6 fold variation in the susceptibility of *E. Vitella* to Cry 1 Aa, Cry 1Ab and Cry 1Ac.

In the present study, even through variation occurred in the toxicity of Cry 1Aa and Cry 1Ab, the overlapping fiducial limits at LC_{50} indicated there was no significant difference in their toxicity to *H. armigera* (Table 2 and 3). These results corroborate that of Mahapatro e*et al.* (2001). In the present study the toxicity of Cry toxins to different instars of *H. armigera* was studied and the findings could be useful to maintain the toxicity levels in transgenic cotton that will manage all the instars.

Table3. Relative toxicity of *B. thuringiensis* insecticidal crystal proteins against second instar *Helicoverpa armigera* neonates

Toxin	LC_{50} (ng/ cm^2)	95% fiducial limits Lower	Upper	LC_{95} (ng/ cm^2)	95% fiducial limits Lower	Upper	'b'(±SE)	Chi Square (n-2)
Cry1Aa	218.32	197.32	239.32	730.12	583.21	1030.61	3.14±0.40	3.33
Cry1Ab	202.61	182.03	221.12	633.21	519.72	849.32	3.31±0.40	3.29
Cry1Ac	174.07	164.32	186.03	395.07	336.21	509.21	4.59±0.57	2.95

*In each case, the Chi square value from the goodness of fit test was less than tabular value (p =0.05), indicating that the data fit the probit model

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Sushil Kumar and Harish Kumar*

Department of Entomology, CCS Haryana Agricultural University
Hisar-125 004, Haryana, India
Department of Biotechnology and Molecular Biology
Correspondence address : F-631, Nai Basti (Satwari)
Jammu-180 003, J& K, India
E-mail- sushil_ento@yahoo.com

Acute and Selective Toxicity of Profenofos Against the Shoot and Capsule Borer C*onogethes punctiferalis* Guenee of Small Cardamom

ABSTRACT: The acute toxicity of profenofos (Curacron®) was assessed to target pests *viz.*, small cardamom shoot and capsule borer C*onogethes punctiferalis* Guenee and non-target major pollinator, Indian bee *viz.,Apsi cerana indica* (Fab.) in terms of LD_{50}/LC_{50} and LD_{95}/LC_{95}. The LD_{50} values of profenofos and endosulfan to *C. punctiferalis* by topical application was 0.082 and 0.105 µg respectively. Profenofos was 1.27 times more toxic than endosulfan. The LC_{50} values of profenofos and endosulfan to *A.cerana indica* were 4.243 and 6.499 ppm respectively. Safety index of profenofos worked out for *A. cerana indica* in comparison with endosulfan was 0.653.

INTRODUCTION

Cardamom (*Elettaria cardamomum* Maton) ,the queen of spices cultivated in a self sustainable tropical evergreen forest of Western Ghats in South India, is attacked by more than thirty species of insect pests (Nayar *et al.,* 1976; Kumaresan *et al.,* 1988). Among them considerable damage is done by the shoot and capsule borer *Conogethes punctiferalis* Guen. in Tamil Nadu. It not only causes direct crop loss in terms of quantity and quality, but also results in a decline in the area of the plants over a period of years. A number of insecticides have been evaluated against the pests on cardamom. Insecticides evaluated against cardamom pests had been reviewed by Valarmathi, (1997) and subsequently updated by Renuka (2001) and Rajabaskar (2003). Endosulfan, fenthion, phosalone, monocrotophos, quinalphos, carbaryl and malathion and dimethoate at 0.1 percent, were found to be effective against shoot borer (Kumaresan and Joseph, 1982; Regupathy *et al.,* 2003*).* Application of quinalphos at 0.025 percent (Kumaresan *et al.,* 1978; Kumaresan, 1988; Varadarajan and Kumaresan, 1984; Valarmathi and Regupathy, 1999,2004 a,b), lambda cyhalothrin at 20 ppm a.i./ha (Sureshkumar *et al*.,2004), and diafenthiuron 50 WP at 240 g/acre (Regupathy et.al., 2003) were found more effective against *C. punctiferaliso* on cardamom. In endemic areas, application of any of the insecticide(5-7 rounds) such as fenthion (0.05 percent), phosalone (0.07 percent), dimethoate (0.05 percent), acephate (0.075 percent), triazophos (0.04 percent), or monocrotophos (0.025 percent) is suggested as a schedule during February, March, April, May, August, Sept.- October, and October- November by Spices Board (2001) and Regupathy et.al. (2003).

Though ecofriendly neem has been evaluated (Rajabaskar and Regupathy, 2005, 2007) and suggested, the major focus falls on chemical control with insecticides among the planters. To avoid developing resistance to insecticide, repeated use of same insecticide is to be avoided. For this newer molecules are needed. Recently, profenofos (Curacron®) 50 EC is one of the insecticides registered for use in India and was found to be effective against *C.punctiferalis* on cardamom (Renuka, 2001; Renuka, *et al*.,2004). The effect of pesticides on bees assumes special significance in crops like cardamom and where pollination by bees is of tremendous importance. It is therefore considered necessary to generate the baseline toxicity of profenofos for future monitoring of the development of resistsnce and safety to the honey bees.

MATERIALS AND METHODS

Insecticides: The insecticide dilutions required for assays were prepared from technical grade profenofos of 90.5 percent purity (M/S Syngenta India Ltd., Mumbai) and from endosulfan of 98.75 purity (Aventis Crop Science India Ltd., Mumbai) by diluting with analytical grade acetone.

Bio assay

C. punctiferalis: Topical application method was followed for *C. punctiferalis* considering the internal feeding habit of the pest and that avoidance of treated surface is not possible. Field collected *C. punctiferalis* larvae were reared on castor. Once they attained the required size of weighing 18 - 22 mg (length 1.2 cm), they were used for the bioassay. An aliquot of 1 µl of a known dilution of an insecticide was placed on the thoracic dorsum of each larva using a 1 µl repeating dispenser (PB 600 -01, Hamilton Co. Ltd.,) fitted with a 50 µl syringe and Rheodyne needle. The control was treated with acetone alone. No less than 30 larvae per dose were used per treatment. Mortality counts were taken 24 hours after treatment. After treatment, larvae were held individually in 12-well tissue culture plates and provided with cardamom capsules for feeding in a 12 cell plate at 25± 2ºC for 24 hours when mortality was recorded. Larvae were considered dead if they were unable to move in a co-ordinated manner when prodded.

Apis cerana indica Fab: The dry film contact toxicity method used by Rajathi et. al., (2006) was followed. One to three day old Indian bee (*A. c. indica*) workers obtained from the apiary of Tamil Nadu Agricultural University were conditioned for 20 hours at the standard experimental conditions. Filter paper of 7 cm diameter were impregnated with 0.5 ml of insecticide test solution and dried. The filter paper was then placed in a plastic containers with sufficient aeration for bees. Worker bees collected from a single frame from a single colony, at about 2.00 p.m. were anaesthetized by keeping it in refrigerator for few minutes.

Approximately 30 bees were quickly transferred with the help of paper used like a spoon into each of the containers. They were then covered with black cloth in order to reduce their flying or "balling" so as to bring about maximum contact with the treated surface of filter papers. The bees were kept in contact with the treated surface of filter paper for one hour, after which the bees were transferred into cages (40x40x40 cm) made of muslin cloth over iron frame and provided with cotton soaked in 40 percent sucrose solution as a source of food. The bees were observed for mortality 20 hours later. Moribund honeybees were counted as dead. Acetone was used as a control. Endosulfan was included as a standard check for comparison.

The median lethal dose/concentration (LD_{50}/LC_{50}) of insecticides used were determined (Regupathy and Dhamu, 2001). The safety index was worked by the following formula.

$$\textbf{Safety index (S.I.)} = \frac{LD_{50}/LC_{50} \text{ of test compound (Profenofos)}}{LD_{50}/LC_{50} \text{ of standard (Endosulfan)}}$$

REASULTS AND DISCUSSION

The LD_{50} values of profenofos and endosulfan estimated by topical application to the third instar larvae of *C. punctiferalis* was 0.082 and 0.105 µg/larva respectively (Table 1). Profenofos was found 1.27 times more toxic than endosulfan. Bishara and Shakeel (1984/85) reported LD_{50} of profenofos by topical application to larvae of *P. gossypiella* as 12.49. The lowest LD_{50} was recorded by Kosugi (1999) against smaller tea tortrix, *A. honmai*.

Table 1. LD_{50} values of profenofos and endosulfan to third instar larvae of *C. punctiferalis* -Topical method (µg/larva)

Chemical	LD_{50} (µg)	95% fiducial limits UL	LL	Y= a + bx	x^2 at P= 0.05	Relative Toxicity
enofos	0.082	0.091	0.074	0.3826+2.4119x	10.036^{NS}	1.27
osulfan	0.105	0.094	0.116	0.5686+2.1942x	7.955^{NS}	1.00

n= number of larvae used (30);NS= Not significant;

The LC_{50} values of profenofos and endosulfan to honey bee was 4.243 and 6.499 ppm respectively (Table 2). The toxicity of profenofos was 1.53 times more than endosulfan. This is low when considering the toxicity of other insecticides used in cardamom. In comparison with endosulfan, phosalone and chlorpyriphos were 1.96 and 38.25 more toxic, respectively, by topical application (Mishra and Verma, 1982), and quinalphos was 13.25 times more toxic by the dry film method (Deshmukh, 1991) and 31.2 times more toxic by potter's tower (Singh *et al.*, 1997). Permethrin was 791.13 times more toxic than endosulfan by dry film (Reddy, 1997), and fenvalerate was 2.4 times more toxic than endosulfan by potter's tower (Singh *et al.*, 1997).

Table 2. LC_{50} values of profenofos and endosulfan to honey bee, *A. cerana indica*

Sl. No	Chemical	LC_{50} (ppm)	95% fiducial limits UL	LL	Y= a + bx	x^2 at P= 0.05	S.I.
1.	Profenofos	4.243	4.802	3.750	Y= 1.6648+2.0490x	8.713^{NS}	0.653
2.	Endosulfan	6.499	7.764	5.441	Y= 2.2226+1.5321x	2.582^{NS}	1.0

n = number of honey bees used (30);NS = Not significant;S.I.=Safety index

Profenofos was found moderately toxic to honey bees as earlier reported by El-Banby and Kansouh (1981) and Hameed and Singh (1997 - 98) and was found toxic in nectar only on the day of application while pollen was the least toxic material tested when compared to leaves and petals in cotton.

Pollination is partly entomophilic in cardamom being a highly cross pollinated crop where the honey bee is the principal pollinating agent, and it increases fruit set considerably when compared to flowers prevented from bee visits (Chandran et *al.*, 1983). Cardamom flowers remain open for 15 - 18 hours and stigma receptivity to pollen is at its peak for just 3 - 4 hours (i.e. between 8.00 a.m. to 12.00p.m.) (Krishnamurthy *et al.*, 1989). This is the most critical period for pollination. If pollination does not occur, by late evening the flower withers away and dies. Maximum foraging activity of bees has been found in the morning hours of the day. Therefore, emphasis should be given to control the pests effectively with minimal hazard to honey bees. Profenofos is one of the insecticides with low residual toxicity within 8 hours (Hameed and Singh, 1997 - 98) and profenofos 50 EC being an emulsion will be less hazardous when compared to dusts and wettable powders as bees are less likely to be in contact with emulsions because they are readily absorbed in plant tissues (Mishra and Sharma, 1988).

The profenofos could be effectively used in a rotation of chemistries in IRM programme with less risk to honey bees by exploiting ecological selectivity. This could be accomplished by applying profenofos during early morning/late evening hours when there is no bee activity or by using tank mix with neem oil/neem based formulations. The repellent effect of neem formulation on honeybee visits/day in cardamom was to the extent of 76.69 percent (Rajabaskar and Regupathy, 2005), and subsequently, the bee visits with the lapse of time (2-3 days) increased aiding the pollination.

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S.Renuka and A.Regupathy[1]
Department of Entomology,Tamil Nadu Agricultural University, Coimbatore, India-641003, India
[1]Present address:Expert-Science Advisory Board, Biofuels Research & Development Centre,
Enhanced Biofuels and Technologies (I)
#5/10-C, Alankar Gardens, G.N. Mills Post,
Coimbatore- 641 029, India,
www.ebtiplc.com
E.mail: regupathya@yahoo.com

Susceptibility of some Canadian Colorado potato beetle populations to imidacloprid and metaflumizone, 2007

INTRODUCTION

Since 1995, imidacloprid, a neonicotinoid insecticide, has been used extensively by growers to control the Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say). It continues to provide effective control in most cases, but resistant beetle populations have been identified across North America (e.g. Mota-Satchez et al. 2000, Olson et al. 2000, Tolman et al. 2005, Mota-Satchez et al. 2006, Cutler et al. 2006). Tolman et al. (2005) surveyed some Canadian

populations in 2003 and found low levels of resistance to imidacloprid, but the control failures in the field were not reported by growers. Subsequently, in 2006, Cutler et al. (2006) reported that CPB populations from several Canadian provinces were highly variable in susceptibility to imidacloprid with some populations showing resistance levels <4X, while others were as high as 22X, indicating hot spots where future control failures would be foreseeable. Cutler et al. (2006) also reported that the semicarbazone insecticide, metaflumizone, was highly toxic to CPB and suggested that, based on a limited amount of data, it would not show cross-resistance to imidacloprid and could be an effective control and resistance management option for potato growers. The objectives of our 2007 study were to determine the current resistance levels of 7 field collected CPB populations from Ontario, Quebec, New Brunswick and Prince Edward Island to imidacloprid, the toxicity of metaflumizone to CPB and the extent, if any, to which cross-resistance to imidacloprid will occur.

MATERIALS AND METHODS

Insects

The reference strain was an insecticide-susceptible CPB strain reared for 85+ generations on insecticide-free potato at the Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada (London, Ontario). Standardized collection and shipping kits were forwarded to collectors in early May, shortly before CPB adults were expected to emerge from reproductive diapause. Research or extension personnel collected 7 field populations in the 4 major potato growing provinces of Prince Edward Island (2), New Brunswick (3), Quebec (2) and Ontario(6), and immediately shipped them by courier to the University of Guelph. On arrival of each population, 2 oviposition cages (30 *l* x 31 *w* x 47 *h* cm) were promptly set up in an insect rearing room at 25ºC and 16:8 [L:D]. Each contained 25-30 adults (60:40 ♀:♂ ratio) and three 4-6 week old potted potato plants. Remaining adults were placed in a growth chamber at 12ºC, 16L:8D as a backup colony.

Colony maintenance was conducted every Monday, Wednesday and Friday to prevent cannibalism and ensure optimal feeding and reproduction. Egg masses from each population were excised from plants and placed on a sheet of Whatman No. 1 filter paper (12.5 cm diameter), in labeled 14 cm diameter Petri dishes. Eggs collected on Monday and Friday were held on a shelf in the 25ºC rearing room, which provided optimal conditions for eggs to hatch within 5 days. To minimize cannibalism, hatching was monitored daily, and larvae were separated from unhatched eggs and placed in a separate Petri dish using a small paintbrush. Eggs collected on Wednesday and all remaining unhatched eggs were held at 12ºC, 16:8 [L:D] until Friday to slow development and synchronize hatch. Bioassays were conducted on 12-24 hours old 1st instar larvae from the F1 generation.

Chemicals

Formulated imidacloprid (Admire® 240F, 240 g AI L^{-1}) was supplied by Bayer CropScience Canada Inc. (Guelph, ON). Formulated metaflumizone (Alverde™) was supplied by BASF Canada Inc. (Mississauga, ON).

Bioassays

Residual leaf-dip bioassays were used to determine the susceptibility of the CPB populations to imidacloprid and metaflumizone using the protocol described by Cutler et al. (2006). A stock solution of 1000 ppm in deionized water was prepared, from which serial dilutions of concentrations ranging from 0.1-100 ppm were prepared. Concentrations causing 5-95% mortality were used in bioassays, as determined in preliminary tests. Using a stainless steel cork borer, 1.5 cm diameter discs were cut from the leaves of fresh potato plants grown in an insecticide-free greenhouse. The leaf discs were fully immersed in the insecticide solution for approximately 4 seconds, and were then placed on a wire rack in a fume hood to air dry. Two dry discs were placed on a Whatman No. 1 1.5 cm diameter filter paper in each of 32 wells in the bioassay tray (Oliver Products Company, Grand Rapids, Michigan). Five 1st instar CPB larvae of a given population were transferred to each well using a clean, soft-bristled paint brush. The larvae were gently dropped onto the potato leaf, avoiding residual contamination of the paint brush. When all wells were infested, each bioassay tray was sealed with a thin, transparent polyester cover using Spray Mount™ (3M, London, ON). A dissection probe was used to puncture 4 pin holes in the cover of each of the 32 wells for ventilation. Bioassay trays were labeled with the date, time and population, and transferred to a climate-controlled holding room (25ºC, 16:8 [L:D]).

For each CPB population, at least 3 bioassays (one tray = one bioassay) were conducted, each on a separate day. Each assay consisted of 7 insecticide concentrations with 3 replicates per concentration, i.e., a minimum of 315 1st instar larvae per population. Bioassays were typically set-up on Mondays, Tuesdays and Wednesdays with mortality assessments completed 48 hours later. Larvae were scored dead if they were unable to move after gentle probing with a small paint brush.

Data Analysis

A standard probit analysis using PROC PROBIT (SAS Institute 2001) was used to model the concentration-mortality responses. Concentrations lethal to 50

percent (LC_{50}) of the CPB 1st instar larvae, 95% confidence limits, regression line slopes, and chi-squared goodness-of-fit test results were determined. Resistance ratios of each population compared to that of the insecticide susceptible laboratory strain were calculated at the LC_{50}.

RESULTS AND DISCUSSION

The National Potato Council (2005) defines resistance as "an inherited change in an insect's susceptibility to an insecticide arising through overuse, extensive use or misuse of the pesticide against a pest species. The result is a resistant form of the pest". Growers' heavy reliance on imidacloprid to control CPB over the past 12 years, limited availability of alternative control options and the inherent ability of CPB to develop resistance to insecticides have led to development of imidacloprid resistance in North American CPB populations (Wise, 2006).

Compared to the insecticide susceptible laboratory strain, the 7 field populations varied considerably in susceptibility to imidacloprid (Table 1). The PEI and ON 4 strains were as, or slightly less susceptible than the laboratory strain, while resistance levels among the other 5 strains ranged from x 6.5 (NB1) to x 27.8 (QC2). CPB from the organic farm (NB1) showed 6.5 fold resistance to imidacloprid, possibly as a result of previous management history.

Metaflumizone was significantly more toxic to 1st instar larvae of the insecticide susceptible CPB laboratory strain than was imidacloprid (Table 1). All 7 field strains were significantly less susceptible to metaflumizone than the laboratory strain, however, resistance levels were very low and similar ranging from x 2.33 to x 3.74 (Table 1).

Table 1. Susceptibility of 1st instar Colorado potato beetles (CPB) originating from Prince Edward Island (PEI), New Brunswick (NB), Quebec (QC), and Ontario (ON) to imidacloprid (Admire® 240F), and metaflumizone (Alverde™), 2007.

Population	N	Region (County)	Slope	χ^2	LC50(ppm)(95% C.L)	Resistance Ratio[a] at LC_{50}
Imidacloprid						
Insecticide-Susceptible	426	Laboratory strain	1.49	8.15	1.04 (0.82-1.31)	1.00
PEI	373	Kings	1.41	2.84	1.08 (0.80-1.40)	1.04
ON4	588	Dufferin	1.48	5.85	1.82 (1.44-2.24)	1.75
NB1[b]	460	Woodstock	1.23	6.06	6.76 (5.23-9.03)	6.50
NB3	561	Florenceville	1.46	10.7	12.4 (10.0-16.2)	12.0
ON6	456	Brant	1.46	8.07	17.8 (14.1-23.7)	17.1
QC1[c]	599	Lavaltrie	1.25	5.70	26.1 (20.8-34.2)	25.1
QC2[c]	351	Lavaltrie	1.31	2.23	28.9 (22.1-39.6)	27.8
Metaflumizone						
Insecticide-Susceptible	422	Laboratory strain	1.83	1.54	0.42 (0.31-0.56)	1.00
PEI	411	Kings	1.83	3.97	1.25 (1.00-1.54)	2.95
ON4	347	Dufferin	2.43	6.26	1.37 (1.12-1.63)	3.26
NB1[b]	318	Woodstock	1.98	4.99	1.57 (1.28-1.96)	3.74
NB3	329	Florenceville	2.01	3.98	0.98 (0.78-1.21)	2.33
ON6	428	Brant	2.10	2.71	1.24 (1.02-1.48)	2.95
QC1[c]	328	Lavaltrie	1.57	3.51	0.98 (0.73-1.26)	2.33
QC2[c]	363	Lavaltrie	1.83	4.00	1.25 (1.00-1.54)	2.98

[a] Resistance ratio = LC_{50} of field population/LC_{50} of insecticide-susceptible population
[b] Organic field site
[c] Bayer CropScience Research Farm

Regardless of level of imidacloprid resistance, there was no indication of cross resistance to metaflumizone (Fig. 1). However, the low resistance levels shown by the field strains suggest that CPB has the potential to develop higher levels of resistance to metaflumizone, with intensive use. To avoid or delay resistance development, this very effective chemical should be used as one component of an integrated chemical/non-chemical CPB management program.

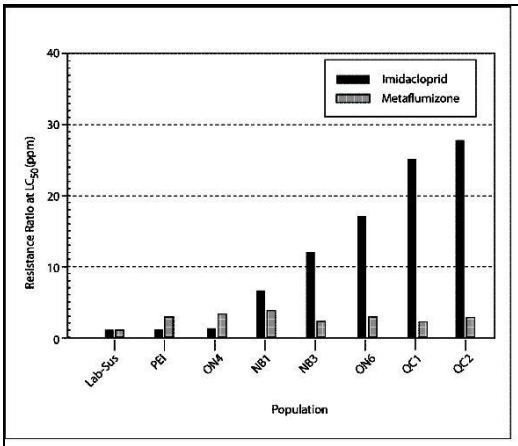

Figure 1. Comparison of imidacloprid (Admire® 240F) and metaflumizone (Alverde™) resistance levels shown by 1st instar Colorado potato beetle populations from Prince Edward Island (PEI), New Brunswick (NB), Ontario (ON), Quebec (QC) with an insecticide-susceptible laboratory strain (Lab-Sus).

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Cynthia D. Scott-Dupree*, C. Ron Harris, Melissa L. Dugas and Sarah Pirani

Department of Environmental Biology, University of Guelph, Guelph, Ontario, Canada N1G 2W1
* To whom correspondence should be addressed
Phone: 519 824-4120 ext. 52477
Fax: 519 837-0442
Email: cscottdu@uoguelph.ca

SITUATION OF PYRETHROID RESISTANCE IN SPINY BOLLWORM, *EARIAS INSULANA*, (BOISD) AND CARBARYL JOINT TOXIC EFFECT

ABSTRACT

Tests were conducted to detect the situation of pyrethroid resistance in Kafr El-Dawar field populations of spiny bollworm, *Earias insulana* (Boisd), using the attracticide resistance monitoring technique (ARMT) in the absence of the intensive exposure to pyrethroid insecticides which may be used one time in the control of major cotton pests throughout the cotton season under the Ministry of Agriculture insecticides rotation program.

The field and laboratory toxicity studies indicated that, deltamethrin was the most toxic to both field and laboratory populations with LC_{50} of 1.36 and 0.18 ppm, respectively; while fenpropathrin was the least toxic to the laboratory population with LC_{50} of 1.51 ppm, but for the field population α-cypermethrin was the least toxic pyrethroid insecticide with LC_{50} of 10.50 ppm.

Results from field trails associated with the absence of the intensive use of pyrethroids in controlling such pest lead to low resistance levels between 4.84 fold (to fenpropathrin) and 13.29 fold (to α-cypermethrin). These results pointed to the fact that, pyrethroids may play a major role in resistance management programs which are based on activate the use of pyrethroids in bollworm control programs.

The role of carbaryl in activating pyrethroids and assisting in reducing resistance levels was studied and the data shows that all the treatments had a potentiation effect with a co-toxicity factor equal to 55.27%, 34.62%, 44.77% and 40.37% for deltamethrin and carbaryl, cypermethrin and carbaryl, cyfluthrin and carbaryl and α-cypermethrin and carbaryl respectively. Also, fenpropathrin + carbaryl had an additive effect with co-toxicity factor of 15.33% and all resistance levels were reduced by rates of 2.2 to 8.85 fold.

Key words: Resistance, monitoring, mixtures, resistance management, joint toxicity, spiny bollworm, pyrethroid, carbamate, cotton.

INTRODUCTION

Spiny bollworm (SBW), *Earias insulana* (Boisd), and pink bollworm (PBW), *Pectinophora gossypiella* (Saunders), are considered to be the two most destructive pests of cotton bolls in Egypt. They are worldwide in distribution and cotton is considered to be the preferred host for these insects. Each species can affect quality and quantity of cotton seed and lint yield. Egypt spends about 15-20 million dollars to combat cotton bollworms (CBW) on an area of about 800 000 acres every year. (Temerak, S.A., 2003a.).Spiny bollworm is considered to be more dangerous pest than pink bollworm for several reasons: **a)** Spiny bollworm has no diapause in the seeds to be controlled in the factories at 55-58°C as in case of pink bollworm. **b)** Spiny bollworm can attack more than one boll and have one generation more than pink bollworm (6 versus 5 generations) (Abdel-Hamid *et al* 1999, Hossain *et al* 1999). **c)** Pink bollworm larvae can not attack very old bolls (7 week old) but spiny bollworm can (Kalifa et al 1980, Ragab 1999). **d)** One unit pink bollworm infestation in the early cotton growing season caused 5% reduction of total yield weight while in case of spiny bollworm caused 6-9% loss (El-Saadany *et al* 1975). Resistance is a genetic change in response to selection by toxicants that may impair control in field (Sawicki 1987). Therefore, the development of insect resistance is an unwanted effect of insecticides. The practical importance of insect resistance may cause a reduction in the period of insecticide use and marketing, and increase the insecticide field rates for successful control, so controlling insects becomes very difficult and increases cost of farmers production, public and private scientific research and human health and environmental protection (Gazzoni 1998).

Monitoring and managing insecticide resistance in spiny bollworm had already been taken into consideration when an adult insecticide resistance bioassay that uses males trapped with pheromones was developed. The management of insect resistance to reduce the harmful effects of insecticides, delay the widespread development of insecticide resistance by spiny bollworm and decrease the probability of severe yield loss by using more than one insecticide includes sequences, mixtures, rotations and mosaics (Tabashnik, 1989 and Leonard *et al*, 1994). Therefore, for resistance management tactics to be effective, resistance must be detected in its early stage. The attracticide resistance monitoring technique can rapidly monitor weekly LC_{50} variations within a single population; also, it can detect resistance values 24 hours before insecticides application to avoid control failure using the simple discriminating dose method (Roush and Miller, 1986). Recently, many investigators have used this technique either in complete dose-response test or in discriminating dose test, and sometimes both tests have been used together (Chu, Chang.Chi. *et al* 1996, Naik et al (1998a,b), Al-Beltagy *et al* 2001a, Shekeban 2002a). New uses for this technique are to manage resistance and to study the insecticides joint toxic effect and synergism (Shekeban *et al* 2003 a,b, 2004, 2005 and Shekeban 2007).

Generally, if two insecticides are jointly applied to an insect, one may interfere with the

activation of the other (in this case antagonism would occur), or may interfere with its detoxication reactions or with the detoxifying enzyme of the other (in this case potentiation would occur), or with both (in this third case additive effect, depending on the degree of interference with different reactions, would occur) (Watson *et al*, 1986).

The aim of the present manuscript is to focus on the situation of spiny bollworm resistance to pyrethroid insecticides and the role of carbaryl – pyrethroid mixtures in managing and reducing resistance levels. Carbaryl is used in this study in sub lethal doses to act as pyrethroid synergist, which may increase activity sufficiently to allow use lower doses of pyrethroids and thus decrease cost.

MATERIALS AND METHODS

I) Attracticide Resistance Monitoring Technique (ARMT):

This technique eliminates insects handling, allows a rapid determination of the male moth population response and enables the collection of large field sample without laboratory rearing. It's also repeatable, usable for field and greenhouse populations and provides stable LC_{50}'s with low control mortality (Haynes *et al,* 1986 and 1987).The attracticide resistance monitoring technique as described by Miller (1986) and modified by Shekeban, (2000) was used to measure insect population dynamics, insecticides toxicity and to monitor for resistance (detection and documentation). Also, it was used to manage resistance to pyrethroids with joint toxic effects of pyrethroid – carbamate, organophosphate mixtures or synergistic effect with pyrethroid - synergist.

a) Insect used: Spiny bollworm (SBW) *Earias insulana* (Boisd.) (Lepidoptera: Noctuidae) was involved in this study as follow:

1-Field male moth population of SBW was locally used in Kombanit Lokeen cotton fields, Kafr El- Dawar District, El-Bohaira Governorate, Egypt, late 2005 cotton season using pheromone baited yellow delta traps.

2-Laboratory population has been reared according to Abdel Hafez et al, (1982) rearing procedure for several generations without insecticides exposure. The male moths were taken for the bioassay experiment.

b) Insecticides used: Formulated forms were supplied by the Ministry of Agriculture.

**Deltamethrin: Decis 2.5% EC, 750 cm3 / feddan. Chemical name: (S) - α - cyano – 3 – phenoxybenzyl (1R , 3R) – 3 – (2 , 2 – dibromovinyl) 2 , 2 - dimethyl cyclopropane carboxylate. (IUPAC)

**Fenpropathrin: Meothrin 20% EC, 750 cm^3 / feddan. Chemical name: (RS) – α – cyano – 3 – phenoxybenzyl – 2,2,3,3 – tetramethylvinyl cyclopropane carboxylate. (IUPAC)

**Cypermethrin: Cymbush 10% EC, 600 cm^3 / feddan. Chemical name: (RS) α – cyano –3 – phenoxybenzyl (1RS) – *cis* – *trans* – 3 – (2 , 2 – dichlorovinyl) 2 , 2 - dimethyl cyclopropane carboxylate. (IUPAC)

**Cyfluthrin: Baythroid 5% EC, 750 cm3 / feddan. Chemical name: (RS) - α – cyano – 4 – flouro - 3 – phenoxy benzyl (1RS) – *cis* – *trans* – 3 – (2 , 2 – dichlorovinyl) 2 , 2 - dimethyl cyclopropane carboxylate. (IUPAC)

** α-Cypermethrin: Fastac, 10% EC, 250 cm^3 / feddan. Chemical name: (S and R) - α – cyano – 3 – phenoxy benzyl (1R and 1S) – *cis* – 3 – (2 , 2 – dichlorovinyl) 2 , 2 - dimethyl cyclopropane carboxylate. (IUPAC)

**Carbaryl: Sevin 85% WP, 1 kg / feddan. Chemical name: 1 – naphthyl –methyl carbamate. (IUPAC)

c) Toxicity procedure: The yellow delta traps were used with a sticky adhesive – coated cards insert containing the insecticide concentrations or insecticides mixtures placed in the trap bottom. A white rubber septum with 2 mg Rialure (EE -10, 12-hexadecadienal), provided by Ministry of Agriculture, acted as the source of pheromone. Toxicity of the tested individuals and resistance ratio were measured.

II) Joint toxic effect procedure:

Mixtures of different concentrations from each tested pyrethroid and carbaryl were prepared at the ratio of 1:5 to compare the efficacy of each mixture and the joint toxicity, this ratio was selected according to Metcalf (1967). These experiments were designed on assumption that carbaryl plays a role as esterase inhibitor which may result in different increases in insecticidal activity against spiny bollworm which was subjected for this study using ARMT. The toxicity of the binary mixtures, its relative toxicity and co-toxicity factor were measured.

III) Statistical analysis:

1-Regression equation, LC_{50}, LC_{95} and confidence limits were calculated according to Finney (1971) probit analysis computer program.

2-Toxicity Index (TI): The toxicity index values were calculated according to Sun (1950) as follow: TI = (LC_{50} of the most toxic insecticide / LC_{50} of the tested insecticide) X 100

3-Relative Toxicity (RT): These values were measured according to the equation of Metcalf (1967) as follows: RT = (LC_{50} of the lowest toxic insecticide / LC_{50} of the tested insecticide) or = (LC_{50} of insecticide alone / LC_{50} of insecticide in the mixture) (fold).

4-Resistance Ratio (R R) was calculated as follow: RR = LC_{50} of the field strain / LC_{50} of the lab strain (fold).

5-According to Salem (1970), the co-toxicity factor was used as an analytical criterion for the joint action of insecticide mixtures as follow: Co-toxicity factor (CTF) = [1 – (actual dose of A in mixture / estimated dose of A single + actual dose of B in

mixture / estimated dose of B single)] X 100. This factor was used to differentiate the results into three categories: Any value of +25% or more was considered potentiation. Any value of -25% or less means antagonism. Any intermediate values between –25% and +25% indicate only additive effect.

6-Reduction of the resistance ratio (RR Red.) was determined according to Shekeban 2003a as follows: RR Red. = Field population RR – Field population RR after joint action.

RESULTS AND DISCUSSION

The laboratory population of male spiny bollworm, *Earias insulana* (Boisd.), moth toxicity study data using the attracticide resistance monitoring technique (ARMT) were evaluated and tabulated in table (1). These data indicated that , deltamethrin was the most toxic among the tested pyrethroids with a LC_{50} value of 0.18 ppm and relative toxicity of 8.39 fold, while fenpropathrin was the least toxic with a LC_{50} value of 1.51 ppm and relative toxicity of 1.0 fold. Cypermethrin, cyfluthrin and α-cypermethrin have got LC_{50} values of 0.54, 0.95 and 0.79 ppm, respectively. The corresponding LC_{95} values were: 2.37, 26.60, 21.89, 16.92 and 31.19 ppm for deltamethrin, fenpropathrin, cypermethrin, cyfluthrin and α-cypermethrin, respectively. When comparing the toxicity values of the major three substitutions on C_3 of the cyclopropane; the dibromovinyl (deltamethrin), the dichlorovinyl (cypermethrin and related compounds cyfluthrin and α-cypermethrin) and the dimethyl (fenpropathrin); data pointed out to the superiority of the dibromovinyl (toxicity index of 100) followed by the dichlorovinyl (toxicity index of 33.3, 18.9 and 22.8, respectively) and then the dimethyl (toxicity index of 11.9), these data were in harmony with those obtained by several investigators (i.e. Angelini *et al* 1982, Shekeban 1989, Marei et al 1991, El-Bassiony, 2001, Shekeban *et al* 2002a and Shekeban 2007).

Tablel: Toxicity parameters of certain pyrethroids against spiny bollworm, *Earias insulana*, male moths of laboratory population using ARMT.

Insecticide	Reg. Equation Y=a + bx	Slope	LC_{50} (ppm) (95%C L)	LC95 (ppm) (95%C L)	RT[1]	TI[2]
Deltamethrin	Y=1.09+1.48x	1.48 ±0 .01	0.18 (0.22 -0.15)	2.37 (3.59 -1.64)	8.39	100
Fenpropathrin	Y=-0.24+1.32x	1.32±0.01	1.51 (1.84-1.23)	26.60 (45.12-17.55)	1.0	11.9
Cypermethrin	Y=0.27+1.02x	1.02±0.007	0.54 (0.69-0.42)	21.89 (45.34-12.34)	2.8	33.3
Cyfluthrin	Y=.032+1.31x	1.31±.01	0.95 (1.16-0.78)	16.92 (31.29-10.69)	1.59	18.9
α- cypermethrin	Y=0.10+1.03x	1.03±0.01	0.79 (1.01-.062)	31.19 (76.79-16.47)	1.91	22.8

1- RT= relative toxicity 2- TI= toxicity index

The toxicity of the tested pyrethroids and the n-methyl carbamate insecticide, carbaryl, was compared against the male field population moth of SBW and the results presented in table (2). The data show that, the LC_{50} values were 1.36, 7.31, 4.41, 8.34, 10.50 and 65.84 ppm for deltamethrin, fenpropathrin, cypermethrin, cyfluthrin, α-cypermethrin and carbaryl, respectively; while the corresponding LC_{95} values were 16.36, 81.05, 65.60, 173.57, 372.08 and 301.79 ppm, respectively. The toxicity index and the relative toxicity were calculated and the results show that all the tested pyrethroids were more toxic than carbaryl and the most toxic pyrethroid was deltamethrin with toxicity index of 100 and relative toxicity of 46.94 fold, while the lowest toxic pyrethroid was α- cypermethrin with toxicity index of 13.0 and relative toxicity of 6.27 fold.

Table 2. LC_{50} values of profenofos and endosulfan to honey bee, *A. cerana indica*

S1. No	Chemical	LC_{50} (ppm)	95% fiducial limits UL	LL	Y= a + bx	x^2 at P= 0.05	S.I.
1.	Profenofos	4.243	4.802	3.750	Y= 1.6648+2.0490x	8.713^{NS}	0.653
2.	Endosulfan	6.499	7.764	5.441	Y= 2.2226+1.5321x	2.582^{NS}	1.0

n = number of honey bees used (30);NS = Not significant;S.I.=Safety index

The resistance levels were calculated by comparing the LC_{50} or the LC_{95} values of the laboratory and the field populations and the results were tabulated in table (3). These data reported that the wise and controlled use of pyrethroids, only one time per season in the rotation of insecticide spraying schedule which was established by the Egyptian Ministry of Agriculture, keep it more potent, as measured by successful control and long life in the controlling market with LC_{50} resistance ratio (RR_{50}) of 7.56, 4.84, 8.17, 8.78 and 13.29 fold for deltamethrin, fenpropathrin, cypermethrin, cyfluthrin, and α-cypermethrin, respectively. The related LC_{95} resistance ratios (RR_{95}) were 8.9, 3.04, 3.0, 10.26, 11.93 fold, respectively.

Table 3: Comparing the LC_{50} and the LC_{95} values of different pyrethroids against spiny bollworm, *Earias insulana*, male moths of both laboratory and field populations and their resistance levels using ARMT.

Insecticide	Population	LC_{50} (ppm)	LC_{95} (ppm)	RR_{50}[1]	RR_{95}[2]
Deltamethrin	Laboratory	0.18	2.37	-	-
	Field	1.36	16.36	7.56	8.9
Fenpropathrin	Laboratory	1.51	26.60	-	-
	Field	7.31	81.05	4.84	3.04
Cypermethrin	Laboratory	0.54	21.89	-	-
	Field	4.41	65.60	8.17	3.0
Cyfluthrin	Laboratory	0.95	16.92	-	-
	Field	8.34	173.57	8.78	10.26
α-cypermethrin	Laboratory	0.79	31.19	-	-
	Field	10.50	372.08	13.29	11.93

1- RR_{50} = Resistance Ratio based on LC_{50} 2- RR_{95} = Resistance Ratio based on LC_{95}

The joint toxic effect of the tested pyrethroids mixed with carbaryl at the ratio of 1-5 against the male moth of spiny bollworm was evaluated and the results were tabulated in table (4). These data show that, the toxicity of the binary combinations could be arranged as follow: deltamethrin and carbaryl (LC_{50} = 0.57 ppm

and LC_{95} = 4.86 ppm), cypermethrin and carbaryl (LC_{50} = 2.16 ppm and LC_{95} = 28.04 ppm), cyfluthrin + carbaryl (LC_{50} = 2.82ppm and LC_{95} = 53.29 ppm), α-cypermethrin and carbaryl (LC_{50} = 3.51 ppm and LC_{95} = 56.81 ppm), and then the latest one was fenpropathrin and carbaryl (LC_{50} = 3.98 ppm and LC_{95} = 73.14 ppm). The results also indicated that, the toxicity of deltamethrin, cypermethrin, cyfluthrin, and α-cypermethrin was potentiated with co-toxicity factors (CTF) of 55.27%, 34.62%, 44.77% and 40.37%, respectively. The only additive effect case was obtained with fenpropathrin (CTF =15.33%).

Table 4: The effect of joint action of insecticide mixtures on pyrethroids toxicity against spiny bollworm, *Earias insulana*, male moths of field population using ARMT.

Insecticide	Reg. Equation Y=a + bx	Slope	LC_{50} (ppm) (95%C L)	LC95 (ppm) (95%C L)	RT[1]	CTF[2]
Deltamethrin + Carbaryl	Y=0.43+1.77x	1.77±0.033	0.57 (0.71-0.43)	4.86 (7.48-3.57)	2.39	55.27
Fenpropathrin + Carbaryl	Y=-0.78+1.3x	1.30±0.012	3.98 (4.95-3.20)	73.14 (130.69-44.29)	1.84	15.33
Cypermethrin + Carbaryl	Y=-0.5+1.48x	1.48±0.013	2.16 (2.65-1.75)	28.04 (43.36-19.34)	2.04	34.62
Cyfluthrin + Carbaryl	Y=-0.58+1.29	1.29±0.012	2.82 (3.51-2.25)	53.29 (92.86-33.24)	2.96	44.77
α-cypermethrin + Carbaryl	Y=-0.74+1.36x	1.36±0.019	3.51 (4.5-2.78)	56.81 (118.9-31.4)	2.99	40.37

1- RT= relative toxicity 2- CTF=Co-Toxicity Factor

Field control experiments conducted with mixtures indicated variations between pyrethroid alone and pyrethroid in the mixture and the relative toxicity (RT) parameters explain this foundation. The RTs of the tested pyrethroids were 2.39, 1.84, 2.04, 2.96 and 2.99 fold for deltamethrin, fenpropathrin, cypermethrin, cyfluthrin, and α-cypermethrin, respectively. The data pointed to the potentiation effect which may occurred as a result of the interference of each insecticide with the detoxication reaction of the other or with the detoxifying enzyme of the other. In the same manner, this superior power may referee to the presence of two modes of action in the mixture which may disrupt the insect mechanism system to overcome the toxic effect of the insecticide leading to stop its tactics to build resistance.

Table (5) presented the effect of the joint toxicity of the tested combinations (pyrethroid + carbaryl) on resistance levels of male field strain moths of spiny bollworm *Earias insulana*, using the attracticide resistance monitoring technique (ARMT). The resistance ratios based on the LC_{50} value against pyrethroids in their mixtures after joint action effect were 3.16, 2.64, 4.0, 2.97 and 4.44 fold for deltamethrin, fenpropathrin, cypermethrin, cyfluthrin and α-cypermethrin, respectively; while they were 7.56, 4.84, 8.17, 8.78 and 13.29 fold, respectively before the effect of joint action with the corresponding resistance reduction rate of 4.4, 2.2, 4.17, 5.81 and 8.85 fold, respectively. In the same manner based on the LC_{95} value after joint toxicity, the levels of resistance declined. It declined 2.05 fold for deltamethrin with reduction rate of 6.85 fold; for fenpropathrin, it declined 2.75 fold with slight reduction rate of 0.29 fold; and for the three dichlorovinyl pyrethroids, cypermethrin cyfluthrin and α-cypermethrin, the decline was 1.28, 3.15 and 1.82 fold, respectively; with resistance reduction rates of 1.72, 7.11 and 10.11 fold, respectively. Mixing the tested pyrethroids with carbaryl at the ratio of 1:5 increased the power of their toxicities to the degree that the field population becomes more susceptible with tolerant levels closed to the laboratory reference population. The role of carbaryl as a potentiator for the tested pyrethroids against spiny bollworm was supported with El-Dahan (1983) results when reported that methomyl showed the maximum potentiation effect when mixed with all tested pyrethroids against susceptible strain of cotton leafworm. Marei et al (1991) indicated that propoxur when combined with the different pyrethroid groups at 1:5 levels to the 4th instar larvae of cotton leafworm resulted in potentiation. Also, Shekeban (2003a, 2004) found that, thiodicarb synergized deltamethrin, fenpropathrin, cypermethrin and α-cypermethrin against PBW using ARMT and the pervious results were in harmony with the results obtained by Shekeban (2005, 2007) when studied beforetime the role of carbaryl in enhancing to manage resistance of PBW to pyrethroids and reducing resistance levels and finally, it may be supported by Busvine (1971) who reported that, not only the mixing of chemicals offers many possibilities in the search for better and more potent uses of toxicants but also it could theoretically prevent the emergence of resistant populations.

From the economic side, these obtained results pointed to the possibility of using sublethal doses of esterase inhibitor insecticides (i.e., carbamates and organophosphates) successfully to manage resistance to pyrethroid insecticides and this possibility lead to decrease the pyrethroid required doses and in the same time it decrease the economic cost, the resistance build up by the target insect and chances to control program failure.

Table 5: The effect of joint toxicity of insecticide mixtures on resistance levels of spiny bollworm, *Eartas insulana*, male moth of field population using ARMT.

Insecticide	Population	LC_{50} (ppm) (95%C L)	LC_{95} (ppm) (95%C L)	LC_{50} RR	RR* Red.	LC_{95} RR	RR Red.
Deltamethrin	Laboratory	0.18 (0.22 -0.15)	2.37 (3.59 -1.64)	-	-	-	-
	Field	1.36 (2.15-0.81)	16.36 (31.42-9.21)	7.56	-	8.90	-
	Field after JTE***	0.57 (0.71-0.43)	4.86 (7.48-3.57)	3.16	4.40	2.05	6.85
Fenpropathrin	Laboratory	1.51 (1.84-1.23)	26.60 (45.12-17.55)	-	-	-	-
	Field	7.31 (8.86-6.03)	81.05 (152.8-44.79)	4.84	-	3.04	-
	Field after JTE	3.98 (4.95-3.20)	73.14 (130.7-44.3)	2.64	2.20	2.75	0.29
Cypermethrin	Laboratory	0.54 (0.69-0.42)	21.89 (45.34-12.34)	-	-	-	-
	Field	4.41 (5.55-3.47)	65.60 (129.2-35.15)	8.17	-	3.0	-
	Field after JTE	2.16 (2.65-1.75)	28.04 (43.36-19.34)	4.0	4.17	1.28	1.72
Cyfluthrin	Laboratory	0.95 (1.16-0.78)	16.92 (31.29-10.69)	-	-	-	-
	Field	8.34 (10.66-6.56)	173.57 (492.42-69.17)	8.78	-	10.26	-
	Field after JTE	2.82 (3.51-2.25)	53.29 (92.86-33.24)	2.97	5.81	3.15	7.11
α-cypermethrin	Laboratory	0.79 (1.01-.062)	31.19 (76.79-16.47)	-	-	-	-
	Field	10.50 (14.39-7.76)	372.08 (1565.1 -101.3)	13.29	-	11.93	-
	Field after JTE	3.51 (4.5-2.78)	56.81 (118.9-31.4)	4.44	8.85	1.82	10.11

*RR Red = resistance ratio reduction rate (fold) = field strain RR – observed RR after joint toxic effect .
**JTE = joint toxic effect

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MAGDY M.K. SHEKEBAN
Plant Protection Research Station, (P P R I, A R C)
Sobhia, Baccous, Alexandria, Egypt.
E-mail: Magdy_shekeban@yahoo.com

THE SELECTION INFLUENCE OF INSECTICIDES ON ADULT WEIGHT IN HOUSE FLY (*MUSCA DOMESTICA* L)

Abstract. The change of adult weight of the house fly in the process of resistance formation to two organophosphates (the phoxim and phosmet), three pyrethroids (deltamethrin, fenvalerat and ethophenprox) and one chitin synthesis inhibitor (chlorfluazuron) was studied in this investigation. Correlation was found in almost all selected strains between level of resistance, adult weight and fecundity.

Introduction

Using pesticides in agriculture causes resistance formation in vermin. Treatments by insecticides can touch upon the structure and qualitative composition of a population and influence many biological parameters. For instance, sub-lethal doses of organochlorides (DDT, dieldrin, and paration) increased the amount of ova positioning by Colorado beetles 30-65% (Pimentel, Andow, 1984). The reduction of the indexes of the coupling and ovipositor was observed by treating butterfly cotton moths with some organophosphates (azinphosmethyl, methylparathion, and trichlorphon).

Treatment of one-day old butterflies by pyrethroids cyfluthrin, fenpropathrin, fenvalerat, flucytrinat, and permethrin reduced the intensity of the coupling and amount of ova positioning during the first day after treatment (Bariola, 1984). The increased doses of pyrethroids (cypermethrin, deltamethrin, and permethrin) added in food to larvae *Tribolium castaneum* prolonged the development of larvae and reduced the percent of the imago yield (Ishaaya et al., 1983). The chitin synthesis inhibitors (diflubenzuron, trifluron, and alsistin) rendered many of the imago insects sterile (Grigoryan, Halpahchan, 1986; Sehnal et al., 1986). Alos, diflubenzuron considerable reduced ova viability in the desert locust *Schistocerca gregaria* Forskal (Mariy, et al., 1981). All of this data was taken directly after insecticide treatment. There is far less data about the influence of the prolonged treatments (the selection) upon the biotic indexes of insect in literature.

There is data that populations *Drosophila melanogaster* resistant to malathion had decreased fecundity and reduced development (Halpern, Morton, 1987). The reduction of general viability and fecundity of the Colorado beetle imago was observed in azinphosmethyl resistant strain (Argentine et al., 1989).

In this investigation, the change of adult weight in the housefly in the process of resistance formation to two organophosphates (OP), three pyrethroids and one chitin synthesis inhibitor was studied. The determination of resistance level and measurement of adult weight were conducted through each of six generations, the sixth, twelfth, eighteenth, twenty fourth and thirtieth.

Material and Methods

The object of this study was imago house fly. For the study of resistance formation, imago flies of the sensitive (S) strain were divided into groups, and each group was selected by the corresponding insecticide. The selection and determination of level of the resistance to insecticides were conducted as it was describe earler (Sokolyanskaya, 2007). Anesthetized by CO_2, the flies were weighted before the topical treatment.

The criterion of sensitivity of imago flies to the preparations was determined by the lethal concentration which kills 50% of the individuals (LC_{50}, %), which was defined by the method of probit-analysis. The LD_{50} values (in mcg/g alive mass) were calculated by the following formula using the mass of the imago flies processed from the OP and pyrethroid treatments:

$$LD_{50} = LC_{50}\ 1000\ V/\ P$$

where *V* is the volume of the insecticide and *P* is the weight of the insect.

The degree of gained resistance by the housefly was characterized by the resistance index (RI), which is calculated by dividing the LC_{50} value of the resistant strain by the LC_{50} value of the sensitive strain. The statistical analysis of data was conducted using the Student t-test (Lakin, 1990).

Results and Discussion

The results of the toxicological estimation sensitive (S) and selected (R) strains of the housefly are provided in Table 1.

Table 1. The dynamic of the resistance forming in housefly

Strain and insecticide	Index	Generation				
		F_6	F_{12}	F_{18}	F_{24}	F_{30}
R-v, phoxim	LD_{50}, mcg/g	8,92±0,4	20,37±1,7	36,04±4,7	50,31±6,5	55,87±8,6
	RI	0,8	1,75	3,1	4,32	4,8
R-pht, phosmet	LD_{50},mcg/g	16,76±0,9	32,45±3,9	31,66±1,4	36,18±3,1	35,74±1,8
	RI	0,9	1,88	1,83	2,1	2,07
R-d,delta-methrin	LD_{50}, mcg/g	0,09±0,03	0,32±0,022	0,38±0,052	1,06±0,12	2,43±0,27
	RI	1,6	5,6	6,7	18,6	42,6
R-tr,ethophenprox	LD_{50}, mcg/g	5,4±0,5	6,13±1,39	21,09±2,86	26,28±3,2	27,66±2,7
	RI	1,0	1,1	3,9	4,8	5,0
R-fv, fenvalerat	LD_{50}, mcg/g	0,63±0,15	5,36±0,64	6,13±0,57	7,84±0,61	8,15±0,89
	RI	2,5	21,4	24,5	31,4	32,6
R-e, chlorflu-azuron	EC_{50}, %	0,00002± 0,000004	0,00003± 0,0000023	0,000045± 0,0000051	0,000058± 0,0000034	0,000048± 0,0000013
	RI	0,74	0,97	1,14	1,87	1,55

Note – in dark, difference selected strain with sensitive is reliable, P>0,95.

It is seen from this table that resistance to both organophosphate preparations developed slowly: to the 30th generation RI= 4,8 for the strain selected with phocsim (R-v) and RI= 2,07 for the strain selected with phosmet (R-pht). Thereby, these strains only conditionally may be named the resistant. At the same time, resistance to volaton formed several generations quicker. Apparently, both selected strains during the first 30 generations pass only Stage I of resistance formation: selection within the standard of the reaction.

Resistance to deltamethrin developed slowly in the initial stages. Up to the 6th generation, the RI almost did not change (RI=1,58); in the 12th generation, a small leap occurred (RI=5,61), and then resistance grew slowly; in the 18th generation the RI=6,67. In the 24th and 30th generations, it showed an uneven formation of resistance in spite of the fact that the select concentrations changed insignificantly.

In the trebon-selected strain (R-tr), resistance formation occurred considerably slower, even in the 30th generation RI=5. In the strain R-fv, unlike the two previous strains, resistance on the initial stage developed quickly. Already in the 12th generation, the uneven formation of resistance occurred (RI=21,44), but then resistance increased slowly.

In chlorfluazuron selected strain (R-e), formation of resistance was very slow (RI=1,55 even in the 30th generation.

Change of the adult weight of the flies in selection process is shown in Figure 1.

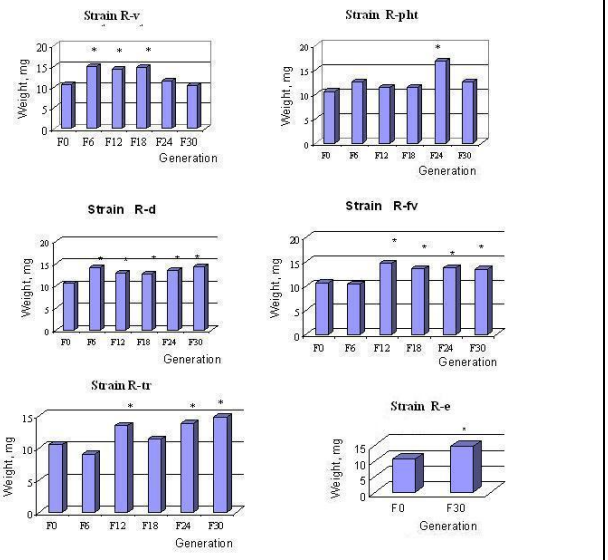

Fig. 1. The change of adult weight houseflies at selection by insecticides.
* -difference with sensitive strain is reliable

In the foxim-selected strain in the initial stage of selection, the adult weight increased, but then it decreased and did not differ from the sensitive strain. In phosmet-selected flies, the adult weight in the selection process practically was not changed. In imago selected with fenvalerat and ethophenprox, reliable and stable increase of the adult weight was fixed after the 12th generation. The adult weight of individuals of the strain R-d was reliably above the sensitive flies, in the process of selection. The flies of the 30th generation had adult weight comparable with the adult weight of the fenvalerat- and ethophenprox-selected strains. In spite of this, visually these flies in all generations were larger then the rest, and this affords ground to assume that increase of their size is connected with greater production of lipids in organism. In the strain selected chlorfluazuron, the adult weight also increased in the selection process.

Number of ovipositor and ova was not counted especially in this work; however fecundity of selected flies was obviously differed. The highest fecundity existed in the strain R-d (also, with the highest RI), and selection was conducted at a rate of $LK_{80\text{-}90}$. Fecundity of the fenvalerat- and ethophenprox-selected strains was smaller so selection was conducted at a rate of $LK_{60\text{-}80}$. In flies, selected by phoxim-and

phosmet-, fecundity was less, and selection was conducted at a rate of LK_{50-60}. Chlorfluazuron selected flies had lowest fecundity, and they were conducted at a rate $LK_{40-50.}$ Thereby, these data confirm broadly wide-spread opinion (Leather, 1988) that in arthropod fecundity increases with increase of the adult weight.

The data collected in this work supports already published data. I.M. Sikura and O.P. Borsuk (1984) found that populations of the Colorado potato beetle resistant to organochloride insecticides, differed by greater weight, bigger of fat content and greater fecundity. Treatment of the caterpillar's lightbrown apple moth *Epiphyas postvittana* by insect growth regulator fenoxycarb increased their weight and size in contrast with control individuals (Mc Ghie, Tompkins, 1988). Methiokarb and methoprene increased the growth of the caterpillars' fall armyworm *Spodoptera frugiperda*. In addition, resistance of these armyworms to permethrin, methomil, methylparathion, diflubenzuron, and prophenphos did not influence their growth, but fenvalerat, permethrin, chlorpiriphos and sulprophos noticeably oppressed the growth of the caterpillars (Ross, Brown, 1982).

Conclusion

In strains of the housefly selected by organophosphates phoxim and phosmet under a small level of fecundity to the 30-th generation, the adult weight did not differ realistically from the sensitive strain. In all pyrethroid selected strains to 30^{th} generation, resistance growth was parallel reliable increases of the adult weight and fecundity. Though the level of resistance reached differed greatly in those strains, it was above the OP-selected flies. In the strain of the housefly selected by chlorfluazuron, the adult weight increased although the resistance index was very small.

Consequently, in strains of the housefly selected by OP insecticides and pyrethroids, correlation exists between resistance index, adult weight and fecundity. Therefore in field conditions, the long use of insecticides (particularly pyrethroids) can increase the growth of the populations and the reproduction of the vermin.

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M. P. Sokolyanskaya
Institute of Biochemistry and Genetics of RAS, Ufa, Russia
e-mail: sokolyanskaya-m@yandex.ru

SUSCEPTIBILITY OF MEXICAN POPULATIONS OF Helicoverpa zea (Boddie) (LEPIDOPTERA: NOCTUIDAE) TO CONVENTIONAL INSECTICIDES

ABSTRACT

The level of resistance reached by the heliothine species to conventional insecticides has caused serious economic losses in cotton production. As an alternative for resistance management, transgenic cotton or Bt–cotton, which express the Bacillus thuringiensis (Berliner) Cry1Ac toxin, has been developed. Unfortunately, the cotton bollworm Helicoverpa zea (Boddie) is naturally tolerant to this toxin and, therefore, conventional insecticides are currently being applied in Bt-cotton to assist in its control. Due to the bollworm's ability to develop insecticide resistance and Bt-cotton selection pressure, an evaluation of susceptibility to conventional insecticides is necessary. The LD_{50} and LD_{95} of Cypermethrin, Spinosad and Chlorpyrifos were evaluated by the topical application method. Cypermethrin was the most toxic insecticide followed by Spinosad and Chlorpyrifos, respectively. The evaluated insecticides were effective for H. zea control; however,

these products are exerting selection pressure in Bt-cotton. Consequently, a systematic resistance management program to detect changes in bollworm population susceptibility is needed in order to preserve the effectiveness of chemical products if Cry1Ac resistance is developed.
Keywords: Helicoverpa zea, Bacillus thuringiensis, Bt-cotton, response base lines, remedial action plan.

INTRODUCTION

Cotton bollworm Helicoverpa zea (Boddie) and tobacco budworm Heliothis virescens (Fabricius) attacks a wide variety of crops such as cotton, maize, tobacco, tomato and chickpea (Fitt, 1989; Pacheco, 1994). In Mexico, H. zea is an important pest of cotton, maize and tomato (Pacheco, 1994). Due to numerous applications of conventional insecticides to reduce H. zea populations over the past decades, strong selection pressure was exerted, and resistance to organophosphates, organochlorines and pyrethroids developed (Wolfenbarger et al., 1971; Stadelbacher et al., 1990). This situation caused many producers in traditional cotton growing areas to abandon production of the crop (Bottrell and Adkisson, 1977).

As an alternative for H. zea control and other pests that have developed resistance to conventional insecticides, the gene that encodes Bacillus thuringensis (Berliner) var. kurstaki Cry1Ac δ-endotoxin has been inserted into certain crops, such as cotton, and was introduced into the market of various countries in 1996 (Edge et al., 2001; Traxler et al., 2002). In Mexico, Bollgard® transgenic cotton or Bt-cotton cultivated area gradually reached 60.2% from 1996 to 2005 (SIAP, 2005).

The use of transgenic cotton brought numerous benefits including low negative impact on human and animal health, beneficial fauna and notoriously on the environment, avoiding the use of conventional pesticides (Edge et al., 2001: Shelton et al., 2002: Bennet et al., 2004). In the Huasteca region of Mexico, because of the use of Bt-cotton, the application of more than twelve thousand liters of insecticide intended for H. virescens control in 1999 was avoided (Monsanto, 2000). Producers of Comarca Lagunera were also saved from applying over 659 thousand kilograms of the active ingredient (Traxler et al., 2002). Moreover, there is no cross resistance between insecticides and Bt-cotton toxin action (Tabashnik, 1994; Wu and Gou, 2004; Wu et al., 2005), and a reversion of resistance by H. virescens and Helicoverpa armigera (Hübner) to insecticides since the introduction of Bt-cotton has been documented (Wu and Gou, 2004; Teran et al., 2005: Wu et al., 2005).

However, the transgenic cotton benefits are threatened by possible development of resistance to the B. thuringiensis endotoxin by target pests (Sims et al., 1996; Liu et al., 1999; Siegfried et al., 2000). The natural tolerance of Helicoverpa sp. to the Cry1Ac δ-endotoxin provides a strong basis to develop resistance (Akhurst et al., 2003). Moreover, Cry1Ac is not as effective for H. zea and H. armigera as it is for H. virescens (Agi et al., 2001; Akin et al., 2001). The natural tolerance of H. zea towards Bt-cotton is three times greater than that of H. virescens (Stone and Sims, 1993; Traxler et al., 2002) as a consequence, conventional insecticides have been applied to control bollworm populations (Brickle et al., 2001, Stewart et al., 2001).

To delay the development of Bt-resistance, the Environmental Protection Agency (EPA) has required Bt-cotton registrants in the USA to develop and implement a Resistance Management Program (Matten and Reynolds, 2003), which includes, among other elements, a remedial action plan (Gould and Tabashnik, 1998: USEPA, 2001). This suggests that, after confirming suspected resistant individuals, conventional insecticides would then be used to eliminate the B. thurgingiensis resistance genes in the affected region (Matten and Reynolds, 2003). Hence, in northern China, insect susceptibility studies of H. armigera populations are being conducted to obtain effective measures if resistance to Bt-crops is developed (Wu and Gou, 2004). In Mexico, the Genetically Modified Organisms Bio-safety Law, considers evaluating the possible risks to human health, the environment and the country's biodiversity by using Genetically Modified Organisms (GMOs) (CIBIOGEM, 2005), but a strategy to counteract the development of resistance to Bt-cotton or a remedial action plan has not been contemplated if the situation presents itself.

The aim of this research was to evaluate the susceptibility of H. zea populations to conventional insecticides of three different toxicological groups in order to obtain information that permits the implementation of a remedial action plan in case this pest develops resistance to the B. thuringienis CryAc toxin, which Bt-cotton express.

MATERIALS AND METHODS

This research was conducted during 2005 in the Entomology Laboratory of Experimental Station Southern Tamaulipas (CESTAM) of the National Institute of Forestry, Agriculture and Livestock (INIFAP) and the Entomology laboratory of the Acarology Program of the College of Postgraduates (Colegio de Postgraduados).

Susceptibility of three H. zea populations was evaluated. The Comarca Lagunera population (LAG) was established by collecting 300 larvae from transgenic cotton fields in the Ejido Patrocinio, Municipio de San Pedro, Coahuila. The CESTAM

population was initiated with 200 larvae collected from maize in the Estación Cuauhtémoc, Municipio de Altamira, Tamaulipas (CESTAM). The susceptible Stoneville population (STON) came from the USDA-ARS Southern Insect Management Research Unit (SIMRU), which has been sustained without exposure to insecticides for over 25 years. It has also been maintained as a breeding colony since 2003 in the Entomology laboratory of CESTAM and was used as a reference population.

Collected larvae were placed in 30mL plastic cups with 15mL of artificial diet (Southland Products Incorporated, Lake Village, Arkansas), taken to the laboratory, treated with a 0.1% chlorine solution, and placed in new cups with fresh diet. Larvae were reared in these recipients until pupae developed. Pupae were placed in a Petri dish with blotting paper, and then placed in wire frame entomology cages lined with organza fabric. Upon emergence, adults were fed with a 10% sugar-water solution. After mating and oviposition, the eggs deposited on the fabric were collected in a 2 L plastic box (19x 15x 13 cm) until the eggs hatched. The first instar larvae (L1) were placed individually in plastic cups with 3-5 mL of artificial diet to allow development of third instar, with which the bioassays were conducted. All insects life cycle were maintained in a room with controlled temperature of 25 ± 3 ºC, relative humidity (RH) of 60–80% and a 12:12 –(L:D) photoperiod.

Technical grade insecticides were evaluated: Chlorpyrifos 98.6% (OP) (Dow Agrosciences of Mexico S.A. of C.V.), Cypermethrin 92% (PYR) (Trident S.A. of C.V.) and Spinosad 44.2% commercial formulation (SPIN) (Dow Agrosciences of Mexico S.A. of C.V.).

Bioassays were conducted according to The American Entomological Society topical application method (Anonymous, 1970). One microliter (µL) of a known concentration of the insecticide diluted in technical grade acetone, was applied in the pronotum of each third instar larva (L3) which weighed 20 to 30 mg. For Spinosad, concentrations were diluted with distilled water plus adherent solution (285 mL Zeta Adherent in 200 L of water) since the commercial formulation was not miscible in acetone. Applications were performed with an electric micro-applicator (ISCO model M, serial No. 510; Instrumentation Specialties Company, Inc. Lincoln 7, Nebraska) and a 500 µL micro-syringe (Hamilton Company, Reno, NV).

Each dose-mortality line was determined with seven doses with a minimum of ten larvae of the F2-F3 generation and five to seven replications per concentration, including a control in which only acetone was applied. Treated larvae were kept individually in their respective containers and under the same controlled conditions until mortality quantification (72 hours after application). Any larva, which did not respond when prodded with a blunt probe, was considered dead. When control mortality exceeded 10%, the treatment was eliminated, other wise, control mortality was corrected using the Abbott´s formula (Abbott, 1925).

Data were analyzed with POLO-PC (1987) to obtain the log dosage–probit response line. LD_{50} and LD_{95} were expressed in micrograms per larva (µg/larva). Field population response was considered susceptible when confidence limits (CL) of LD_{50} or LD_{95} overlapped with the LD´s reference population values. The relative response (RR) was obtained by dividing the LD_{50} of the field population by LD_{50} of the susceptible population.

RESULTS AND DISCUSSION

Cypermethrin showed the highest LD_{50} toxicity level (0.009 µg/ larva), followed by Spinosad (0.015 µg/ larva) and Chlorpyrifos (0.40 µg/ larva) for the susceptible STON population. Interpreting by toxicological groups, pyrethroid (Cypermethrin) demonstrated the highest level of toxicity followed by spinosin (Spinosad) and organophosphate represented by Chlorpyrifos. The regression line indicates that the most heterogeneous response for resistance development was obtained with Spinosad (0.9), while the most homogeneous responses were to Chlorpyrifos (3.2) and Cypmerhrin (2.0) (Table 1).

Table 1. Toxicity of three insecticides evaluated on field and laboratory populations of *Helicoverpa zea*.

Insecticide	Poblation	n	LD_{50}	CL_{50}	RR_{50}	LD_{95}	CL_{95}	RR_{95}	X^2	b ± s
hlorpyrifos	STON	342	0.40	0.3–0.5	1	1.3	0.9–2.5	1	5.8	3.2+0.3
OP)	CESTAM	245	0.35	0.3–0.4	0.9	1.6	1.2–2.5	1.2	0.4	2.5+0.3
	LAG	453	0.40	0.3–0.6	1	1.6	1.1–2.8	1.2	7.3	2.9±0.2
pinosad	STON	342	0.015	0.01–0.02	1	1.00	0.50–2.70	1	1.9	0.90+0.08
SPIN)	CESTAM	488	0.050	0.04–0.07	3.3	3.00	1.60–6.60	3	5.0	0.90±0.07
	LAG	360	0.035	0.02–0.05	2.3	3.84	1.71–12.0	3.8	1.5	0.81+0.08
ypermethrin	STON	584	0.009	0.008–0.01	1	0.06	0.05–0.08	1	5.9	2.0±0.1
PYR)	CESTAM	546	0.026	0.02–0.03	2.9	0.10	0.09–0.10	1.7	5.7	2.6+0.2
	LAG	390	0.015	0.01–0.02	1.7	0.11	0.07–0.24	1.8	7.4	1.9±0.2

LD = lethal dosage; CL = Confidence limits; RR = relative response (LD for the field population/LD for susceptible population); b = regression line slope; s = standard Error

For the CESTAM population, like the STON strain, Cypermethrin was the most toxic according to the LD_{50} value (0.026 µg/ larva), while Chlorpyrifos showed the lowest toxicity (0.35 µg/ larva). The pyrethroid group proved to be the most effective while the organophosphate group was the least effective, as occurred in the reference colony. The low slope value (0.9) of Spinosad, showed the most heterogeneous response. The slope found for Chlorpyrifos and Cypermethrin were above 2.5, which indicates greater uniformity of the colony in response to selection by any of the evaluated products (Table 1).

In the case of the Comarca Lagunera (LAG) population, Cypermethrin (0.015 µg/ larva) showed the highest toxicity, while Spinosad (0.035 µg/ larva) and Chlorpyrifos (0.40 µg/ larva) proved to be less effective. The heterogeneity of the response was the same as in the other populations (Table 1).

The relative response (RR) of Chlorpyrifos values in CESTAM population, based on LD_{50} and LD_{95}, were 0.9 and 1.2, respectively. This indicates similar values to the susceptible colony, confirmed by overlapping of confidence limits (CL). As well, Spinosad and Cypermethrin performance was similar: since RR_{50}=3.3 and 2.9. Although RR_{50} values showed slight separation, there was a marked overlap of CESTAM population CL values and those of the STON population, indicating susceptibility of the field population to the evaluated insecticides

The RR_{50} and RR_{95} estimated for each insecticide in LAG population implied that the response was similar to that of the reference colony (STON) and was confirmed by the overlapping CL values, indicating that both populations were susceptible to the three evaluated insecticides.

Cypermethrin proved to be the most toxic insecticide against the three H. zea populations, as Leonard et al. (1988) and Usmani and Knowles (2001) documented. Kanga et al. (1996) reported Cypermethrin RR_{50} values lower than 2, and concluded that it was not different from the susceptible strain, as occurred with the LAG field population. Although RR_{50} values for the CESTAM and LAG colonies with Cypermethrin indicated a slight separation in confidence limits, they are small when compared with the RR_{50} values developed by H. armigera in the 1980s in India, where this species reached RR_{50} values of 115, 34, and 700 (Regupathy and Ayyasamy, 2003). However, it is necessary to conduct more studies to detect the presence of pyrethroids resistant genes in these populations.

The pyrethroid group was the most effective for all three evaluated populations, as Nava et al. (1990) documented. They observed that pyrethroids (Permethrin and Deltamethrin) were more toxic for H. zea and H. virescens than the other organophosphates, carbamates and organochlorines.

Cook et al. (2002) observed that the LD_{50} and LD_{90} values of Spinosad were greater when compared with their reference population, as in this work, the response of the CESTAM and LAG field populations indicated more tolerance than the susceptible STON population. It is possible that field populations do not have the resistant genes in a detectable frequency to determine if susceptibility is decreasing. Even the slope suggested a tendency towards resistance, it is probably that the obtained heterogeneity was due to the fact that the insecticide is more effective when ingested and not by contact (Sparks et al., 1995 and 1998). In addition, Spinosad was diluted with distilled water plus adherent solution and not with acetone since the commercial formulation did not allow it; therefore, reducing the penetration on the larvae integument and as a consequence, the heterogeneous response.

The CESTAM and LAG field populations were susceptible to the evaluated insecticides. The susceptibility of the CESTAM population might be attributed to the fact that cotton has not been grown in southern Tamaulipas since 2001 because of the low price of the fiber, making cotton production unprofitable. Thus, it was likely that the absence of selection pressure favored the current response of H. zea. The Comarca Lagunera (LAG) population was also susceptible, however, this was most likely because the region's major pest was Pectinophora gosypiella (Saunders), thus, H. zea was not the target pest and was not directly selected. The susceptibility of the bollworm in the studied populations, can also be attributed to the reversion of resistance to conventional insecticides, as occurred with H. virescens in southern Tamaulipas, Mexico (Terán et al., 2005) and H. armigera in northern China (Wu and Gou, 2004; Wu et al., 2005), due to the use of transgenic cotton. Furthermore, H. zea has a wide range of hosts, including many wild plants (Sudbrink and Grant, 1995) where there was not any selection pressure.

The evaluated insecticides have been shown to be effective in controlling bollworm population that developed in Bt-cotton and can be included in a remedial action plan if H. zea develops resistance to the B. thuringiensis toxin in the short term. Nevertheless, we must realize that there are four important facts: 1) the evaluated insecticides, and others with similar mode of action, are being applied on Bt-cotton to control bollworm populations, 2) the H. zea ability to develop resistance to conventional insecticides (Wolfenbarger et al., 1971), 3) the possibility of development of resistance to B. thuringiensis (Siegfried et al., 2000) and 4) H. zea was a secondary pest in cotton, and now, by opening new niches with the introduction of Bollgard® cotton, conditions favored bollworm to become a major pest.

If traditional insecticide control practices against H. zea continues, and Bt-cotton and other transgenic crops like Bt-corn are widely use, is possible that the selected agent (insecticide or Bt resistance) may increase to unmanageable levels for which there would be no effective tools for controlling resistant H. zea

populations. This situation is a matter of concern given that the development of new products with different modes of action to what is currently available has not kept up with the demand, and those that are developed will be progressively less effective.

CONCLUSIONS

The pyrethroid Cypermethrin was the most effective insecticide with the lowest LD_{50} and LD_{95}, followed by the spinosin Spinosad and the organophosphate Chlorpyrifos, respectively, for the CESTAM and LAG colonies as well as for the STON reference colony.

The confidence limits of the CESTAM and LAG strains overlapped with those of the STON reference strain, indicating that the field colonies were susceptible to the three evaluated insecticides.

Even when the studied populations of H. zea exhibited susceptibility to the evaluated insecticides, it is essential to create resistance management programs in every agricultural region and susceptibility monitoring programs. This would permit timely detection of resistance levels and the mechanisms involved. It is also necessary to create remedial action plans that effectively deal with a contingency of resistance to B. thuringiensis. The present study provides information on H. zea susceptibility in the Comarca Lagunera and in Southern Tamaulipas, Mexico, contributing to initiate a resistance monitoring program in both regions.

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Katina Stamatiu-Sánchez[1§]; Laura D. Ortega-Arenas[1]; Celina Llanderal-Cázares[1]; Hussein Sánchez-Arroyo[1]; Urbano Nava-Camberos[2]; Antonio P. Terán-Vargas[3]

[1]Programa de Entomología y Acarología, Instituto de Fitosanidad del Colegio de Postgraduados, Campus Montecillo, Km. 36.5 Carretera México- Texcoco, 56230, Montecillo, Texcoco; Edo. de México, México. [2]Campo Experimental La Laguna, INIFAP, Km. 17.5 Carretera Torreón- Matamoros, 27000, Torreón, Coahuila, México. [3]Campo Experimental Sur de Tamaulipas, INIFAP, Km. 55 Carretera Tampico- Mante, 89619, Est. Cuauhtémoc, Tamaulipas, México. [§]Author for e-mail communication: katina@colpos.mx.

Ovipositional and feeding preferences of *Helicoverpa armigera* towards putative transgenic and non-transgenic pigeonpeas

ABSTRACT: *Helicoverpa armigera* is the major constraint for pigeonpea production, and therefore, efforts are being made to develop transgenic pigeonpeas with *Bt* and *SBTI* genes to minimize the losses due to this pest. The oviposition behavior of *H. armigera* on transgenic and non-transgenic plants was studied under no-choice, dual-choice, and multi-choice conditions. No differences were observed in the number of eggs laid on the inflorescences of the transgenic pigeonpeas with *cry1Ab* or *SBTI* genes and with the non-transgenic plants. In dual-choice feeding tests, there were no differences in leaf damage, larval weights, and the number of larvae between transgenic and non-transgenic plants. The results suggested that transgenic plants have no influence on the oviposition and feeding preferences of *H. armigera*.

Pigeonpea (*Cajanus cajan* (L.) Millsp.) plays an important role in nutritional security as an important source of high quality dietary proteins. It is damaged by over 150 insect species, of which *Helicoverpa armigera* (Hubner) is the most important pest, which causes an estimated annual loss of US$ 317 million in the semi-arid tropics in pigeonpea (ICRISAT, 1992). In an effort to minimize the *H. armigera* damage, transgenic pigeonpea plants with *Bacillus tthuringiensis* (*Bt cry1Ab*) and soybean trypsin inhibitor (*SBTI*) genes have been developed recently (Sharma *et al*., 2006). Genetic transformation of crops leads to slight changes in the chemical composition, which might influence host selection and colonization by the insects. Therefore, we studied the oviposition preference by females and feeding preference by the *H. armigera* larvae on transgenic and non-transgenic plants of pigeonpea.

MATERIALS AND METHODS

The pigeonpea varieties, ICPL 88039 and ICPL 87 that were transformed using the constructs pHS 723: *Bt cry1Ab* and pHS 737: *SBTI* through *Agrobacterium tumefaciens*-mediated transformation (Sharma *et al.* 2006) were raised in a containment (P_2 level) green house at 24 to 28°C, 70 to 80% RH. The

H. armigera culture was maintained under laboratory conditions of 24°C and 70% RH (Armes *et al.*, 1992).

Oviposition preference

The oviposition behavior of *H. armigera* was studied under no-choice, dual-choice (in comparison to the non-transgenic control), and multi-choice conditions (all the test genotypes placed inside the cage). Fresh inflorescences (20 cm long) with flowers and tender leaves were collected from the greenhouse, and placed in a conical flask (150 ml) filled with water. A cotton swab was wrapped around the stem to keep the inflorescence in upright position. For no-choice tests three pairs, and for dual- and multi-choice tests four pairs of two-day old moths were released inside the cage. Sucrose solution (10%) in a cotton swab was offered to the adults as a food, changed on alternate days. The number of eggs laid by the moths was recorded, and the inflorescences were replaced daily. The experiments were replicated six times in a completely randomized design. Percentage of eggs laid on each plant was calculated from the total number of eggs laid. The data was subjected to analysis of variance. A Student "T" Test was used to test significance of difference in dual-choice tests.

Neonate feeding preference assay

Fully expanded tender leaves of equal size from transformed and non-transformed pigeonpea plants were collected and placed one centimeter apart in a Petri dish arena (9 cm dia) lined with moistened filter paper. Ten neonate larvae were placed in the middle of Petri dish arena. Data on leaf feeding was recorded after 72 hours on a 1 to 9 scale (1 = <10% leaf area damaged and 9 = >80% leaf area damaged). The number of larvae on each leaf and their weights were recorded separately. Each treatment was replicated five times in a completely randomized design.

RESULTS AND DISCUSSION

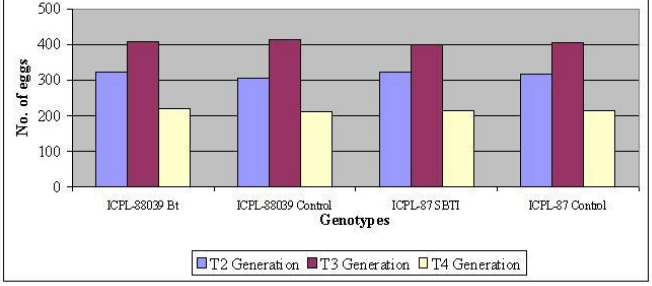
There were no significant differences in the numbers of eggs laid on the inflorescences of transgenic and non-transgenic control plants under no-choice, multi-choice (Fig. 1), and dual-choice conditions (Table 1). Egg densities of the tobacco budworm (*Heliothis virescens*) (Parker and Luttrell, 1998) and cotton bollworm (*H. armigera*) (Sharma and Pampapathy, 2006) have not been found to be significantly different on transgenic and non-transgenic cottons. The lack of differences in oviposition preference indicated that there are no major changes in the physico-chemical characteristics of the transgenic plants that influence oviposition behavior. This corroborates the earlier observations that the oviposition behaviour of *H. armigera* moths was independent of the presence of transgenes (MacIntosh *et al.*, 1990; Orr and Landis, 1997; Ramachandran *et al.*, 1998).

Table 1 Oviposition preference of *H. armigera* females towards transgenic and non-transgenic pigeonpeas under dual-choice conditions.

Genotype		No. of eggs/twig	
		Transgenic	Non-transgenic
T_2 Generation			
SBTI	ICPL 87	244.2[a]	215.3[a]
Bt	ICPL 88039	202.8[a]	201.0[a]
T_3 Generation			
SBTI	ICPL 87	112.2[a]	128.2[b]
Bt	ICPL 88039	123.8[a]	132.5[a]
T_4 Generation			
SBTI	ICPL 87	166.2[a]	159.8[a]
Bt	ICPL 88039	164.5[a]	156.8[a]

Figures followed by the same letter in a row are not significantly different at *Fp* 0.05

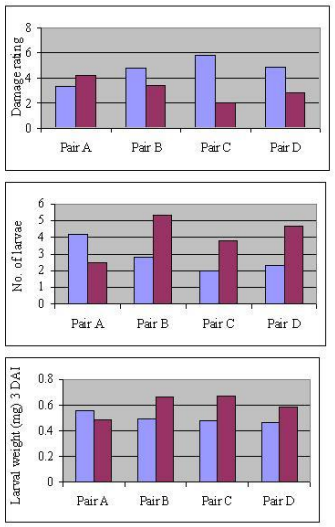
Fig. 1 Oviposition preference of *H. armigera* females towards transgenic and non-transgenic pigeonpeas



T2 and T3 generations were tested under no-choice conditions.
T4 generation was tested under multi-choice conditions.

There are no significant differences in leaf damage, larval weights, and the number of larvae that settled on leaves of transgenic and non-transgenic plants (Fig. 2). Gould *et al.* (1991) observed that *H. virescens* larvae were able to detect and avoid high levels of *B. thuringiensis* toxins in diet. Increased movement and dispersal of *H. virescens* larvae has also been observed on transgenic cotton lines (Benedict *et al.*, 1993; Parker and Luttrell, 1999). Lack of feeding preference by *H. armigera* larvae on transgenic and non-transgenic pigeonpea plants may be because of low levels of expression of toxin proteins in transgenic pigeonpeas, which do not result in perceptible changes in insect behaviour and development.

Fig. 2: Feeding preference of neonate larvae of *H. armigera* towards leaves of transgenic and non-transgenic pigeonpea plants in dual-choice tests (2002 rainy season)



Pair A : Bt ICPL 88039 and its non-transgenic control
Pair B : SBTI ICPL 87 and its non-transgenic control
Pair A : Bt ICPL 88039 and SBTI ICPL 87
Pair A : Non-transgenic controls of ICPL 88039 and ICPL 87

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S V S Gopala Swamy[1], H C Sharma[2], G V Subbaratnam[1] and M Peter Vijay[2]

[1] Acharya N G Ranga Agricultural University, Rajendranagar, 500 030, Andhra Pradesh

[2] International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, 502 324, Andhra Pradesh

Abstracts in Resistance Management

Genetics and Management of Whitefly Resistance to Pyriproxyfen

Selective insecticides, such as insect growth regulators, that kill pests but cause little or no harm to non-target organisms have become increasingly important in crop production systems worldwide. The insect growth regulator pyriproxyfen has been successfully used for the last decade in Arizona as part of an integrated pest management (IPM) program for the sweetpotato whitefly, *Bemisia tabaci*. *B. tabaci*, a problematic pest in Arizona and other sub-tropical regions throughout the world, damages crops due to direct feeding, transmission of plant viruses, and production of honeydew. The use of pyriproxyfen for *B. tabaci* control has decreased use of broad-spectrum insecticides, preserved natural enemies and beneficial organisms, and increased farmer profits.

A serious threat to the continued success of the IPM program in Arizona is the evolution of insecticide resistance in *B. tabaci*. Despite implementation of a rotation program designed to preserve efficacy of pyriproxyfen, laboratory bioassays tracking the evolution of resistance reveal an area-wide decline in susceptibility to this insecticide, threatening

the sustainability of *B. tabaci* control. To enhance the ability to design sound strategies for managing whitefly resistance to pyriproxyfen, we conducted laboratory and field studies to evaluate insecticide resistance traits in male and female *B. tabaci*. We used results from these studies to develop resistance management models. These objectives were designed to provide a comprehensive understanding of the underlying factors affecting *B. tabaci* resistance to pyriproxyfen and to improve the current IPM program for *B. tabaci*.

Laboratory and field bioassays investigated the population genetics of pyriproxyfen resistance in males and females from a susceptible strain of *B. tabaci* and a laboratory-selected resistant strain (>1000 fold resistance). Results showed that male and female *B. tabaci* did not differ in susceptibility to pyriproxyfen, resistance was partially to completely dominant under approximated field conditions, and fitness costs were not associated with resistance. These results indicated that pyriproxyfen susceptibility could be threatened if individuals in field populations have traits similar to those of the laboratory-selected strain. Models were used to identify key factors, both biological and operational (i.e. factors that can be modified by farmers), on the evolution of pyriproxyfen resistance. Model results indicated that the current IPM program for *B. tabaci* can be improved by curtailing the use of pyriproxyfen in cotton-intensive regions, synchronizing the use of pyriproxyfen with key crop production stages in the field, and applying low pyriproxyfen concentrations.

Knowledge generated from this research has provided fundamental insight into factors affecting *B. tabaci* resistance to pyriproxyfen, which will be used to improve management in Arizona cotton and other crops. Due to the life history and ecology of *B. tabaci*, insecticides remain essential for protecting many crops in the United States and abroad against this pest. Sustaining efficacy of the selective insecticides currently used against the whitefly would be a major boon to agriculture in many countries.

David Crowder*, Christa Ellers-Kirk, Timothy Dennehy, Bruce Tabashnik, and Yves Carriere
Department of Entomology, University of Arizona, Tucson, AZ (dcrowder@ag.arizona.edu; contact for more info and/or links to full publications)

Pyrethroid Resistance in Populations of the Annual Bluegrass Weevils, *Listronotus maculicollis* (Dietz), (Coleoptera, Curculionidae) from Connecticut Golf Courses.

ABSTRACT. The annual bluegrass weevil *Listronotus maculicollis* (Dietz) (Coleoptera, Curculionidae), is a pest of highly maintained annual bluegrass, *Poa annua* L. (*Poaceae*) on golf courses and tennis courts throughout the Northeast. The species was first seen on turfgrass in Connecticut in 1931 and by the late 1950's and early 1960's, it was responsible for severe turf damage on several golf courses in the state. Today, annual bluegrass weevils have remained prevalent on many Connecticut golf courses despite widespread insecticide use for their control. Throughout the past decade, pyrethroids had offered excellent weevil management, demonstrating an average of 90% reduction in pest populations. Within the past few years however, several golf course superintendents in Connecticut have observed a noticeable decline in the effectiveness of pyrethroid based products against the species. The objective of this study was to obtain toxicity data on the susceptibility of annual bluegrass weevil populations from several golf courses in Connecticut to the most commonly used pyrethroids, bifenthrin and lambda-cyhalothrin, and to substantiate or refute reports of resistance to these insecticides.

Topical application bioassays of bifenthrin and λ-cyhalothrin were conducted on seven field collected populations of adult annual bluegrass weevils. Weevils were collected from one golf course with no history of pyrethroid use (Enfield), one with historically low pyrethroid use (New Haven) (one to two applications per year for at least three years) and from five golf courses with historically high pyrethroid use (Willimantic, Norwich, Stamford, Bloomfield, Hartford) (more than two applications per year for at least three years). The LD_{50} for each insecticide and each population was determined by log-concentration vs. mortality regression lines for adult weevils during the summer months (June-August) when they were most abundant. The estimated LD_{50}'s were then used to calculate resistance ratios relative to the most susceptible population for each insecticide (Enfield).

The resulting toxicity data showed that weevils collected from the high pyrethroid use golf courses (Hartford, Norwich, Stamford, Willimantic and Bloomfield) were significantly more resistant to bifenthrin and λ-cyhalothrin than the populations from the low pyrethroid use golf courses (Enfield and New Haven). Among the high pyrethroid use courses, bifenthrin resistance ratios ranged from 37.2 to 94, and λ-cyhalothrin resistance ratios ranged from 15.5 to 113, when compared to the most susceptible course (Enfield). According to these data, the annual bluegrass weevil populations from high pyrethroid use golf courses researched here have developed resistance

to both bifenthrin and λ-cyhalothrin. This is the first report of insecticide resistance for *L. maculicollis.*

Darryl Ramoutar[1], Steven R. Alm[1] and Richard Cowles[2]
[1]Dept. of Plant Sciences and Entomology,
University of Rhode Island,
Kingston, RI. 02881
[2]Valley Laboratory
The Connecticut Agricultural Experiment Station
153 Cook Hill Road
Windsor, CT 06095

Selecting for Resistance to the Cry3Bb1 Protein in a Genetically Diverse Population of Non-diapausing Western Corn Rootworm

The western corn rootworm (WCR, *Diabrotica virgifera virgifera)* is an economically important pest of maize in North America. Larvae inflict the most damage by feeding on maize roots. Feeding damage can disrupt nutrient flow, thereby reducing seed production, and may also cause plant lodging that can disrupt harvesting. In 2003, the U. S. Environmental Protection Agency approved the commercial use of Monsanto's Bt maize expressing the Cry3Bb1 protein. When ingested, this protein is toxic to these beetles. However, given the previous adaptability of western corn rootworm beetles to control measures, evolution of resistance to the toxin is a concern. To understand how resistance might evolve to the Cry3Bb1 we created a genetically diverse base population (NDBP) of non-diapausing WCR. Genetic variation from four geographically distinct diapausing western corn rootworm populations was introduced into a non-diapausing colony through assigned and random matings. From this genetically diverse population we created three experimental and two control lines. Our goal was to achieve 80% larval mortality for each generation of selection. Therefore, prior to selecting for Cry3Bb1 resistance we established the optimum Bt exposure duration to achieve ~80% mortality due to Bt selection while minimizing mortality from other factors. After several preliminary trials comparing survivorship for 1st, 2nd, and 3rd larval instars on MON863, MON863 isoline, and a no maize control (i.e. starved), we began our selection experiments with 24 hour old neonate larvae as the initial exposure stage. The neonate larvae from each experimental line were exposed to Bt maize seedlings in a Petri dish. The time of exposure to the Bt maize seedlings increased each generation for several generations. Initial exposure time was 24 hour, which increased in 12 h increments for five generations, and then larvae were exposed in 24 h increments for another five generations. Control lines were treated identically to experimental lines except for the Bt exposure. Beginning with the fourth generation and alternating every other generation, we reared larvae from each line entirely on Bt maize to assess the degree of resistance. From the NDBP we currently selected three experimental colonies (EC1, EC2, EC3) for resistance to the Cry3Bb1 protein for nine generations, increasing exposure time with each generation. We have also maintained two control lines (CC1, CC2) on MON863 isoline. There was little difference in emergence for the EC and CC lines on isoline corn, indicating there may be little cost to selection for Cry3Bb1 resistance. We also selected for rapid Cry3Bb1 resistance from both the EC and CC lines starting at the fourth generation. Intense selection pressure to survive produced completely resistant CC lines, which were never exposed to Cry3Bb1, as quickly as EC lines. This exemplifies the adaptability of WCR to plant incorporated protectants and the importance of resistance management plans for WCR and other corn rootworm species. We will continue to investigate the genes involved in Cry3Bb1 resistance and potential fitness costs associated with resistance. Understanding how resistance to a transgenic crop can evolve could help in insect resistance management guidelines.

B. Wade French[1], Mark Bagley[2], and Chad Nielson[1]
[1]North Central Agricultural Research Laboratory
USDA-ARS
2923 Medary Avenue
Brookings, SD 57006
[2]Molecular Ecology Research Branch
National Exposure Research Laboratory
U.S. Environmental Protection Agency
26 Martin Luther King Dr. West
Cincinnati, OH 57751

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