Resistant Pest Management Newsletter

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

Vol. 22, No. 2 (Spring/Fall 2013)

Table of Contents

Letter from the Editors

Dear Subscribers,

I would first like to thank all of our subscribers, contributing authors and supporters for their continued support of the RPM newsletter. It is with bitter sweet regards that after 24 wonderful years, this will be the final edition of the newsletter. The success of this newsletter has been attributed greatly to the hardwork and diligence seen throughout academia, industry and government in reporting cases of observed pesticide resistance across the world. From its first publication in January of 1989, the newsletter has continued to grow and expand with the ever developing world of resistance and was fortunate to provid a platform for international communication amongst the scientific community.

Though there will not be future publications of the RPM newsletter, the online archive will continue to be maintained at http://whalonlab.msu.edu/rpm-newsletter/. Any inquires regarding the newsletter's availability as a hard copy can be emailed directly to rpmnews@msu.edu.

Many Thanks and Best Regards,

Brittany Harrison
Newsletter Coordinator
Pesticides Alternatives Laboratory
Michigan State Univeristy
rpmnews@msu.edu

Abstracts in Resistance Management

RESISTANCE DEVELOPMENT AND IMPORTANCE OF BIOTIC FACTORS IN THE LOCAL POPULATIONS OF COLORADO POTATO BEETLE LEPTINOTARSA DECEMLINEATA SAY IN THE SOUTHERN URALS

The rate of resistance development in insect species is an overwhelmingly important problem (Sukhoruchenko, Dolzhenko, 2008). Unfortunately, complex analysis of all the factors concerned in this process is far from widely adaptation. To discuss the problem we produce the results of investigations, including not only the toxicological and molecular genetic analysis data for Colorado beetle populations in the South Urals (Republic of Bashkortostan), but estimation of biotic factors effect on the resistance development.

Investigations of resistance population spreading combined the procedures of ecological monitoring of population state, phenetic, toxicological and molecular analysis carried out in 2007-2012. These measures confirmed the existing complicated subdivided structure of Colorado beetle populations in the South Urals and showed the distinctions between the groups of local populations (Udalov, Benkovskaya, 2008; Udalov et al., 2010; Benkovskaya, Udalov, 2011; Udalov, Benkovskaya, 2011). The distinctions revealed a border passing between the areas of large species population complexes, determined by habitation of at least two sympatric forms diverged at the ecotype level. These peculiarities of Colorado beetle population structures complicated the problem of evaluating the impact of biotic factors to the resistance development.

In our recently published article (Surina et al., 2013), we insisted that the correlation between geographical location and level of susceptibility to the entomopathogenic fungi has not been observed. At the same time we established the compound dependence between the level of resistance to insecticides and susceptibility to the mycopathogens.

Analysis of acetylcholinesterase (AChE) activity in haemolymph, fat bodies and head tissues of Colorado beetle mature females showed significantly increased activity level in females resistant to mycopathogen (*Beauveria bassiana* isolate Ufa-2). Molecular analysis (Udalov, Benkovskaya, 2008) established the belonging of all these individuals to homozygotes with *AChE* gene mutation caused the resistance to organophosphorous insecticides.

However, during the registration number of mycopathogen infected beetles in the excerpts from resistant to the other insecticides populations we elucidated that not always insecticide resistance is closely connected with increased resistance to mycopathogens. In the local Colorado beetle populations in Republic of Bashkortostan, we revealed the mycopathogens from genus *Beauveria* occurring everywhere. We presume that mycopathogens effects and the natural selection of mycopathogen-resistant individuals even if are not unidirectional with the selection on insecticide resistance, can influence on the resistance development rate.

Estimation of spectrum of predators able to feed on Colorado beetle eggs and larva established the most species of entomophages (Kitaev et al., 2011; Kitaev et al., 2012). Results of toxicological evaluation of susceptibility to insecticides in one of carabus species demonstrated their capability to develop the resistance. Since resistance development in the local populations confined to potato ecosystems pass most likely simultaneously in Colorado beetles and their predators, we can assume that the presence in ecosystem of insecticide-resistant predators makes its contribution to the regulation of pest number.

Among the factors influencing on resistance development in the Colorado beetle populations is the potato varieties specificity. Significantly differing effectiveness of insecticides under the treatment of the potato varieties distinguishing from each other by the resistance to Colorado beetle (Mardanshin et al., 2012) evidencing the necessity of taking into account of this factor.

All our findings allow asserting that the impact of biotic factors on the Colorado beetles number in the local population is doubtless. Their importance corresponds to the conception of integration to natural and agricultural ecosystem completion stage. The biotic factors cause the acceleration of differentiation in local populations and thus influencing upon the resistance development.

ACKNOWLEDGEMENTS

Investigations funded by RFBR 11-04-01886-a, 11-04-97022-r_povolzye_a, 12-04-31106-mol_a and 12-04-01450-a.

REFERENCES

Benkovskaya G.V., Udalov M.B. Colorado Potato Beetles Investigations in the South Urals. Nova Science Publishers, Inc. New York. 2011.60 p. ISBN: 978-1-61728-951-4

Kitaev K.A., Udalov M.B., Benkovskaya G.V., Choosing predators for biocontrol of Colorado potato beetle in the South Ural // Resistant Pest Management Newsletter. 2011. Vol. 21, No. 1. P 2–3.

Kitaev K., Udalov M., Benkovskaya G. Population genetics of interactions Leptinotarsa decemlineata and Coccinella septempunctata // Abstracts of XXIV International Congress of Entomology. Aug.19-25, 2012. Daegu, Korea. S1402TU04.

Mardanshin I.S., Benkovskaya G.V., Surina E.V., Kitaev K.A., Udalov M.B. Comparative estimation of efficiencies of different insecticides in experiments on potato protection against Colorado potato beetle // Agricultural chemistry. 2012. No.9. P. 58-63.

Sukhoruchenko G.I., Dolzhenko V.I. Problems of resistance development in arthropod pests of agricultural crops in Russia // OEPP/EPPO Bulletin. 2008. V. 38. P. 119-126.

Surina E.V., Udalov M.B., Benkovskaya G.V. Population genetic aspects of susceptibility of the Colorado potato beetle to mycopathogens in the Republic of Bashkortostan // Russian journal of Ecology. 2013. V. 44. No.3. P. 227-232.

Udalov M.B., Benkovskaya G.V. 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 // Resistant Pest Management Newsletter. 2008. Vol.17, No. 2 (Spring 2008). P. 15–16.

Udalov M. B., Benkovskaya G. V., and Khusnutdinova E. K. Population structure of the Colorado Potato Beetle in the Southern Urals // Russian Journal of Ecology, 2010, Vol. 41, No. 2, p. 126–133.

Udalov M. B. and Benkovskaya G. V. Change in the Polymorphism Level in Populations of the Colorado Potato Beetle // Russian Journal of Genetics: Applied Research, 2011, Vol. 1, No. 5, p. 390–395.

G. Benkovskaya, E. Surina, K. Kitaev, T. Leontieva, M. Udalov, I. Mardanshin, L. Syrtlanova, T. Akhmetkireeva.
Institute of Biochemistry and Genetics, Ufa Scientific Centre of Russian Academy of Sciences
450054, Ufa, prospect Oktyabrya, 71
e-mail: bengal2@yandex.ru

CURRENT RESISTANCE LEVELS TO PERMETHRIN AND OXAMYL IN *LEPTINOTARSA DECEMLINEATA* (SAY) (COLEOPTERA: CHRYSOMELIDAE) POPULATIONS FROM THE EASTERN SHORE OF VIRGINIA (USA)

Since the 1950s, Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), has developed resistance to over 50 different insecticides (Alyokhin et al. 2008). One region that has been notorious for resistance problems in *L. decemlineata* is the Eastern Shore of Virginia (Tisler and Zehnder 1990), which has been an important potato producing area in the U.S. since the 1800s. The insecticide resistance status of Virginia's *L. decemlineata* populations is out of date and needs to be re-evaluated. Providing growers with an updated insecticide resistance profile for *L. decemlineata* populations will help growers select the proper management tools and ensure potato production remains profitable for growers in the state.

Herein, we evaluated the current resistance levels of populations of *L. decemlineata* from eastern Virginia to the pyrethroid permethrin and the carbamate oxamyl, which represent two insecticides that *L. decemlineata* had previously developed resistance to (Tisler and Zehnder 1990).

In 2012, *L. decemlineata* populations from three locations from the Eastern Shore of Virginia (New Church, Painter, and Cheriton) were exposed to serial

dilution concentrations of oxamyl and permethrin to determine the LC_{50} values. Commercial insecticides formulated for agricultural use were used for all experiments and included the following: 1) permethrin (Permethrin 3.2 EC (38.4% ai) Helena Chemical Company, Collierville, TN; and 2) oxamyl (Vydate 2L (24% ai) DuPont Crop Protection, Wilmington, DE. The concentrations of each compound used in the bioassays can be seen in Table 1.

Table 1: Insecticide concentrations evaluated in larval and adult *L. decemelineata* insecticide toxicity bioassays conducted in Painter, VA 2012.

Permethrin (g ai/L)	Oxamyl (g ai/L)
0.0	0.0
0.06	0.09
0.31	0.47
1.53	2.37
7.67	11.86
38.34	59.3

Toxicity bioassays for adult and first instars of *L. decemlineata* were set up following the methods of Tisler and Zehnder (1990). Adult *L. decemlineata* were exposed by completely submerging groups of ten beetles into various concentrations of permethrin and oxamyl for 30 sec. After 30 sec, the exposed adults were placed onto clean paper towels to absorb excess moisture and then placed into Petri dishes and evaluated for mortality 24 hrs after exposure. Adults pooled from each location (n = 120) were exposed to each concentration of permethrin and oxamyl.

First instar larvae of *L. decemelineata* collected from the same three Virginia locations were exposed to permethrin and oxamyl through a contact bioassay via treated filter paper according to the methods described by Tisler and Zehnder (1990). Egg masses were collected from multiple locations and observed for hatching. After hatching the neonates were isolated for 24 hrs prior to being utilized in a bioassay. Serial concentrations of oxamyl and permethrin were prepared with acetone (Table 1). A 0.5-mL volume of each compound at each concentration was pipetted onto filter paper discs and allowed to dry. A non-treated control of acetone was used in all larval assays. Each treated filter paper was fitted into Petri dishes, with an additional 0.5 mL of water pipetted onto each treated filter paper once, and ten *L. decemlineata* neonates were added to each dish. Mortality was assessed 24 hrs after insecticide exposure. For the larval bioassays, the LC_{50} values were calculated based on mortality levels of 340 and 330 individuals, pooled from each location, and exposed to each concentration of permethrin and oxamyl, respectively.

Toxicity bioassays were analyzed with standard Probit analysis using statistical software, GraphPad Prism, version 5 (Motulsky 2007), to determine the permethrin and oxamyl LC_{50} values for small larvae and adult *L. decemlineata*. Differences in LC_{50} values between present day populations and those from 1990 were determined based on whether or not there were overlapping standard errors.

Results from the bioassays comparing LC_{50} levels of present-day *L. decemlineata* populations from the Eastern Shore of Virginia to the 1990 populations for permethrin and oxamyl are shown in Figure 1. The LC_{50} values of *L. decemlineata* adults and larvae from each population in 2012 were averaged and compared to the average LC_{50} values reported in 1990.

Adult LC_{50} values for oxamyl in 2012 were not significantly different from those reported by Tisler and Zehnder (1990). However, there was a significant decrease in the oxamyl LC_{50} value in 2012 larvae compared with the LC_{50} value for the larvae in 1990 (Fig. 1A). Results from the permethrin bioassays showed that *L. decemlineata* adults collected in 2012 had a significantly lower LC_{50} value compared to the adults in 1990. However, there was no significant difference in LC_{50} value for larvae exposed to permethrin in 2012 compared to the larvae LC_{50} value in 1990 (Fig. 1B).

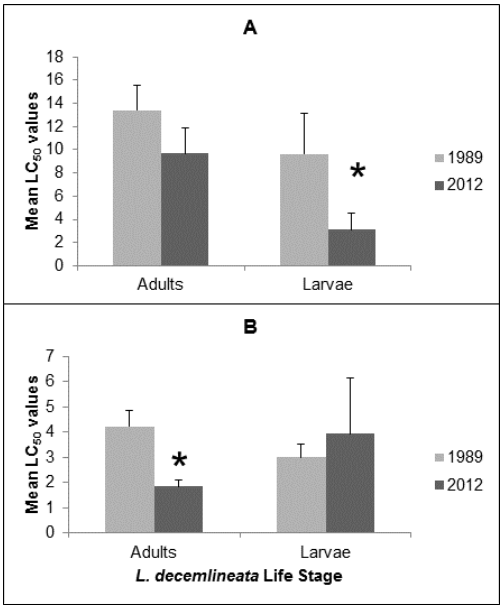

Figure 1: A.) Mean LC_{50} values (g ai/L) of oxamyl for Eastern Shore of VA populations of *L. decemlineata* in 1990 and 2012, (*) indicates significance. B.) Mean LC_{50} values (g ai/L) of permethrin for Eastern Shore of VA populations of *L. decemlineata* in 1990 and 2012, (*) indicates significance.

Leptinotarsa decemlineata has the ability to overcome insecticides labeled for its control. Current management practices of *L. decemlineata* in potato almost solely rely on the utilization of chemical insecticides. This has led to the development of resistance in this pest to almost every compound labeled for its control (Alyokhin et al. 2008). Without an adequate management program, *L. decemlineata* populations can cause up to 50% loss of yield in potato in Virginia (Kuhar et al. 2006, 2008, Kuhar and Doughty 2009, 2010). A responsible pest management program for *L. decemlineata* should include insecticide resistance management practices to minimize insecticide resistance development. Detection of resistant populations and then understanding the mechanism of resistance are vital for managing *L. decemlineata* when resistant populations are suspected. The purpose of this research was to evaluate the current resistance levels of *L. decemlineata* populations in the Eastern Shore of VA compared to the resistance levels in 1990.

Results obtained from the toxicity assays, comparing the LC_{50} values of Eastern Shore *L. decemlineata* populations from 2012 and 1990, varied by the compound and beetle life stage. The results indicated a significant decrease of LC_{50} value in adult beetles exposed to permethrin in 2012 compared to 1990. However, the LC_{50} value of the permethrin-exposed larvae in 2012 was not significantly different from the LC_{50} value in 1990; most likely due to the high amount of variability observed within those particular assays (Fig 1 B). In the toxicity assays measuring the LC_{50} value of oxamyl, there was a significant decrease in the larval LC_{50} value in 2012 compared to 1990. The results from the 2012 oxamyl-exposed adults did not show a significant difference in LC_{50} value compared to the 1990 LC_{50} value. One possible explanation of why we saw a decrease in LC_{50} value of oxamyl exposed larvae and not adults is that adults typically are more robust than the larvae and also may have more developed detoxification systems (Silcox et al. (1985). Oxamyl and permethrin are two compounds that *L. decemlineata* populations on the Eastern Shore of VA have historically shown resistance to (Tisler and Zehnder 1990). The resistance to permethrin and oxamyl coupled with the introduction of the neonicotinoid insecticides allowed growers in Virginia and elsewhere to stop using these compounds. The lack of selection pressure from these compounds should logically result in *L. decemlineata* populations that are more susceptible to oxamyl and permethrin than when these chemicals were frequently applied to potatoes. Alyokhin et al. (2008) suggested that once growers stop using a particular insecticide that a population of *L. decemlineata* is resistant to, there would most likely be a decline in the alleles that confer that resistance; however, the rate of that decline is unknown.

One of the recommendations for managing resistance in this pest is to include an untreated area in a potato field to allow susceptible beetles to survive and contribute to the genetic diversity of the population, preventing the development of homozygously-resistant populations (Alyokhin et al. 2008). This resistance management strategy is based on the need for keeping susceptible populations of *L. decemlineata* in the gene pool of a given field population. Research by Whalon and Ferro (1998), suggested 20% of a potato field untreated with insecticides should provide enough survival of a susceptible population to decrease mating between resistant beetles. Although it is unlikely that potato growers on the Eastern Shore of Virginia incorporated untreated portions in their fields, it is likely permethrin and oxamyl were not applied to potato fields because the beetle had become resistant to those compounds and newer more effective insecticides became available. The results from our toxicity assays have shown that the toxicity levels to permethrin and oxamyl have increased in *L. decemlineata* populations from the Eastern Shore of Virginia after 20 years of reduced use of these insecticides on potatoes.

REFERENCES

Alyokhin, A. V., M. Baker, D. Mota-Sanchez, G. Dively, and E. Grafius. 2008. Colorado potato beetle resistance to insecticides. Am. J. Potato Res. 85: 395–413.

Kuhar, T. P., and H. Doughty. 2009. Evaluation of foliar insecticides for the control of Colorado potato beetle in potatoes, 2008. Arthropod Manage. Tests 34: E71.

Kuhar, T. P., and H. Doughty. 2010. Evaluation of foliar insecticides for control of Colorado potato beetle and potato leafhopper in potatoes in Virginia 2009. Arthropod Manage. Tests 34: E71.

Kuhar, T. P., E. Hitchner, and A. Chapman. 2006. Evaluation of foliar insecticides on potatoes, 2005. Arthropod Manage. Tests 31: E56.

Kuhar, T. P., H. Doughty, E. Hitchner, and M. Cassell. 2008. Evaluation of foliar insecticides for the control of Colorado potato beetle in potatoes in Virginia, 2007. Arthropod Manage. Tests 33: E8.

Motulsky, H. J. 2007. Prism 5 Statistics Guide. Graph Pad Software Inc., San Diego, CA.

Silcox, C. A., G. M. Ghidiu, and A. J. Forgash. 1985. Laboratory and field-evaluation of piperonyl butoxide as a pyrethroid synergist against the Colorado potato beetle (Coleoptera: Chrysomelidae). J. Econ. Entomol. 78: 1399-1405.

Tisler, A. M., and G. W. Zehnder. 1990. Insecticide resistance in the Colorado potato beetle (Coleoptera: Chrysomelidae) on the eastern shore of Virginia. J. Econ. Entomol. 83: 666-671.

Whalon, M. E., and D. N. Ferro. 1998. Bt-potato resistance management., pp. 107-136. In M. Mellon and J. Rissler (eds.), Now or never: Serious new plans to save a natural pest control. Cambridge: UCS.

Adam Wimer and Thomas Kuhar
Department of Entomology, Virginia Tech, Blacksburg, VA 24061-0319

CUCURBIT POWDERY MILDEW FUNGUS (*PODOSPHAERA XANTHII*) EXHIBITS CROSS RESISTANCE TO SOME SDHI FUNGICIDES

ABSTRACT
A leaf disk bioassay was used to determine if isolates of the cucurbit powdery mildew pathogen, *Podosphaera xanthii*, that were resistant to boscalid were also resistant to other succinate dehydrogenase inhibitor (SDHI) fungicides. Boscalid is the first SDHI (FRAC code 7) fungicide registered for this use in the USA. Resistant isolates tolerate 500 ppm boscalid (formulated as Endura). The seven boscalid-resistant isolates tested were also able to grow on disks cut from cotyledon leaves sprayed with 500 ppm penthiopyrad (Fontelis) and on disks with 500 ppm fluxapyroxad (Merivon). These isolates were more sensitive to fluopyram (Luna Privilege), being able to tolerate 10 ppm but not 50 ppm fluopyram. Only one of the seven boscalid-sensitive isolates tested was able to grow on leaf disks treated with 500 ppm penthiopyrad and 500 ppm fluxapyroxad. Based on these results, boscalid-resistant isolates are cross resistant to penthiopyrad and fluxapyroxad, but not to fluopyram. Therefore Luna fungicides are recommended for managing powdery mildew in labeled cucurbit crops.

INTRODUCTION

Succinate dehydrogenase inhibitor (SDHI) fungicides (FRAC code 7) are important for managing cucurbit powdery mildew because their mobility in leaves enables control on the lower leaf surface where the pathogen, *Podosphaera xanthii*, develops best. Due to high potential for resistance development in the pathogen, applying an SDHI fungicide in alternation with other mobile fungicides also at risk for resistance development is recommended for managing resistance. Boscalid, the first SDHI fungicide developed, was registered in 2003. Strains of *P. xanthii* resistant to 500 ppm boscalid were first detected in the US in 2008. This dose is in the range of the field application rate. Boscalid-resistant strains were associated with control failure with the fungicide Pristine, which has boscalid as an active ingredient.

The goal of this study was to determine if sensitivity to new SDHI fungicides was correlated with sensitivity to boscalid (phenomenon called cross resistance). New SDHI fungicides include penthiopyrad (formulated as Fontelis), registered in the US for cucurbit powdery mildew in March 2012, fluopyram (Luna fungicides), registered in February 2012, and fluxapyroxad (Merivon) which has not yet been registered. Knowledge about occurrence of cross resistance is needed to be able to provide growers with sound recommendations about fungicides to use to manage cucurbit powdery mildew.

MATERIALS AND METHODS

Isolates of *Podosphaera xanthii* were collected in September 2012 from research and commercial cucurbit crops that had been treated with fungicides to manage powdery mildew. For this study, 14 isolates were selected from this collection based on their previously determined sensitivity to boscalid: half were sensitive and half were resistant (able to grow on leaf disks treated with 500 ppm boscalid). Isolates were maintained on detached pumpkin cotyledon leaves kept on water agar in petri dishes.

Fungicide sensitivity was determined with a leaf disk bioassay (McGrath et al 1996) (Figure 1). Pumpkin seedlings (*Cucurbita pepo* 'Sorcerer') at the cotyledon leaf stage (about seven-day-old) were sprayed with fungicide solutions using a DeVilbiss atomizer bottle attached to a compressed air source (30 psi). Treated plants were allowed to dry overnight, then disks were cut from the cotyledons using a #9 cork borer (9 mm diameter). Six disks of each treatment were placed on water agar in sectioned Petri plates. Each plate had four sections thus there were three treatments per plate plus a non-treated control. Disks were inoculated by transferring about 10-20 conidia to each disk center. Each disk in a plate was inoculated with each *P. xanthii* isolate and plates were incubated for at least 7 days at room temperature on a laboratory shelf under constant light supplied by aquarium bulbs. Pathogen growth was assessed when the control treatment showed good growth, with sporulating mildew covering an average of about 50% of leaf disk area. The percent leaf disk area colonized by sporulating mildew was recorded for each disk and averaged for each treatment. A *P. xanthii* isolate was considered to be insensitive (resistant) to a particular fungicide concentration if it was able to grow and sporulate on at least half of the treated disks. Usually, isolates that were able to grow on at least half of the disks exhibited little reduction in growth. An

isolate was considered to be sensitive if there was no growth on most disks. Usually there was no growth on any disks. Disks with no evident growth at the first assessment were re-examined about 7 days later to determine if there was any change in isolate sensitivity. In extremely rare circumstances, a very limited amount of mycelial growth has been observed with no sporulation at 10 days. In these situations sometimes conidial chains have formed several days later.

RESULTS AND DISCUSSION

Boscalid-resistant isolates were able to grow on disks cut from cotyledon leaves sprayed with 50 and 500 ppm penthiopyrad (formulated as Fontelis) and on disks with 50 and 500 ppm fluxapyroxad (Merivon) (Table 1; Figure 1). These isolates were more sensitive to fluopyram (Luna Privilege), being able to tolerate 10 ppm but not 50 ppm fluopyram. Only one of the seven boscalid-sensitive isolates tested was able to grow on leaf disks treated with 500 ppm penthiopyrad and 500 ppm fluxapyroxad. Most boscalid-sensitive isolates were unable to grow on leaf disks treated with 50 ppm fluxapyroxad or 50 ppm penthiopyrad (Figure 2). Based on these results, fluopyram appears to differ in activity from other SDHI fungicides. Therefore Luna fungicides are recommended over other SDHI fungicides for managing powdery mildew in labeled cucurbit crops. Currently only watermelon is labeled. Until Luna labels are expanded to include other cucurbit crops, either Fontelis or Pristine is recommended for use sparingly in a fungicide program with other mobile fungicides in FRAC group 3, 13, and U6.

Table 1: Sensitivity of 14 isolates of *Podosphaera xanthii* to SDHI fungicides. Isolate name is in column one, next two columns contain information about the isolate, then their sensitivity to four SDHI fungicides. R=resistant and S=sensitive.

			Sensitivity to SDHI Fungicides						
			Boscalid	Penthiopyrad		Fluxapyroxad		Fluopyram	
Isolate	Location	Date Collected	500 ppm	50 ppm	500 ppm	50 ppm	500 ppm	10 ppm	50 ppm
7-2	Research Field 1	9/26/12	S	S	-	S	-	-	S
3e5	Research Field 2	10/23/12	S	S	-	S	-	-	S
A-12	Long Island Farm 1	9/24/12	S	S	-	S	-	-	S
S-10	Long Island Farm 4	9/24/12	S	S	-	S	-	-	S
Z-2	Long Island Farm 5	9/24/12	S	S	-	S	-	-	S
A-6	Long Island Farm 1	9/24/12	S	S	-	R	-	-	S
D-3	Long Island Farm 2	10/5/12	S	R	R	R	R	R	S
7-4	Research Field 1	9/26/12	R	R	R	R	R	R	S
7-6	Research Field 1	9/26/12	R	-	R	-	R	R	-
3e2	Research Field 2	10/23/12	R	R	R	R	R	R	S
6e3	Research Field 3	10/23/12	R	R	R	R	R	R	S
L-1	Long Island Farm 3	10/5/12	R	R	R	R	R	R	S
S-2	Long Island Farm 4	9/24/12	R	R	R	R	R	R	S
Z-3	Long Island Farm 5	9/24/12	R	R	R	R	R	R	S

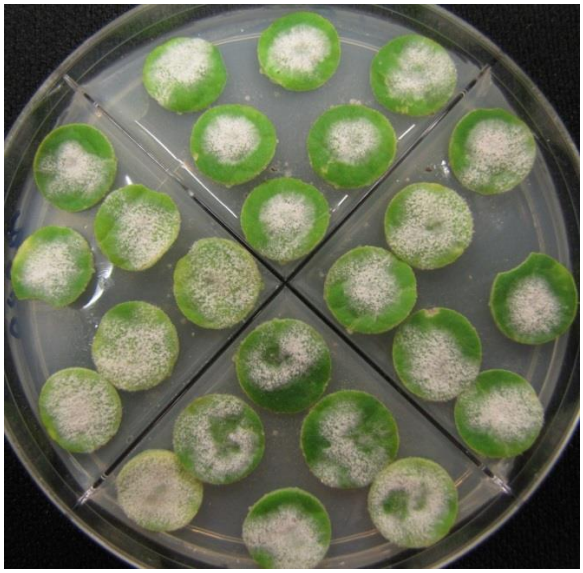

Figure 1: Leaf disk bioassay plate with boscalid-resistant isolate 6e3 tested at 500 ppm fluxapyroxad (left section), 500 ppm penthiopyrad (top section), and 10 ppm fluopyram. Similar to other boscalid-resistant isolates tested, this one grew as well on these fungicide-treated leaf disks as on the non-treated disks, which are in the bottom section.

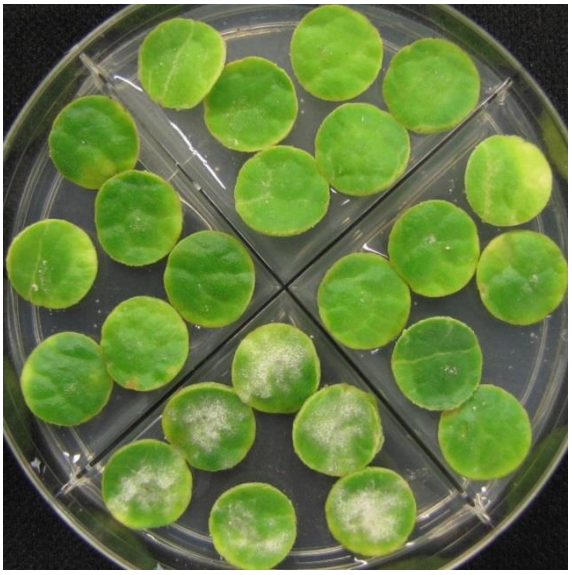

Figure 2: Leaf disk bioassay plate with boscalid-sensitive isolate S-10 tested at 50 ppm fluxapyroxad (left section), 50 ppm penthiopyrad (top section), and 50 ppm fluopyram. Similar to most boscalid-sensitive isolates tested, this one was only able to grow on the non-treated disks, which are in the bottom section.

REFERENCES

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

Margaret T. McGrath
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
631-727-3695 (phone)
mtm3@cornell.edu

WAYS OF OVERCOMING INSECTICIDE RESISTANCE: USE OF MIXTURES AND ROTATIONS OF INSECTICIDES

The number of species of arthropods having a resistant population is steadily increasing. Moreover, if pests are subjected to long and intensive breeding the frequency of resistance may stabilize at a high level over a wide area. For example, the hop aphid in England, green rice leafhopper in Japan, the Philippines, Taiwan and Vietnam, malarial mosquitoes almost all over the world. Today the three main chemical ways of preventing the emergence and development of resistance in pests that are offered are: the use of synergists, mixtures of insecticides and the rotation of drugs. In this article the most widely used methods that will be discussed are the use of mixtures of insecticides and their rotation. The use of mixtures of insecticides has found wide application not only in laboratory experiments, but also in field conditions. It is possible this approach produces such significant results because of the difficulty for insects to develop a few of adaptive reactions at the same time. In mixtures of insecticides as drugs of the same class are used as well as, more often, drugs of different classes with different mechanisms of action.

S.F. Barroga and B. Morallo-Rejesus (1981) studied the effectiveness of mixtures malathion with several organophosphorus compounds (OP) in two strains of *Plutella xylostella* highly resistant to malathion (RI=305-736). For both strains the mixture with potenziruty action containing mercury with mevinphos was founded. Potentiation of this mixture is connected with increasing of inhibition of cholinesterase inhibition in both strains of *P. xylostella*. A mixture of malathion with kitazin p proved to be effective against resistant to malathion resistant green rice leafhopper *Nephhotettix cincticeps* (Miyata et al., 1981). This mixture called kumichon was applied and against several types of rice leafhopper, multi-resistant to organophosphorus and carbamate insecticides. Kitazin p showed a high synergism in mixtures with fenthoat, diazinon, fenvalerate, dimethylvinilphosphat and carbaryl (CS from 83 to 3656) (Japan pesticide information, 1985). The binary mixtures of actellik with fozalon, gardona with chlorophos, gardona with actellik, actellik with ripcord or with reldan (1:1) were proposed by L.M. Didenko with co-authors (1983) for pest management reserves wheat weevil *Sitophylus granarius* and red flour beetles *Tribolium castaneum*.

Mixtures of fenvalerate with malathion (CS=113-120) and some carbamates (CS =81-357) showed big synergistic effect in the experiments with green rice leafhopper *N. cincticeps* resistant to OP and carbamates, and with the increase of the number of carbamates CS decreased (Ozaki et al.,1984). In the experiments on the pink box worm cotton *Pectinophora gossypiella* a mixtures of chlorpyriphos with deltamethrin and cypermethrin showed synergism (El-Guindy et al., 1982).

Researches of M. Auda and D. Degheele (1985) on the study of joint actions of the pyrethroids with chitin synthesis inhibitors (CSI), OP and carbamates on the sensitive and resistant strains *Spodoptera littoralis* showed the following. The synergism of the toxicity was for sensitive caterpillars when λ -cyhalothrin, cypermethrin and deltamethrin were mixed with metomyl and profenphos in doses appropriate $ЛК_{25}$ (1:1). A similar effect was observed in the resistant strains for mixing λ-cyhalothrin with profenphos and monocrotophos. For both strains the synergism showed a mixture of pyrethroids with chlorfluazuron and ftorfluazuron in the same doses and ratio. On caterpillars of IV age budworm *Choristoneura occidentalis* synergism showed a mixture of chlorfluazuron with permethrin in a ratio 9:1 (Robertson, Smith, 1984). To fight with budworms *Trichoplusia ni, Heliothis virescens, S. exigna* a mixture of insecticides phosmet and diflubenzuron was offered, the ratio of the components was from 3:1 to 1:3, preference was given to the ratio of 1:1. It has been shown in laboratory experiments, the LD_{50} of the mixture decreased approximately 10 times compared with each of the components used (Esmeralda, 1988). According to M. Auda and D. Degheele (1986) with feeding to caterpillars *S. littoralis* of sensitive and resistant to monocrotophos strains of leaves of castor, processed mixtures CHI chlorfluazuron and diflubenzuron with profenphos, monocrotophos and metomyl in the ratio of 1:1 ($ЛК_{25}$:$ЛК_{25}$) 70-100-fold increased toxicity of insecticides was noted. The

exception was the mixture of diflubenzuron with monocrotophos for sensitive strain. The greatest increased toxicity was observed for resistant strains for all combinations of insecticides with diflubenzuron. A synergistic effect manifested by a mixture of dimilin with juvenile hormone on *Anthonomus grandis.* This mixture increased frequency of failed outputs of adults in the processing of pupae in the later stages by 7 times (Leopold et al., 1985). Mixture of chlorpyriphos (47.5%) with lufenuron (2.5%) was effective against the 2nd instar larvae of *S. littoralis* after a feeding period for 48 h on treated leaves (Ahmed E.M. Abd El-Mageed, Shehata E.M. Shalaby, 2011).

Different mixtures of fenvalerate and avermectin also had synergistic action for *Myzus persicae* the mixture with a ratio of 24:1 was particularly effective (ratio of co-toxicity 277.84). (Lan et al., 2002). The coefficient of co-toxicity of mixtures abamectin and chlorpyriphos for *Liriomyza spp.* were 165-234. In field experiments these mixtures have shown high speed action and a good persistence (Zeng et al., 2002).

For joint use mixtures of bacterial preparations with insecticides are propose. For example, mixtures of some pyrethroids (fenvalerate, cypermethrin, permethrin), and many of organophosphorus compounds with *Bacillus thuringiensis var. galleriae* against *S. littoralis* (Salama et al.,1984), a mixture of 0.1% of the bacterial preparation dipel with ambush, decis and ripcord in sublethal doses (Кузманова, Лечева, 1984), suspensions of microbial insecticides (the virus of nuclear polyedros, *B.thuringiensis*, *Beauveria bassiana*) and chemical ones (fenvalerate, monocrotophos) (Jayanthi, Padmavathamma, 2001). In laboratory experiments synergistic action took place in relation of mortality of larvae II age of the Colorado potato beetle when they were fed with potatoes leaves, processed together by subletal doses of imidacloprid and number of doses of *Beauveria bassiana*. Similar results were obtained in the version when the larvae were directly sprayed with suspension of conidia and immediately fed leaves treated with imidacloprid (Furlong, Groden, 2001).

Naturally, not all of the mixtures have synergistic effect. Some insecticides form antagonistic mixtures, for example: chlorpyriphos with metomyl, miotrin, profenphos, fenvalerate; monocrotophos with pyrethroids; cypermethrin with deltamethrin, miotrin, profenphos and metomyl and some other (El-Guindy et al., 1982). In addition insects can also form resistance and to the mixture of insecticides. For example, house flies, processed with mixture of permethrin and dichlorvos (DDVF) for 8 generations formed 6-7-fold resistance to each of the drugs (Mac-Donald, 1983a).

Use of mixtures is effective when resistance is determined by not completely dominant gene, while they may significantly slow down the development of resistance. In the case of dominant genes, there is a rapid development of resistance (Curtis, 1985).

The most common measure to overcome the resistance is rotation of insecticides. However not ever rotation slows down the development of resistance. It is observed in those cases when it is possible to choose drugs for alternation, genes of resistance to which belong to the same group of adhesion (Zilbermints, Smirnova, 1979). Selection of drugs for rotation must be made for each type of pests.

R. Mac-Donald with co-authors (1983a) studied the development of resistance to permethrin and dichlorvos in the house flies with constant or alternating application of insecticides on the farms of the Ontario province (Canada). At alternating application of insecticides resistance to both almost did not change, at constant processing of manure by one of the drugs resistance increased in several times. The same authors (1983b) carried out a similar study in the laboratory conditions on the same object with permethrin and dichlorvos. In the strain selected, permethrin for 8 generations RI increased in 73 times, and the strain selected alternating two insecticides - in 31 times. The replacement of preparations: carbamates, OP and pyrethroids prevented the emergence of resistance in the Colorado potato beetle (Cooper, Lindquist, 1983).

In the fight against cotton bollworm in Tajikistan there was a system for rotation of the following drugs: organochlorine (OC), organophosphates compounds, carbamates, pyrethroids, microbiological means, acaricides and aficides. The check has shown, that within 5 years the resistance of bollworm to OC decreased from 98- to 7-fold, to the sevin from 23 to 5, to fozalon for a number of years does not exceed 2, 2-5. The development of resistance of bollworm to thiodan and pyrethroids has not marked, as well as in aphids to the applied preparations (Suchorucenco, Smirnova, 1984).To stop the process of the development of resistance in the Colorado potato beetle to the applicable control measures in Tajikistan tactics of application of insecticides in the seasonal schemes struggle is based on alternation of drugs with different mechanism of action on the basis of sensitivity to him of the pest. To prevent the formation of resistance to pyrethroids the best combination is with neonicotinoids or microbiological preparations. In this case, the processing in a phase of budding should be conducted pyrethroid to which the population is sensitive, and in the flowering phase – confidor or bitoxibacillin to reduce the risk of accumulation of toxic residues in the tubers (Kakharov, 2008).

In Russia, for struggles with resistant to organophosphates compounds *Tortricoidea* family the schemes of rotation of insecticides were introduced into practice, which included new OP, pyrethroids and biological products. Five years of application of these schemes has shown that the level of resistance to the previous OP reduced (Tolstova, Burkova, 1988). When used in the North-Caucasian region of the pyrethroids and organophosphates in rotation with actara and mospilan there is the containment of resistance in bug *Eurygaster integriceps* within 4-26, while at treatment only pyrethroids and organophosphates resistant indicator varies from 33 to 260 (Kovalenkov et al., 2007).

At the same time, the studies of G. Georghiou with co-authors (1982) on *Culex quinquefasciatus* on rotation of insecticides have shown that replacement of selectant (permethrin, temephos, propoxur and diflubenzuron) in each generation quickly caused resistance to permethrin, and the faster, the more often it was used. A small resistance appeared also in relation to the propoxur.

Thus, the use of rotation of insecticides deserves attention as a tactic that prevents the development of resistance. The rotation of select insecticides with a different mechanism of action is needed to prevent the rapid development of cross-resistance.

REFERENCES

Ahmed E.M. Abd El-Mageed and Shehata E.M. Shalaby. 2011. Toxicity and Biochemical Impacts of Some New Insecticide Mixtures on Cotton Leafworm *Spodoptera littoralis* (Boisd.). Plant Protect. Sci. V. 47. No. 4. P. 166–175.

Auda M., Degheele D. 1986. Joint action of critin synthesis inhibitors with profenofos, monocrotopnos or methomyl on a susceptible and resistant strain of *Spodoptera littoralis* (Boisd) // Meded. Fac. Landbouwwetensch. Rijksuniv. gent. - V.51. N3B. Deel 4. P.1239-1243.

Auda M., Degheele D. 1985. Joint action of pyrethroids with critin syntesis inhibitors, organophosphorous and carbamate insecticides on a susceptible and resistant strain of *Spodoptera littoralis* (Boisd) // Meded. Fac. landbowwetensch. Rijksuniv gent. V.50. N26. P.751-760.

Barroga S.F., Morallo-Rejesus B. 1981. Mechanism of joint action of insecticides on malathion-resistant diamondback moth (*Plutella xylostella* L.) // Philipp. Entomol. V.5. N1. P.115-137.

Cooper R.M., Lindquist R.K. 1983. Seek solution for problem with *Colorado potato beetle* // Ohio Rept. Res. and Dev. Agr., Home Econ., and Natur. Resour. V. 68. N. 3. P. 45-47.

Curtis C.F. 1985. Theoretical models of the use of insecticide mixtures for the management of resistance // Bull. Entomol. Res. V.75. N. 2. P. 259-265.

Didenko L.M., Roslavceva S.A., Agashcova T.M. 1983. The toxicity of binary mixtures of insecticides for insects // Chem.in the agr. V.21. N.12. P. 32-34.

El-Guindy M., Abdel-Sattar M., Dogheim S., Madi S., Issa Y. 1982a. The joint action of certaion insecticides on a field strain of the pink bollworm *Pectinophora gossypiella* Saund // Int. pest. Congr. V. 24. N. 6. P. 154-155.

El-Guindy M., El-Refai A.-P., Saleh W. 1982b. The role of esterases in the defence mechanism against intoxication by fenitrothion in susceptible and field tolerant strains of *Spodoptera littoralis* Boisd // Int. Pest cjntr. 4. N 4. P. 100-106.

Esmeralda R. 1981. Sherman steufleer chemical Co. Phosmet-diflubenzuron insecticidal composition // Pat. 4382927, USA. 13.07.81 N2T2469, publ. 10.05.88, ЛЕНИ А 01 N57/10, А 01 N47/28, НКИ 424/200.

Furlong M.J., Groden E. 2001. Evaluation of synergistic interactions between the Colorado potato beetle (Coleoptera: Chrysomelidae) pathogen *Beauveria bassiana* and the insecticides, imidacloprid, and cyromazine // J. Econ. Entomol. V. 94. N.2. P. 344-356.

Georghiou G.P., Lagunes A., Baker J.D. 1982. Effect of insecticide rotations on evolution of resistance // The fifth intern. congr. of Pestic. Chemistry. Hum. Welfare-Environ.-Pesticides Abstr. Aug. 29.-Sept.4. Kioto, Japan.

Japan pesticide information. 1985. On the control of insecticide resistance rice leafhoppers and planthoppers // Japan pesticide information. 47. P.14-16.

Jayanthi P.D.K., Padmavathamma K. 2001. Join action of microbial and chemical insecticides on *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae) // J. Trop. Agr. V. 39. N 2. P. 142-144.

Kakharov K. C. 2008. Toxicological peculiarities of Colorado beetle (*Leptinotarsa decemlineata*, Say) and measures to prevent it in the conditions of Tajikistan. Abstract of diss. ... doctor of agric. Sci. St. Petersburg. 40 p.

Kovalenkov V., Tyurina N.M., Kozadaeva S.V. 2007. The resistance of phytophages to insectoacaricides // Achievements of entomology at the service of the agro-industrial complex, forestry and medicine: Abs. of the 13th Congress of the Russian entomological society, Krasnodar, 9-15 St., 2007. Krasnodar. P.101.

Kuzmanova Й., Lecheva И. 1984. В'зможности за с'вместно използуване на препарата дипел с

намалени дози пиретроиди // Градин. и Лозар. наука. Т..21. N. 5. P. 35-39.

Lan Y.-q., Zhao S.i-x., Liu X., Lin W. 2002. Синергизм действия смесей авермектина и фенвалерата в отношении Myzus *persicae* // Jiangxi nongye daxue xuebao = Acta Agr. Univ. Jiangxi. V. 24, N. 3. P. 383-385.

Leopold R.A., Marks E.P., Eaton G.K., Knoper J. 1985. Ecdysial failures in the cotton boll weevil; synergism of diflubenzuron with jiwenile hormone // Pestic. Biochem. and Physiol. V. 24, N. 2. P. 267-283.

Mac-Donald R.S. 1983a. Development of resistance to permethrin and dichlorvos by the house fly (Diptera: Muscidae) following continous and alternating insecticide use on four farms // Can. Entomol. V.115. N.12. P. 1555-1561.

Mac-Donald R.S., Surgeoner G.A., Solomon K.R., Harris C.R. 1983b. Effect of four spray regimes on the development of permethrin and dichlovos resistance, in the laboratory, by the house fly (Diptera: Muscidae) // J. Econ. Entomol. V.76. N3. P.417-422.

Miyata T., Sacai H., Saito T., Voshioka K., Ozaki K., Sasaki Y., Tsuboi A. 1981. Mechanism of joint toxic action of kitazin p with malathion resistant green rice leafhopper, *Nephotettix cincticeps* Uhler (Hemiptera: Deltocephalidae) // Appl. Entomol. and Zool. V.16. N. 3. P. 258-263.

Ozaki K., Sasaki Y., Kassai T. 1984. The insecticidal activity of mixtures of pyrethroids and organophospates or carbamates against the insecticideresistant green rice leafhopper *Nephotettux cincticeps* // J. Pest. Sc. V. 9. N. 1. P. 67-72.

Robertson J.L., Smith K.C. 1984. Western spruce budworm: joint action of pyrethroids and insect growth regulators by contact or inqestion // J. Ga. Entomol. Soc. V.19. N.4. P.454-462.

Salama H.S., Foda M.S., Zaki F.N., Moawand S. 1984. Potency of combinations of *Bacillus thuringiensis* and chemical insecticides on *Spodoptera littoralis* (Lepidoptera: Noctuidae) // J. Econ. Entomol. V.77. N. 4. P. 885-890.

Suchoruchenco G.I. 2000. Monitoring, strategy and tactics of struggle with resistance to pesticides in the UIS countries (the results of research in the 90-ies) // Modern state of the problem of resistance of pests, pathogens and weeds to pesticides in Russia and the neighbouring countries at the turn of the XXI century. Mater. 9-th meeting. Saint-Petersburg, 20-22 December 2000. S-Pb. P. 8-9.

Tolstova Y.U., Burkova L.A. 1988. Reversion of the sensitivity of leafrollers on the background of schemes of rotation of insecticides // The problem of the resistance of pests and plant pathogens to the chemical means of protection and its overcoming. Tez. rep. 7-th meeting. 14-18 of Nov.1988. Riga. P.15-16.

Zeng Y.-L., Kang L., Qin X.-W., Wang Da-S., Zhang W.-Ji, Wang J., Wang T.-S., Liao C.-Zu. 2002. Эффективность синергистических смесей хлорпирифоса и абамектина против *Liriomyza spp*// Kunchong xuebao = Acta entomol. Sin. V. 45. N. 5. P. 603-610.

Zilbermints I.V., Smirnova A.A. 1979. The problem of resistance of arthropods to insectoacaricides and methods of its overcoming // Resistance of pests to chemical means of protection of plants. M. P. 3-10.

M.P. Sokolyanskaya
Institute of Biochemistry and Genetics,
Ufa Scientific Center of RAS.
450054, Ufa, October Prospect, 71, Russian Federation
e-mail: sokolyanskaya-m@yandex.ru

Research in Resistance Management

FUNGICIDE SENSITIVITY EXAMINED FOR POPULATIONS OF THE CUCURBIT POWDERY MILDEW FUNGUS (*PODOSPHAERA XANTHII*) ON LONG ISLAND, NY, IN 2011 AND 2012

ABSTRACT

Managing fungicide resistance is an important aspect of controlling powdery mildew of cucurbit crops because fungicides that are most effective, due to their mobility in leaves, are at risk for resistance development and the pathogen has high potential to develop resistance. The goal of this study was to examine the sensitivity to currently-available fungicides in populations of the pathogen in field-grown cucurbit plantings. A seedling bioassay was conducted in these fields. Resistance to FRAC Code 1 fungicide (MBC; Topsin M) was detected in all populations examined at high levels. Resistance to FRAC Code 11 fungicides (QoI) was also detected in all populations examined, typically at high levels. Therefore fungicides in these chemical groups are not recommended for managing cucurbit powdery mildew. Also in both 2011 and 2012, strains of the pathogen were detected with the ability to tolerate 500 ppm boscalid (active ingredient in Pristine; FRAC Code 7), 120 ppm myclobutanil (Rally; Code 3) and 10 ppm quinoxyfen (Quintec; Code 13). Ability to tolerate 500 ppm boscalid is of concern because this concentration is in the range of what would be in the spray tank when Pristine is applied. Only minor differences were detected in

sensitivity among the four FRAC Code 3 (DMI) fungicides tested. Based on these results, Quintec is expected to be the most effective fungicide for cucurbit powdery mildew. Quintec should be applied in alternation with FRAC Code 3 fungicides for resistance management and to comply with label restrictions. Including an application of Pristine in the fungicide program will likely be effective while the frequency of resistant strains is low.

INTRODUCTION

Producing a high-quality cucurbit crop necessitates effectively managing powdery mildew. This foliar, fungal disease is common because the pathogen produces spores easily dispersed by wind. While fruit are not affected directly, powdery mildew causes leaves to die prematurely which results in fewer fruit and/or fruit of low quality (poor flavor, sunscald, poor storability).

Powdery mildew is managed with resistant varieties and fungicides. An integrated program with both management tools is needed to achieve effective control because the pathogen is adept at evolving new strains resistant to individual tools that thus are not controlled as well by the tool. Resistant varieties have not provided as effective control in recent years as before. But they remain an important tool.

The recommended fungicide program for powdery mildew is weekly applications of targeted, mobile fungicides tank mixed with a protectant fungicide beginning very early in powdery mildew development. Mobile fungicides are needed for control on the underside of leaves where the pathogen develops best. The action threshold for starting applications is one leaf with symptoms out of 50 older leaves examined. Powdery mildew usually begins to develop around the start of fruit production. Alternating among targeted fungicides and applying them with a protectant fungicide is recommended to manage resistance development and avoid control failure if resistance occurs, and also to comply with label use restrictions.

Some fungicides are no longer recommended because resistant pathogen strains have been sufficiently common to render them ineffective: Topsin M (FRAC code 1; MBC fungicide) and QoI fungicides (Code 11), which include Quadris, Cabrio and Flint.

Other fungicide chemistry has remained adequately effective to include in a fungicide program although the pathogen has developed some resistance, in particular the DMI fungicides (Code 3), which include Procure, Rally, Tebuzol, Folicur, and Inspire Super. They remain effective partly because resistance to this group is quantitative, whereas to the Code 1 and 11 fungicides it is qualitative (pathogen is sensitive or resistant), and these DMI fungicides are inherently more active than the first DMI fungicide, Bayleton, which is no longer registered for this use because of control failures due to resistance. Highest label rate is recommended when resistance is quantitative or might be (generally assumed to be until known). Procure applied at its highest label rate provides a higher dose of active ingredient than the other Code 3 fungicides.

Quintec (FRAC Code 13) has been the most consistently effective fungicide in fungicide evaluations, therefore it has been recommended as the main mobile fungicide to use on labeled crops (pumpkin, winter squash, gourd, melon) where the crop rotational restriction of 12 months is acceptable. Recent crop additions to the Quintec label have increased the options of what can be planted within 12 months of the last application. The Quintec label specifies no more than two consecutive applications plus a crop maximum of four applications.

FRAC Code 7 is the third fungicide chemistry recommended for managing powdery mildew. Boscalid is the only active ingredient in this fungicide group labeled currently for this use. It is in the product Pristine. While highly resistant pathogen strains have been detected, Pristine has continued to provide some control in fungicide efficacy experiments.

The goal of this study was to examine pathogen populations to obtain information about sensitivity to fungicides that are currently in use. The seedling bioassay procedure used in this study provides estimates of the proportion of a population able to tolerate fungicides at the concentrations applied to the seedlings. It was conducted in commercial crops and research fields during the 2011 and 2012 season.

MATERIALS AND METHODS

Pumpkin seedlings were used in the bioassay to examine fungicide sensitivity of populations of the cucurbit powdery mildew fungus. They were produced in a growth chamber to about the one-leaf stage and then moved to a greenhouse and put in pots. After they had produced two true leaves, they were treated with various doses of different fungicides applied to coverage with a CO_2-pressurized backpack sprayer, the next day put in the field for at least 4 hours (Figure 1), then kept in a greenhouse at LIHREC for about 10 days until powdery mildew was visible and could be assessed. Amount of mildew on leaves of treated plants was compared with leaves on non-treated plants to estimate the proportion of the pathogen population able to tolerate each fungicide concentration. While the seedlings were in the greenhouse before the treatments were applied, powdery mildew started to develop. These symptoms were marked out with a marking pen. Occurrence of the disease before the bioassays started may have confounded results because these fungicides are not effective for established

infections; it is possible there was some additional symptom development after the early symptoms were marked out. Fungicide concentrations tested were 50 ppm trifloxystrobin (formulated as Flint); 50 ppm thiophanate-methyl (formulated as Topsin M), 1 and 10 ppm quinoxyfen (formulated as Quintec); 50, 175, and 500 ppm boscalid (formulated as Endura); 40, 80, and 120 ppm myclobutanil (formulated as Nova 40WP), 40 ppm triflumizole (formulated as Procure), 40 ppm difenconazole (formulated as Inspire), and 40 ppm tebuconazole (formulated as Tebuzol).

RESULTS AND DISCUSSION

In 2011, the first bioassay was conducted on 26th of July in spring-planted summer squash in commercial crops and research fields at LIHREC. The second and third bioassays were conducted on the 13th and 21st September in commercial pumpkin fields and in research plots of pumpkin and squash (Figure 1).

Figure 1: Fungicide-treated seedlings for bioassay in a commercial pumpkin crop in September 2011.

Resistance to FRAC Code 1 and Code 11 fungicides were detected in all populations examined, typically at high levels. Resistance to these chemistries is qualitative and cross resistance occurs amongst all fungicides in each group; thus a pathogen strain able to tolerate 50 ppm of any fungicide in each group is completely resistant and would not be controllable with any fungicide in the group. The bioassay results supported not recommending Topsin M, Quadris, and other fungicides in these groups in 2011; similar results were obtained in previous years.

Strains of the pathogen were detected able to tolerate 500 ppm boscalid (active ingredient in Pristine), 120 ppm myclobutanil (Rally) and 10 ppm quinoxyfen (Quintec). Ability to tolerate 500 ppm boscalid is of concern because this concentration is in the range of what would be in the spray tank when Pristine is applied. Resistance is common to the other active ingredient in Pristine, which is in FRAC code 11. Therefore Pristine would not be expected to be able to control these strains. On average, a lower proportion of the pathogen populations were able to tolerate 10 ppm quinoxyfen than 500 ppm boscalid or 120 ppm myclobutanil. The proportion was lowest for 10 ppm quinoxyfen. Therefore Quintec was expected to be the most effective fungicide in 2011.

Four FRAC Code 3 (DMI) fungicides were included in the bioassays at the same dose (40 ppm) to assess whether there are inherent differences in activity among fungicides in this chemical group. Only minor differences were detected which were not considered to be important. The fungicides tested were Rally, Procure, Tebuzol and Inspire. There are differences in the dose that can be applied to crops with these. The dose in the spray tank when these fungicides are applied at the highest labeled rate at 50 gpa are 263 ppm for Inspire Super, 300 ppm for Rally, 363 ppm for Tebuzol, and 527 ppm for Procure.

In 2012, the bioassay was conducted on the 17th of July in five locations in three research fields at LIHREC. Resistance to FRAC Code 1 fungicide (MBC; Topsin M) was detected in all populations examined at high levels. Resistance to FRAC Code 11 fungicides (QoI) was detected in all populations examined, typically at high levels. The bioassay results supported not recommending Topsin M, Quadris, and other fungicides in these groups in 2012. Also similar to 2011, strains of the pathogen were detected able to tolerate 500 ppm boscalid (active ingredient in Pristine), 120 ppm myclobutanil (Rally) and 10 ppm quinoxyfen (Quintec). Therefore Quintec was expected to be the most effective fungicide in 2012. Quintec was very effective while Pristine was ineffective in a fungicide efficacy experiment conducted at LIHREC in 2012.

ACKNOWLEDGEMENTS:

Project partly funded by Friends of Long Island Horticulture Grant Program and Hatch Act Formula Funds.

Margaret T. McGrath
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
631-727-3695 (phone)
mtm3@cornell.edu

FUNGICIDE SENSITIVITY OF CUCURBIT POWDERY MILDEW FUNGUS (*PODOSPHAERA XANTHII*) ON LONG ISLAND, NY, IN 2010-2012

ABSTRACT

Fungicide resistance can be a major constraint to effectively managing powdery mildew in cucurbit crops. The objective of this study was to determine fungicide sensitivity of isolates of the pathogen, *Podosphaera xanthii*, by testing them in the laboratory on treated leaf disks. Isolates were obtained in September and October at the end of the growing season in 2010, 2011, and 2012 from commercial pumpkin crops and research plantings. Most isolates tested were resistant to FRAC Code 11 fungicides. Resistance to FRAC Code 1 fungicides was also found to be high when examined, which was in 2010 and 2011. Isolates resistant to the FRAC Code 7 fungicide boscalid (able to grow on leaf disks treated with 500 ppm boscalid) were detected in all three years. They were most common in 2010. A low percentage of isolates able to tolerate 80 ppm myclobutanil, a FRAC Code 3 fungicide, were detected in all years. A low percentage of isolates able to tolerate 80 ppm quinoxyfen were detected in 2011 and 2012. One of the 2011 isolates was insensitive to 80 ppm myclobutanil and also to 40 ppm quinoxyfen as well as being fully resistant to boscalid and FRAC Code 1 and 11 fungicides. Existence of pathogen isolates like this one with resistance or elevated insensitivity (compared to other isolates) to all labeled fungicide chemistries is a concern for continued effective management of cucurbit powdery mildew with currently-registered fungicides. One of the 2012 isolates was insensitive to 80 ppm myclobutanil and to 80 ppm quinoxyfen as well as being fully resistant to boscalid and FRAC Code 11 fungicides (resistance to FRAC Code 1 fungicides was not determined).

INTRODUCTION

Fungicide resistance can be a major constraint to effectively managing powdery mildew in cucurbit crops. The most effective fungicides for this disease are prone to resistance because they have single site mode of action. The pathogen, *Podosphaera xanthii*, has a long history of developing resistance, being the first pathogen to have been documented to have done so in the USA. Resistance to benomyl (FRAC Code 1), the first at-risk fungicide registered for this use, was detected in 1967. The next chemical class registered for cucurbit powdery mildew was the DMI (demethylation inhibitor) fungicides (FRAC Code 3) (McGrath, 2001). Triadimefon, the first active ingredient in this group, was registered for cucurbit powdery mildew in the USA in April 1984. Just two years later, the first reported control failure documented through university fungicide efficacy experiments occurred. QoI (quinone outside inhibitor) fungicides (FRAC Code 11) were the next chemical class developed for this disease. Azoxystrobin was registered in the USA in spring 1999. Control failures were reported from several states throughout the USA in 2002, and resistance was detected.

Several fungicides at risk of resistance developing are registered in the USA. There are fungicides in FRAC Groups 1, 3, 7, 11, and 13. Information is needed on pathogen sensitivity to these fungicides in order to be able to develop guidelines to develop an effective fungicide program. The objective of this study was to determine fungicide sensitivity of isolates of the pathogen, *Podosphaera xanthii*, by testing them in the laboratory on treated leaf disks.

MATERIALS AND METHODS

Isolates of *Podosphaera xanthii*, the fungus that causes powdery mildew in cucurbits, were obtained in September and October at the end of the growing season in 2010, 2011, and 2012 from commercial pumpkin crops and research plantings. They were maintained on cotyledons of pumpkin (cv. Sorcerer) on agar media in Petri dishes (culture plates) until tested.

For the leaf disk bioassay, pumpkin seedlings (cv. Sorcerer) at the cotyledon leaf stage (about seven-days-old) were sprayed with various fungicide doses in a laboratory fume hood. The treated plants were left there to dry overnight and disks were cut from the cotyledons and placed on water agar in sectioned Petri plates. Each plate has four sections thus there were three treatments per plate plus a non-treated control. Each plate was used to test one isolate. Six disks with the same treatment were placed in each section. Disks were inoculated by transferring spores from culture plates to each disk center. Then plates were incubated at room temperature on a laboratory shelf under constant light supplied by aquarium bulbs. Amount of pathogen growth on the disks was assessed after 10 days of incubation when the control treatment usually had good growth of the pathogen, with white sporulating pathogen growth covering an average of about 50% of leaf disk area (Figure 1).

Figure 1: Leaf disk bioassay with an isolate of *Podosphaera xanthii* able to tolerate 80 ppm myclobutanil (left section) and 10 ppm quinoxyfen (top section), but not 100 ppm boscalid (right section). The lower section has the non-fungicide-treated control disks.

The percent leaf disk area with symptoms of powdery mildew was recorded for each disk and averaged for each treatment. An isolate was considered to be insensitive (resistant) to a particular fungicide concentration if it was able to grow and produce spores on at least half of the disks. Due to limitations in the number of isolates and fungicide doses that can be done in each bioassay, the procedure was conducted multiple times over many weeks to obtain information on sensitivity to several fungicides. Fungicide concentrations tested were 50 ppm trifloxystrobin (formulated as Flint); 50 ppm thiophanate-methyl (formulated as Topsin M), 10, 40, and 80 ppm quinoxyfen (formulated as Quintec); 25, 100, and 500 ppm boscalid (formulated as Endura); and 10, 20, 40, and 80 ppm myclobutanil (formulated as Nova 40WP).

RESULTS AND DISCUSSION

2010 isolates of *Podosphaera xanthii*. Sensitivity to fungicides was determined for 96 isolates collected in 2010. Almost all isolates (97%) tested with thiophanate-methyl were resistant to this fungicide group (MBC; FRAC code 1). Resistance to QoI fungicides (FRAC code 11) was detected in 98% of the isolates tested. Resistance to both fungicide groups is qualitative, thus pathogen isolates are either sensitive or resistant, and fungicides are ineffective against resistant isolates. These results support the recommendation to no longer use fungicides containing only these chemistries for managing powdery mildew. There is a fungicide (Pristine) with a FRAC code 11 active ingredient that has continued to be recommended because it contains another active ingredient (FRAC code 7). Applying Pristine could select for pathogen strains resistant to FRAC code 11 fungicides, thereby maintaining this resistance in the pathogen population. The very high frequency of resistance to FRAC code 1 fungicides, despite very limited use for other pathogens, indicates that there is no 'fitness cost' of this trait that would cause resistant strains to be at a competitive disadvantage with sensitive strains in the pathogen population.

Ability to grow on leaf disks with a high concentration (500 ppm) of boscalid, an active ingredient in Pristine, was detected in 43% of the pathogen isolates tested. This concentration is in the range of what would be in the spray tank when Pristine is applied at labeled rates, therefore isolates tolerating 500 ppm are likely fully resistant to this fungicide, which means they would not be controlled by Pristine. Isolates collected in 2010 were more sensitive to the other fungicides tested, which represent the other two fungicide chemistries recommended for managing cucurbit powdery mildew.

Myclobutanil, the active ingredient in Rally (aka Nova), a FRAC code 3 fungicide, at 20 ppm was tolerated by 47% of the isolates. Only 11% were insensitive to 80 ppm myclobutanil. Quinoxyfen, the active ingredient in Quintec, a FRAC code 13 fungicide, at 10 ppm was tolerated by 42% of the isolates. One isolate tested was insensitive to 80 ppm myclobutanil and also to 40 ppm quinoxyfen as well as being fully resistant to boscalid and code 1 and 11 fungicides. Existence of pathogen isolates like this one with resistance or elevated insensitivity (compared to other isolates) to all labeled fungicide chemistries is a concern for continued effective management of cucurbit powdery mildew with currently-registered fungicides. Further evolution could result in development of full (practical) resistance to all fungicides.

2011 isolates of *Podosphaera xanthii*. Sensitivity to fungicides at risk for resistance development was determined for 55 isolates collected in 2011. Resistance to MBC fungicides (FRAC code 1) was detected in 50% of the isolates tested (not all isolates were tested with this fungicide). Resistance to QoI fungicides (FRAC code 11) was detected in 79% of the isolates tested (not all isolates were tested with this fungicide). Resistance to this fungicide chemistry is qualitative, thus pathogen isolates are either sensitive or resistant, and fungicides are ineffective against resistant isolates. There is a fungicide (Pristine) with a FRAC code 11 active ingredient that has continued to be recommended because it contains another active ingredient (FRAC code 7). Applying Pristine could select for pathogen strains resistant to FRAC code 11

fungicides, thereby maintaining this resistance in the pathogen population. Resistance to most fungicide chemistry is quantitative, including active ingredients in Pristine, Procure, Rally, and Quintec. With this type of resistance, pathogen isolates exhibit a range in sensitivity. Several concentrations are used in assays to characterize sensitivity. Ability to grow on leaf disks with a high concentration (500 ppm) of boscalid, an active ingredient in Pristine, was detected in only 6% of the pathogen isolates tested. This concentration is in the range of what would be in the spray tank when Pristine is applied at labeled rates, therefore isolates tolerating 500 ppm are likely fully resistant to this fungicide, which means they would not be controlled by Pristine. Each of the three isolates was obtained from a different farm. In contrast, 43% of the isolates collected in 2010 from similar locations were resistant. With myclobutanil, the active ingredient in Rally, a DMI (FRAC code 3) fungicide, 4% of isolates tolerated 80 ppm, 33% tolerated 40 ppm, while 16% were sensitive to 10 ppm. The concentration in the spray tank would be 150 ppm for Rally applied at the lowest label rate (2.5 oz/A) and 50 gpa. With quinoxyfen, the active ingredient in Quintec (FRAC code 13) fungicide, 4% of isolates tolerated 80 ppm, 24% tolerated 40 ppm, while 22% were sensitive to 10 ppm. The concentration in the spray tank would be 141 ppm for Quintec applied at the lowest label rate (4 fl oz/A) and 50 gpa. One of the two isolates able to tolerate 80 ppm quinoxyfen was also resistant to boscalid. Sensitivity to thiophanate-methyl was examined for some isolates to determine if the pathogen is maintaining resistance to this old fungicide. It was found that resistance continues to be common to this fungicide group (MBC; FRAC code 1); however, there were fewer resistant isolates in 2011 (50%) than in previous years when most isolates were resistant (97% in 2010).

2012 isolates of *Podosphaera xanthii*. Sensitivity to fungicides at risk for resistance development was determined for 55 isolates collected in 2012. Resistance to QoI fungicides (FRAC code 11) was detected in 85% of the isolates tested (not all isolates were tested with this fungicide). Resistance to thiophanate-methyl was not determined for 2012 isolates. Resistance to boscalid (tolerant of 500 ppm boscalid) was detected in 16% of the isolates tested. These isolates came from the three research fields and three of the six commercial plantings. All isolates tested that were sensitive to 500 ppm boscalid were able to grow on leaf disks treated with 100 ppm boscalid. With myclobutanil, the active ingredient in Rally, a DMI (FRAC code 3) fungicide, 13% of isolates tolerated 80 ppm and 45% tolerated 40 ppm. All but one of the remaining isolates that were tested further were found to be able to tolerate 10 ppm myclobutanil. With quinoxyfen, the active ingredient in Quintec (FRAC code 13 fungicide), 13% of isolates tolerated 80 ppm and 27% tolerated 40 ppm. All of the remaining isolates that were tested further were found to be able to tolerate 10 ppm quinoxyfen. One isolate was insensitive to 80 ppm myclobutanil and to 80 ppm quinoxyfen as well as being fully resistant to boscalid and FRAC Code 11 fungicides.

ACKNOWLEDGEMENTS:
Project partly funded by Friends of Long Island Horticulture Grant Program and Hatch Act Formula Funds.

Margaret T. McGrath
Dept. of Plant Pathology and Plant-Microbe Biology, Cornell University
Long Island Horticultural Research and Extension Center
3059 Sound Avenue, Riverhead, New York 11901-1098
631-727-3695 (phone)
mtm3@cornell.edu

DISTRIBUTION OF GLYPHOSATE-RESISTANT ITALIAN RYEGRASS (*LOLIUM PERENNE SSP. MULTIFLORUM*) IN THE MID-SOUTH REGION OF THE UNITED STATES

ABSTRACT
Glyphosate-resistant Italian ryegrass was reported in Mississippi in 2007. Its distribution has expanded in recent years and created a problem with land preparation for row crop production in the Delta areas of Arkansas and Mississippi and the adjacent Mississippi hills. Additional resistant populations are suspected in Louisiana and Tennessee. To avert a major increase in tillage, weed scientists in the affected states are recommending a three-stage approach, comprising fall, winter, and spring herbicide applications. Such practices will incur additional herbicide costs and expand the use of herbicides in the Mid-South outside the summer growing season. Should the weed management program fail, due to inefficient implementation by growers, or if the efficacy of the acetyl CoA carboxylase (ACCase) herbicide, clethodim, is compromised by resistance, increased tillage would be essential for row crop production over large areas of the Mississippi Delta and adjacent hill areas.

REPORT

The genus *Lolium* is native to the Mediterranean region (Rakes, 1973). All ryegrasses are diploid (2n = 14) and self-incompatible; however, they readily cross with one another. Perennial ryegrass (*Lolium perenne*) is a major forage crop in western and southern Europe. Annual ryegrasses were exported to the Americas and Australia, incidentally with livestock, and intentionally for use as pasture grasses. Italian ryegrass (*L. perenne ssp. multiflorum*) is an annual used for winter grazing in both hemispheres. *Lolium rigidum*, called rigid ryegrass, is a weedy form of Italian ryegrass that originated in southern Europe. Ryegrasses are the principal forages for sheep in Australia. Rigid ryegrass escaped from Australian sheep paddocks and is now the most important weed in Australian wheat (*Triticum aestivum*). Similarly in North America, Italian ryegrass is over-seeded for winter grazing of cattle in the southern U. S., and it is also used for winter over-seeding of turf and as a fast-growing component of grass seed mixtures. Italian ryegrass is naturalized over large areas in the southern U. S. and is the number one weed in winter wheat in the southern region of the U. S. (Webster and Nichols, 2012).

Rigid and Italian ryegrass both have formidable histories of evolving resistance to herbicide mechanisms of action (Heap, 2013). In Australia, populations of rigid ryegrass have evolved resistance to the ACCase and acetolactate synthase (ALS) mechanisms of action, and to glyphosate (N-(phosphonomethyl) glycine). Several populations are resistant to more than one mechanism of action. Similarly, Italian ryegrass has evolved resistance to the same herbicide mechanisms of action in the U. S., making winter wheat production problematic across a broad geography in the central U. S. because of lack of effective post emergence herbicides to adequately control the weed (Salas et al., 2013). In the U. S. glyphosate-resistant Italian ryegrass was suspected in Mississippi and Oregon in 2004. Suspect populations were assayed and the resistance reported (Perez-Jones et al., 2005, Nandula et al., 2007). In the Mississippi Delta, glyphosate-resistant ryegrass was recognized as a significant problem in 2006-2008 and has been spreading since that time. Shortly thereafter, suspected populations were observed in Arkansas. Louisiana, and Tennessee, and confirmed in Arkansas (Dickson et al., 2011).

The Mississippi Delta is characterized by highly productive alluvial soils. Fields tend to be flat. Row crop production predominantly uses full-tillage. Most fields are planted with corn (*Zea mays*), soybean (*Glycine max*.), cotton (*Gossypium hirsutum*) and rice (*Oryza sativa*). Following stalk shredding, most fields will be disked and bedded in the fall. For almost 30 years, spring tillage for most Delta fields was preceded by an aerial application of glyphosate at 0.84 to 1.12 kg a.e./ha to control winter annual weeds (Williams et al., 2002). Additional spring tillage might include re-bedding, if required, because of erosion following winter rains. However, most field preparation is performed in the fall to facilitate early planting on soils that are often wet in the spring. A modified form of conservation tillage, known as stale seed-bed planting, is practiced in some areas of the Mid-South. In this planting system, the winter vegetation is killed with glyphosate or paraquat (1,1'–dimethyl-4,4'-byridinium ion) and planting proceeds directly on the fall-formed bed. True no-tillage, i. e. direct planting following no fall tillage, is infrequently practiced in the Delta, but is often practiced on the loess soils of the hills of northeast Mississippi and in Tennessee.

Glyphosate use figures prominently in all these tillage systems – as an aerial over-spray preceding spring bed preparation in full-tillage systems, as an over-spray preceding stale seed-bed planting, and as a broad-spectrum component of pre-plant burndown treatments for no-tillage. Thus, failure to control Italian ryegrass with glyphosate, and the consequent shift in the winter weed species composition to glyphosate-resistant Italian ryegrass will adversely affect all three of these tillage systems. If Italian ryegrass is uncontrolled, the fall full tillage system will likely need two spring diskings, possibly followed by inversion, and re-bedding. Similarly, stale seed-bed and no-tillage systems will be inoperable if Italian ryegrass cannot be adequately controlled with herbicides. No-tillage has never been practiced in this area without reliance on glyphosate for broad-spectrum control of winter weeds. Heavy stands of Italian ryegrass form sods in mild winters and would require extensive mechanical tillage.

To date, 11 and 31 counties have confirmed populations of glyphosate-resistant Italian ryegrass in Arkansas and Mississippi, respectively (Table 1 and Figure 1).

Table 1: Counties in Arkansas and Mississippi with confirmed populations of glyphosate-resistant Italian ryegrass.

State	..... Counties .....
Arkansas	Bossier, Chicot, Crittendon, Desha, Greene, Jefferson, Lafayette, Lee, Monroe, Mississippi, Phillips
Mississippi	Bolivar, Calhoun, Carroll, Coahoma, Desoto, Grenada, Hinds, Humphreys, Issaquena, Lafayette, Lee, Leflore, Lowndes, Madison, Montgomery, Oktibbeha, Panola, Rankin, Quitman, Scott, Sharkey, Sunflower, Tallahatchie, Tate, Tippah, Tunica, Union, Washington, Webster, Yalobusha, Yazoo

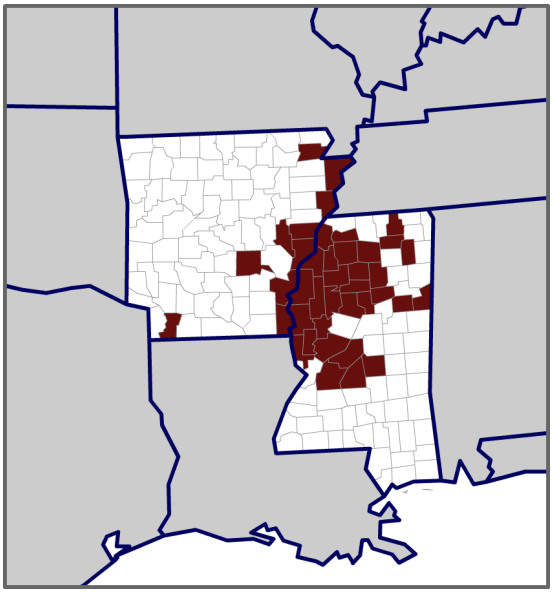

Figure 1: Counties in Arkansas and Mississippi with confirmed populations of glyphosate-resistant Italian ryegrass.

The overall area affected by glyphosate-resistant Italian ryegrass is likely an ellipse with its longer, north-south axis extending south into northeast Louisiana and north into west Tennessee. In both of these states, glyphosate-resistant Italian ryegrass populations are suspected by cooperative extension weed scientists (Drs. Daniel Stephenson, Louisiana State Univ. at Alexandria and Larry Steckel, University of Tennessee – personal communications). Suspected populations will be assayed this fall-winter when greenhouse temperatures are favorable for growth of potted Italian ryegrass.

To avert additional tillage, Mid-South weed scientists have developed alternative herbicide programs to manage glyphosate-resistant Italian ryegrass (Table 2.) (Bond et al. 2012a, Bond et al. 2012b). If a fall herbicide is applied, then a post emergence treatment is needed either in winter or spring. If fall tillage is used without a residual herbicide, then winter and spring treatments are required for optimum control. If no residual herbicide or late-fall tillage is performed, adequate control cannot be achieved with winter and spring herbicide treatments. Italian ryegrass that emerges in late-summer or early-fall would be too large to control with any spring treatment except vigorous tillage. Fall treatment alone is insufficient because additional flushes of Italian ryegrass emerge in late-winter and early-spring.

Table 2: Herbicide Programs for Managing Glyphosate-Resistant Italian Ryegrass before Planting Spring-Summer Row Crops in the Mid-South Region.

Crop	Fall[1]	Winter[2]	Spring[3]
Corn	*s*-metolachlor[4]	clethodim[5]	paraquat + atrazine[6]
Cotton	*s*-metolachlor	clethodim	paraquat + diuron[7]
Soybean	*s*-metolachlor	clethodim	paraquat + metribuzin[8]
Rice	*s*-clomazone[9]	clethodim	paraquat

[1] Apply as a pre-emergence treatment in mid-October to mid-November.
If Italian ryegrass is emerged, add paraquat as a tank-mix partner to control emerged weeds.
[2] Apply to emerged Italian ryegrass in mid-January to mid-February.
[3] Apply paraquat or paraquat plus residual pre-emergence herbicide shortly before or at planting
[4] 2-chloro-N-(2-ethyl-6-methylphenyl) – N-(2-methoxy-1-methylethyl) acetamide
[5] {2-[(E)-1-[[[(2E)-33-chloro-2-propenyl] oxy] imino] propyl]-5-[2-9ethio0propyl]-3-hydroxy-
2-cyclohexen-1-one}.
[6] 6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine-2,4-diamine
[7] N'-(3, 4-dichlorophenyl)-N,N-dimethylurea
[8] 4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one
[9] 2-[(2-chlorophenyl) methyl]-4,4-dimethyl-3-isoxazolidinone

Programs that include fall, winter, and spring treatments have led to an unprecedented increase in the use of off-season herbicides in the Delta and adjacent hill areas. A critical concern for this system is maintaining the efficacy of clethodim. Ryegrass species have developed resistance to the ACCase mechanism of herbicide action on four continents (Heap, 2013). Several ACCase herbicides are not effective for Italian ryegrass control. Of those that might be employed, sethoxydim's (2-[1-(ethoxyimino)butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one) efficacy is only fair, while pinoxaden's (8-(2,6 diethyl-4-methylphenyl)-1,2,4,5-tetrahydro-7-oxo-7H-pyrazolo[1,2-d][1,4,5]oxadiazepin-9-yl] 2,2-dimethylpropanoate) efficacy is good; however, pinoxaden is registered for use in winter wheat, but not for use in fallow fields.

The evolution and expansion of glyphosate-resistant Italian ryegrass in the Mid-South has complicated weed management and increased the costs of production for all major row crops in the region. Should the newly-formulated, three-stage weed management program fail at specific sites because of the lack of timely implementation by growers or should there be a general failure due to evolution of clethodim resistance, tillage would increase in the region. Not only would such a change prove costly, plantings of summer crops could be delayed in a region frequently plagued by wet soils in the spring. The emergence and spread of glyphosate-resistant Italian ryegrass in the Mid-South has had a major negative effect on crop production in the region. Means to remedy this

problem are not apparent to weed scientists at this time given the herbicides that are presently available.

ACKNOWLEDGEMENTS
Some of the information and data in this publication were developed by cooperative agreements between Cotton Incorporated and the Mississippi Agricultural and Forestry Experiment Station (MAFES). The authors thank Ms. Wendy Richardson for her able and amiable assistance with preparation of the map depicting counties affected by glyphosate-resistant Italian ryegrass.

REFERENCES

Bond, J. A., T. W. Eubank, and R. C. Bond. 2012a. Residual herbicides are cornerstone of glyphosate-resistant Italian ryegrass control. p. 1518. Proc. Beltwide Cotton Conferences National Cotton Council. Memphis, TN.

Bond, J. A., T. W. Eubank, and R. C. Bond. 2012. Sequential herbicide programs for control of glyphosate-resistant Italian ryegrass. Proc. Southern Weed Sci. Soc. 65:107

Dickson, J. W., R. C. Scott, N. R. Burgos, R. A. Salas, and K. L. Smith. 2011. Confirmation of Glyphosate-Resistant Italian Ryegrass (Lolium perenne ssp. multiflorum) in Arkansas. Weed Technology 25:674-679.

Frakes, R. V. The Ryegrasses. pp 307-313 in Heath, M. E., D. S. Metcalfe, and R. E. Barnes. 1973. Forages The Science of Grassland Agriculture 3rd ed. Iowa Univ. Press. Ames, IA. 755 p.

Heap, I. 2013. International Survey of Herbicide Resistant Weeds. www.weedscience.org.

Nandula, V. K., D. H. Poston, T. W. Eubank, C. H. Koger, and K. N. Reddy. 2007. Differential response to glyphosate in Italian ryegrass (*Lolium multiflorum*) populations from Mississippi. Weed Technology 21:477-482.

Perez-Jones, A., K. Park, J. Colquhoun, C. Mallory-Smith, and D. L. Shaner. 2005. Identification of glyphosate-resistant Italian ryegrass (*Lolium multiflorum*) in Oregon. Weed Science 43: 12-19.

Salas, R. A., N. R. Burgos, A. Mauromoustakos, R. B. Lassiter, R. C. Scott, and E. A. Alcober. 2013. Resistance to ACCase and ALS inhibitors in Lolium perenne ssp. multiflorum in the United States/ J. Crop and Weed. 9(1):168-183.

Webster, T. M. and R. L. Nichols. 2012. Changes in the Weed Species Composition of the Southern US: 1994/1995 to 2008/2009. Weed Science 60:145-167.

Williams, B., D. Miller, S. Kelley, R. Vidrine, R. Leonard, J. Griffin, D. Sanders, E. Webster, R. Lencse, E. Millhollon, R. Strahan, D. Reynolds, S. Crawford, and D. Jordan. 2002. Guidelines for Managing Winter Vegetation in Northeast Louisiana. Louisiana State University Research Information Sheet 105. 16 p.

Nichols, R. L.[1], J. A. Bond[2], T. W. Eubank[2], J. W. Dickson[3], and R. C. Scott[3]
[1] Senior Director, Cotton Incorporated, Cary, North Carolina
[2] Associate Professor and Assistant Professor, Mississippi State University, Stoneville, Mississippi, respectively
[3] Former Graduate Student and Associate Professor, University of Arkansas, Lonoke, Arkansas, respectively

SELECTIVITY OF *AMBLYSEIUS LARGOENSIS* (MUMA) WITH PRODUCTS PLANT USED IN THE PRODUCTION PROTECTED OF PEPPER (*CAPSICUM ANNUUM L.*).

INTRODUCTION

Bell Pepper (*Capsicum annuum* l.) is the most widely cultivated Solanum, second worldwide, with a performance average 13 t.ha-1. The largest producer is China, with 52, followed by Mexico, Turkey, Indonesia and Spain (1). Areas dedicated to pepper have progressively increased in Cuba, but it has not happened in the same way with the performance (2).

The production of this crop has been depressed due to the high incidence of pest, these include the effects by virus and the presence of aphids (*Mysus persicae* Sulzer), whitefly (*Bemisia tabaci* gene), *Thrips palmi* Karny and the white mite *Polyphagotarsonemus latus* (Banks), this last considered the plague the farming potential to open countryside and in conditions of protected production (3, 4, 5).

In the particular case of this tarsonemido, it is difficult to control through the use of chemicals such as dicofol and abamectin (6) acaricides. This is because applications are not made at the right time, by the location of the mite in the most protected area of the underside of the leaves, so there is no guarantee of the desired effect. This, together with the increase of the resistance of this species to the acaricides (7), has led to the need to develop control tactics that are

compatible with natural enemies present or introduced in the agro-ecosystem (8, 9, 10).

In this regard, it should be mentioned that the protected cultivation currently enjoys a high role in intensive production, with a great commercial value, in the context of the Cuban agriculture. Their participation is essential in the productive response to demand that agricultural commodity is generated, in addition to project itself, as a variant of great prospects for export and other national demands (11, 12).

In these protected vegetable production systems, in most cases there is little information, underpinned by rigorous studies on the behavior of pests in them, the exception being nematodes (4, 11). This is particularly true if you want to design a strategy for management of *P. latus* in protected Pepper production.

Taking into account that control *P. latus* is done almost exclusively with chemicals, the incidence of *P. latus* in the production of peppers (4,5) and bioregulators demonstrated qualities of *Amblyseius largoensis* (Muma) on white mite (13,14). It is indispensable to know selectivity data I compatibility of these control agents, with plant protection products applied in cultivation.

This information has unquestionable value from the practical point of view, so determining the selectivity of *A. largoensis* with plant protection products used in the production of pepper would facilitate and optimize the use of this beneficial arthropod within the management strategies of *P. latus* in protected Pepper production systems.

MATERIALS AND METHODS

The experiments were conducted in the laboratory of Acarology of the national centre of agricultural health (CENSA). The temperature and the relative humidity were 25.2 ± 2.5°C and 72.3 ± 7.50, respectively, measurements with a digital Thermo-hygrometer (Testo 608-H2). Products used in this experiment are listed in table 1.

Table 1: Relación de productos evaluados en el ensayo de selectividad. / *Relation of products evaluated in the trial selectivity.*

Commercial name	Name of the active ingredient	Dose
Acaricidas		
Mitigan CE 18.5	dicofol	0.27 kg ia.ha^{-1}
Comoran Supra SC 72	azufre	4.0 kg ia.ha^{-1}
LBt- 13 1 a 3 * 10^9 esporas/g $^{-ml}$	*Bacillus thuringiensis*	5.0 L.ha^{-1}
Insecticidas		
Polo SC 50	diafentiuron	0.5 L PC.ha^{-1}
Confidor GD 70	imidacloprid	0.40 kg ia.ha^{-1}
Corsario CE (2.71 + 21.69)	cipermetrina + diazinon	1 L PC.ha^{-1}
Fungicidas		
Ridomil Gold MZ PH 68 (4 + 64)	Metalaxil + mancozeb	200g ia + 1.6 kg ia.ha^{-1}
Mersuram PH 80	mancozeb	2.4 kg ia.ha^{-1}
Control		
Distilled water and Tween 80	_	

Extracted gravid females of 2-5 days of age from a breeding of a. largoensis established on Panonychus citri (McGregor) and placed in sections of young leaves of grapefruit (Citrus paradisi Macf var.) Marsh) 9 cm^2 approximately with equal prey in a Petri plate 14 cm in diameter.

For evaluative purposes products were sprayed with a sprinkler manually, ensuring total coverage of the blade, with a discharge of 0.5 ml/cm^2. The Petri dishes were placed at an angle until the liquid evaporated. Once dried leaves were placed in Petri plates, females were transferred with 00 brushes onto another plate, previously prepared Petri, where settled there was another section of grapefruit with abundant population of p. citri, which had not received treatment.

The products were evaluated at the highest doses used in the cultivation systems protected and registered in the official list of pesticides (6), prepared with distilled water and Tween 80 to the 0.001 as wetting agent. Chemicals were obtained from projects "Liliana Dimitrova" horticultural protected Research Institute of production systems and the strain of *B. thuringiensis* from the center of reproduction of Entomofagos and Entomopathogens (CREE) of Guines, the quality was checked in the laboratory of Phytopathology of the national agricultural health center (CENSA).

A control with distilled water and Tween 80 was included. Three replications with 20 females each for a total of 60 females were prepared. A count was collected for the number of females of *a. largoensis* dead at 24, 48 and 72 hours of initial application. A consideration of dead was applied for all mites who did

not carry out any movement after being massaged by 00 brushes. Mortality results were classified according to the scale proposed by the International Organization for Biological Control (IOBC) (15).

RESULTS AND DISCUSSION

Of the acaricides evaluated, only ECA LBt-13 of *Bacillus thuringiensis* (Berliner) at 72 hours was slightly toxic to *a. largoensis*. In the case of the tested insecticides, two of them, diafentiuron and cipermetrinadiazinon were toxic from 24 hours of application, while imidacloprid was classified as harmless, with only a 5.56 mortality at 72 hours, as well as two fungicides, metalaxilmancozeb and mancozeb (table 2).

Table 2: Mortality caused by products on the females of Amblyseius largoensis. Mortality caused by the products evaluated on the females of Amblyseius largoensis.

Active ingredient	Mortality (%)			Comment	Kind of mortality
	24 h	48h	72h		
Acaricides					
dicofol	0	0	5	Harmless	1
azufre	14.7	14.7	14.7	Harmless	1
Bacillus thuringiensis	18.18	18.18	27.27	Slightly toxic	2
Insecticides					
diafentiuron	40.9	72.7	86.4	Tóxic	4
imidacloprid	0	0	5.56	Harmless	1
cipermetrina+diazinon	100	-	-	Tóxic	4
Fungicides					
metalaxil+mancozeb	0	0	0	Harmless	1
mancozeb	0	0	5.27	Harmless	1
Control					
Distilled water and Tween 80	0	0	0	-	-

The acaricide LBt-13 strain was harmless after 24 and 48 hours after being applied and only 72 hours can be considered slightly toxic to females, largoensis. This is a promising result, since it implies that both biological media could be used together in a management program of the white mite in protected systems.

Although it is known that the ss-Exotoxin of B. thuringiensis has a high inhibitory potential of molting in insects and mites and only in high concentrations affecting fertility and longevity of adults, it is required to delve into the possible sublethal effects of this product on a. largoensis.

Fungicides in general are considered moderate action on the predatory mites pesticides. There are multiple studies showing its safety. Fungicides for example, dodine, myclobutanil, mancozeb, metiram and captan were not toxic to the females of Amblyseius fallacis (Garman) and did not affect the number of laid eggs (16). Godwind, (17) reported as harmless to the fungicides bitertanol, bupirimeta, captan, iprodione, mancozeb, triademefon and vinclozolin on Phytoseiulus persimilis (Athias-Henriot).

Imidacloprid is a systemic insecticide that has also a remarkable action of stomach contact and prolonged residual control. It has no effect on mites or nematodes. It has been reported as non-toxic, in at least nine species of Phytoseiidae: Neoseiulus collagae (De Leon), Phytoseiulus macropilis (Banks) and Proprioseiopsis mexicanus (Garman); Amblyseius womersleyi Schicha; (Amblyseius) Euseius victoriensis (Womersley; Typhlodromus pyri (Scheuten); Typhlodromus doreenae Schicha, Typhlodromus dossei Schicha (18).

However, the current experience with the use of this insecticide in the integrated management of pests in different cultures suggests that you should be cautious, considering the mites as a family, since results that indicate high toxicity on other species have been noted. This is the case of Galendromus occidentalis Nesbitt, Amblyseius fallacis (Garman) and Amblyseius andersoni (Chant), for which the imidacloprid was highly toxic (19). These results indicate that species have different degrees of susceptibility and it is likely that some species of phytoseiidae mites are more susceptible to imidacloprid, than the relative few species evaluated. Hence the relevance to demonstrate that a. largoensis is not affected by the application of this insecticide in wide use in the country.

Generally, the consulted literature shows that dicofol has a marked direct toxicity on the predatory mites, for example has been classified as grade IV on the females of Phytoseiulus persimilis (Athias-Henriot), Amblyseius gossipi El-Badry, Iphiseiodes zuluagai Denmark and Muma. However, the residual action of the immature stages of p. persimilis is ranked as slightly toxic. As for imidacloprid, these results may be due to the variability between species (17). On phytophagous mites (Tetranychus urticae Koch, Tetranychus kanzawai Kishida and p. citri) it has been reported that the resistance to dicofol in a population is reached by an incomplete recessive gene. Apparently, this acaricide resistance is associated with an increase in the metabolic detoxification.

Ahmad et. al (20) found a selective mortality of Phytoseius plumifer (Canestrini & Fanzago) opposite the acaricide hexythiazox, fenpyroximate and abamectin. The analysis of the data showed that the hexythiazox can be considered an acaricide harmless; while fenpyroximate and abamectin were toxic to the predator field concentrations.

However, don't forget that in practice, expertise in the management of the factors affecting selectivity, along with the follow-up of the evolution of pests and their natural enemies, favors the compatibility between

pesticides and beneficial organisms, which will give more reliability and success to a system of integrated management that has been adopted.

Applications of chemical products are sometimes necessary to reduce population density of phytophagous mites to acceptable levels, prior to the release of a predator. For this reason, the fact of knowing that a. largoensis is compatible with various chemicals used in protected cultivation, constitutes an additional element which will favour the introduction in terms of production of this predator. These results demonstrate that there are options that enable the integration of the predator. These trials should be extended to a larger number of products thus assessing the possible residual effects or the effects on eggs the predator of the chemicals and their evaluation in field conditions.

REFERENCIAS

Gutiérrez CY. Análisis del mercado para chile pimiento. Proyecto de Desarrollo Productivo. Cadena de Valor Frutícola. Fomilenio, El Salvador. 2009,11 pp.

Rodríguez Yaritza, Depestre T, Gómez Olimpia. Obtención de líneas de pimiento (*Capsicum annuum)* progenitoras de híbridos F1, resistentes a enfermedades virales, a partir del estudio de cuatro sub-poblaciones. Cien Inv Agr. 2007, 34(3): 37-342.

Casanova AS, Gómez Olimpia, Hernández M, Chailloux M, Depestre T, Pupo FR, et al. Manual para la Producción Protegida de Hortalizas. 2da Versión. Instituto de Investigaciones Hortícolas "Liliana Dimitrova". Editorial Liliana, Ministerio de la Agricultura, 2007, 179 pp.

Rodríguez H, Montoya A, Ramos Mayra, Miranda Ileana, Rodríguez Yaritza. Comportamiento poblacional de *Polyphagotarsonemus latus* (Banks) en pimiento (*Capsicum annuum* L.) en cultivo protegido. Fitosanidad. 2008; 12(4):215-220.

Miranda Ileana, Montoya A, Rodríguez Yaritza, Depestre T, Rodríguez, H. Densidad límite para el control de *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae) sobre pimiento (*Capsicum annuum* L.) en cultivo protegido. Rev. Protección Veg. 2010; 24(3):146-151.

CNSV. Lista Oficial de Plaguicidas Autorizados. Registro Central de Plaguicidas. Centro Nacional de Sanidad Vegetal (CNSV). Ministerio de la Agricultura (MINAG). República de Cuba. 2008, 421 pp.

Venzon Madelaine, Rosado MC, Molina-Rugama AJ, Duarte VS, Días R, Pallini A. Acaricidal efficacy of Neem against *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae). Crop Prot. 2008; 27: 869-872.

Gerson U, Weintraub PG. Mites for the control of pests in protected cultivation. Pest Management Sci. 2007; 63: 658-676.

Weintraub P.G. Integrated control of pests in tropical and subtropical sweet pepper production. Pest Management Sci. 2007; 63(8): 753-760.

Weintraub PG, Kleitman S, Alchanatis V, Palevsky, E. Factors affecting the distribution of a predatory mite on greenhouse sweet pepper. Exp Appl Acarol. 2007; 42: 23-35.

Gómez Lucila, Rodríguez M, Enrique R, Miranda I, González E. Factores limitantes de los rendimientos y calidad de las cosechas en la producción protegida de hortalizas en Cuba. Rev Protección Veg. 2009; 24(2):117-122.

Rodríguez Mayra, Sánchez L, Gómez L, Hidalgo L, González E, Gómez M, Diaz-Viruliche L, Casanova A, Cuadra R, Fernández E, Hernández R. *Meloidogyne* spp., plagas de las hortalizas: alternativas para su manejo en sistemas de cultivo protegido. Rev Protección Veg. 2005; 20(1):1-10.

Montoya, A. Miranda Ileana, Ramos Mayra, Rodríguez, H. Cría de *Amblyseius largoensis* (Muma) sobre *Tetranychus tumidus* (Banks) utilizando el método de las bandejas. Rev Protección Veg. 2009; 24(3):191-194.

Rodríguez, H. Ileana Miranda, Mayra Ramos y M. H. Badii. Functional and numerical responses of *Amblyseius largoensis* (Muma) (Acari: Phytoseiidae) on *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae) in Cuba. Internat J Acarol. 2010; 36(5):371-376.

Boller EF, Vogt H, Ternes P, Malavolta C. Working Document on Selectivity of Pesticides. International Organisation for Biological and Integrated Control of Noxious Animals and Plants: West Palaearctic Regional Section. 2005. p. 1-9,

Bostanian NJ, Thistlewood H, Racette G. Effect of five fungicides used in Quebec Apple orchards on *Amblyseius fallacis* (Garman) (Phytoseiidae: Acari). J Hort Sci Biotecnol. 1998; 73(4):527-530.

Goodwin S. Laboratory evaluation of pesticides on an Australian strain of the predatory mite, *Phytoseiulus persimilis* Athias-Henriot. Acarol V. 1982; 2:648-654.

James DG. Toxicity of imidacloprid to *Galendromus occidentalis*, *Neoseiulus fallacis* and *Amblyseius andersoni* (Acari: Phytoseiidae) from hops in Washintong State, USA. Exp Appl Acarol. 2003; 31:275-281.

Ware GW, Whitacre DM. Introducción a los Insecticidas. Extraído de *The Pesticide Book*, 6th ed. Publicado por MeisterPro Information Resources. Una división de Meister Media Worldwide Willoughby, Ohio; 2004.

Ahmad N, Karim K, Masoud A, Fateme, A. Selectivity of Three Miticides to Spider Mite Predator, *Phytoseius plumifer* (Acari: Phytoseiidae) Under Laboratory Conditions. Agric Sci in China. 2009; 8(3):326-331

.

Pedro Posos Ponce**, A. Montoya Ramos* ,Oriela Pino **,**
H. Rodríguez* ,Benito Monroy Reyes****, Enciso Cabral Gustavo******
**** Universidad de Guadalajara, Departamento de Produccion Agricola,m CUCBA
***Departamento Biología-Sanidad Vegetal. Facultad de Agronomía. Universidad Agraria de La Habana
** Grupo Plagas Agrícolas, Dirección de Protección de Plantas. Centro Nacional de Sanidad Agropecuaria (CENSA).
* Departamento Básico-Específico. Facultad Agroforestal de Montaña (FAM). Universidad de Guantánamo

CROSS-RESISTANCE OF THE SELECTED STRAINS OF THE HOUSEFLY TO DIFFERENT INSECTICIDES

INTRODUCTION

The broad application of insecticides has led to the emergence of numerous populations and strains of harmful insects resistant against means of chemical application. The problem of resistance has amplified additional discovery that insects, resistant to one insecticide, are often either resistant to other insecticides or capable of gaining such resistance quickly. This phenomenon is usually described as cross-resistance. The first notification of this phenomenon was conducted by Busvine (1953): house flies, resistant to hexachlorocyclohexane, showed resistance to other cyclodien insecticides too.

Overall picture of cross-resistance is that insects resistant to connections of one group of insecticides, usually show considerable resistance to other connections of this group but they have no resistance to preparations of other groups. However, it was noticed that there are many exceptions of this rule. Insects resistant to organophosphorus compounds often become resistant to the carbamates because the mechanism of action of these groups is similar - they inhibit cholinesterase. Resistance to organochlorine compounds is often accompanied with resistance (but to a lesser extent) to synthetic pyrethroids. This occurs because despite the differences in their chemical structure these groups of compounds have a similar mechanism of action. Now there is numerous data on the existence of intra group and intergroup resistance of insects (Sokolyanskaya, 2012). The target of this work was to determine the possible cross-resistance of the six selected strains of house flies to insecticides of three different classes.

MATERIAL AND METHODS

The objective of the studies was imago of one sensitive strain and six selected strains of housefly. Following insecticides were used for selection: from class OP - phosmet (phtalophos, 20% e.k.), foxim (volaton, 50% e.k.); from class pyrethroids - deltamethrin (decis, 2.5% e.k.), fenvalerat (sumicidin, 20% e.k.), ethophenprox (trebon, 30% e.k.); from derivates of benzylphenylurea - chlorfluazuron (eim, 12% e.k.). The selection and determination of resistance index (RI) to selectants were conducted, as it was described earlier (Sokolyanskaya, 2007). Researches were conducted on an imago of the 30th generation. The strains used in studies were: R-v (RI=4.8), R- pht (RI=2.07), R-d (RI=42.6), R-fv (RI=32.6), R-tr (RI=5), R-e (RI=1.55).

RESULTS AND DISCUSSION

The results of researches on an assessment of sensitivity of selected strains of a house fly to organochlorine compound of DDT and preparations of class organophosphates (OP) are given in table 1.

Table 1: Cross-resistance of selected strains of house fly to DDT and organophosphorus compounds.

Strain	Index	DDT	Foxim	Phosmet	Chlorpyriphos
S	LD_{50}	194.97±20.52	11.64±0.57	17.29±0.94	4.04±0.37
R-v	LD_{50} RI	236.45±16.2 1.21	55.87±8.6 4.8	12.02±0.75 0.7	15.18±1.06 3.76
R-pht	LD_{50} RI	123.34±8.58 0.63	16.25±1.08 1.4	35.74±1.79 2.07	6.4±0.48 1.58
R-d	LD_{50} RI	287.58±21.19 1.47	14.57±2.88 1.17	17.0±1.69 0.98	13.05±1.02 3.23
R-fv	LD_{50} RI	178.7±49.96 0.92	13.33±0.85 1.15	18.36±1.03 1.06	13.9±1.07 3.44
R-tr	LD_{50} RI	307.7±35.01 1.58	12.83±0.89 1.1	24.07±1.87 1.39	15.14±1.17 3.75
R-e	LD_{50} RI	120.62±11.0 0.62	10.59±0.62 0.91	25.17±1.64 1.46	6.62±0.29 1.64

Note –in darkchecks difference of selected strain with sensitive is reliably, P>0,95.

Despite the fact that the strains R-v and R-pht were selected organophosphorus insecticides, they did not show an identical level of cross-resistance in relation to DDT and the three used OP. The strain selected by foxim almost didn't possess cross resistance to DDT, had low resistance to chlorpyriphos and showed negative resistance to phosmet. The strain, selected by phosmet, practically demonstrated no cross-resistance

to foxim and chlorpyriphos but possessed negative resistance to DDT. Flies selected by pyrethroids performed more uniformly. At all three strains, cross-resistance in relation to organophosphorus compounds phosmet and foxim wasn't developed and at the same time insignificant stability to chlorpyriphos was observed. In relation to DDT, the values of indicated resistance at these strains are a little various but nevertheless cross-resistance is not actually present. Flies selected by chlorfluazuron, as well as flies of the R-pht strain, showed negative cross-resistance to DDT and almost total absence of cross resistance to three organophosphorus compounds.

In spite of the fact that in the middle of the last century resistance and cross-resistance to DDT in insects developed quickly enough, in our researches selected strains neither shown by resistance (R-fv strain), or degree of resistance were very insignificant (the strains R-tr, R-d and R-v) and two strains showed negative resistance (R-pht and R-e). In the course of selection, cross-resistance in all selected strains and to organophosphorus insecticides foxim and phosmet wasn't created (PR< 2). Cross-resistance to the third organophosphorus insecticide – chlorpyriphos – is slightly higher, in comparison with the previous preparations but also doesn't go beyond tolerance (PR< 4).

Thus, all selected strains show insignificant cross-resistance to DDT, phosmet, foxim and chlorpyriphos. Moreover strains selected by phosmet and chlorfluazuron showed negative cross-resistance to DDT and the strain selected by foxim - to phosmet.

Similar data for insects, selected by organophosphorus insecticides, were received also by other authors on different species of insects. Malathion resistant populations of Indian meal moths *Plodia interpunctella* (RI=17) didn't show resistance to methylchlorpyriphos (Beeman et al. 1982). In the strain of red flour beetle *Tribolium castaneum*, resistant to malathion (RI =73), cross-resistance to many organochlorine and organophosphorus compounds wasn't revealed (Beeman, 1983). Resistant to azinphosmethyl strains of pear psylla *Cocopsylla pyri* remained sensitive to chlorpyriphos and mevinphos (Buès et al. 2000) and middle-resistant to malathion strains of *Sitophilus zeamais* (RI=10) showed insignificant cross-resistance to seven organophosphorus insecticides (RI=3-9 depending on a preparation) (Li, Li, 1992). Resistant to methamidophos (44x) strain of *Nilaparvata lugens* had an average rate of resistance to malathion and diazinon (Liu et al., 2002).

The moth of *Helicoverpa armigera* has become resistant to several pyrethroids because of their application kept sensitivity to triazophos (Martin et al. 2003) and endosulfan ((Ramasubramanian, Regupathy, 2004). House flies resistant to resmethrin had the intermediate level of resistance to organophosphorus compounds (PR< 10), but at the same time they had high resistance (unlike our strains) to DDT (Funaki, Motoyama, 1986). Resistant to fenvalerate strain *Aphis gossypii* did not have cross-resistance to ometoat, but demonstrated a low resistance to endosulfan (Wang et al., 2001).

In researches on determination of possible cross-resistance of selected strains in relation to two preparations from class chitin synthesis inhibitors (CSI) the following data (Table 2) was obtained.

Table 2: Cross-resistance to chitin synthesis inhibitors of larvae of selected strains of flies.

Strain	Index	Chlorfluazuron	Flufenoxuron
S	EC_{50},%	0.000031±0.0000017	0.000032±0.000008
R-v	EC_{50},% RI	0.0000203±0.0000012 0.63	0.000036±0.000004 1.13
R-pht	EC_{50},% RI	0.000031±0.0000082 1.0	0.000042±0.000005 13
R-d	EC_{50},% RI	0.000019±0.0000014 0.61	0.000033±0.0000017 1.03
R-fv	EC_{50},% RI	0.000022±0.0000022 0.71	0.000044±0.000011 1.34
R-tr	EC_{50},% ПР	0.000032±0.0000019 1.03	0.000041±0.0000041 1.28
R-e	EC_{50},% RI	0.000048±0.0000013 1.55	0.000086±0.000018 2.69

The strain selected by chlorfluazuron showed insignificant cross-resistance only related to the preparation of flufenoxuron (RI=2.69). In relation, chlorfluazuron at three strains showed negative cross-resistance with RI=0.61 for the R-d strain, RI =0.71 in the R-fv strain, and RI =0.63 in the R- v strain.

W. Guyer and R. Neumann (1988) indicated a lack of cross-resistance to chitin synthesis inhibitors chlorfluazuron and diflubenzuron in caterpillars, *Spodoptera littoralis*, resistant to organophosphorus compounds. Caterpillars of the same species, with low resistance to diflubenzuron, didn't show cross-resistance to cypermethrin but showed it to related chitin synthesis inhibitors on a basis of benzoilfenylurea (Ahmed et al., 1987). Similar results are noted for houseflies, resistant and multiresistant to various insecticides in relation to diflubenzuron (Keiding, 1986), and also for the Colorado beetle from the USA with multiple resistance to the majority of insecticides who showed sensitivity to triflumuron (Schroder, 1991). Low resistance to this compound (RI=5) caterpillars of the natural population *S. littoralis* developed, which for a number of years intensively

were processed by traditional insecticides from the classes of the OP and pyrethroids, considerable resistance (RI>100) to them (Ishaaya I., Klein M. 1990). At the same time the caterpillars of *Cydia pomonella*, resistant to azinphosmethyl, were also resistant to insect growth regulators (Reuveny, Cohen, 2004).

On the basis of the above it is possible to draw the following conclusions:

The cross-resistance to related connections is not always higher than to compounds of other class. Along with organophosphorus compounds for the purpose of control of resistance forming it is necessary to include preparations in schemes of alternation of insecticides from class of chitin synthesis inhibitors.

REFERENCES

Ahmed M.T., Ahmed Y.M., Moustafa A.S. 1987. Some studies on the development of resistance to diflubenzuron in the Egyptian cotton leafworm // Meded. Fac. Landbouww. Rijksuniv. Yent. Д.52. N.2в. P. 477-483.

Beeman R.W. 1983. Inheritance and linkage of malathion resistance in the red flour beetle// J. Econ. Entomol. V.76. N4. P. 737-740.

Beeman R.W., Speirs W.E., Schmidt B. 1982. Malathion resistance in indianmeal moths (Lepidoptera: Pyralidae) infesting stored corn and wheat in the North-Central United States // J. Econ. Entomol. V.75. N6. P. 950-954.

Bues R., Toubon J. F., Boudinhon L. 2000. Genetic analysis of resistance to azinphosmethyl in the pear psylla *Cocopsylla pyri* // Entomol. exp. Et appl. V.96. N. 2. P. 159-166.

Busvine J.R. 1953. Forms of insecticide resistance in houseflies and body lice. Nature. N. 171(4342). P.118-119.

Guyer W., Neumann R. 1988. Activity and jute of chlorfluazuron and diflubenzuron in the larvae of *Spodoptera littoralis* and *Heliothis virescens* // Pestic. Biochem. and Physiol. V.30. N2. P. 166-177.

Ishaaya I., Klein M. 1990. Response of susceptible laboratory and resistant field strains of *Spodoptera littoralis* (Lepidoptera Noctuidae) to teflubenzuron // J. Econ. Entomol. V. 83. N. 1. P. 59-62.

Keiding J. 1986. Prediction or Resistance Risk Assessment // Pest. Resist.: Strateg. And Tactics for Management. Washington. D.C.:National Acad. Press. P. 279-283.

Li Y.-s., Li W.-z. 1992. Cross-resistance of malation resistant strains to some organophosphprus insecticides in *Sitophilus zeamais* (L.) and *Tribolium castaneum* (Herbst) in China //19 Int. Congr. Entomol., Beijing, June 28-July 4, Proc: Abstr. Beijing, 1992. P. 604.

Liu Z.-W., Han Z.-J., Zhang L.-C. 2002. Кросс-резистентность линии *Nilaparvata lugens*, резистентной к малатиону, е ее биохимические аспекты // Kunchong xuebao = Acta entomol. Sin. V. 45. N. 4. P. 447-452.

Martin T., Ochou O. G., Vaissayre M., Fournier D. 2003. Oxidases responsible for resistance to pyrethroids sensitize *Helicoverpa armigera* (Hubner) to triazophos in West Africa // Insect Biochem. and Mol. Biol. V. 33. N. 9. P. 883-887.

Ramasubramanian T., Regupathy A. J. 2004. Pattern of cross-resistance in pyrethroid-selected populations of *Helicoverpa armigera* Hübner (lep. Noctuidae) from India. // Appl. Entomol. V. 128. N. 9-10. P . 583-587.

Reuveny H., Cohen E. 2004. Resistance of the codling moth *Cydia pomonella* (L.) (Lep. Totricidae) to pesticides in Israel / // J. Appl. Entomol. V. 128. N. 9-10. P. 645-651.

Schroder F.F.W. 1991. An integrated pest management approach to Colorado potato beetle control in the USA // Bull. OEPP. V. 21. N. 1. P. 9-10.

Sokolyanskaya M.P. 2012. The problem of cross-resistance of insects and mites to insectoacaricides // Resistant Pest Management Newsletter. V. 21. N. 2. P. 31-35.

Sokolyanskaya M.P. 2007. The resistance forming dynamics to different insecticides in housefly // Resistant Pest Management Newsletter. V. 17. N. 1. P. 27-30.

Wang K.-Y., Liu T.-X., Jiang X.-Y., Yi M.-Q. 2001. Cross-resistance of *Aphis gossypii* to selected insecticides on cotton and cucumber// Phytoparasitica. V. 29. N. 5. P. 393-399.

M.P. Sokolyanskaya
Institute of Biochemistry and Genetics,
Ufa Scientific Center of RAS.
450054, Ufa, October Prospect, 71, Russian Federation
e-mail: sokolyanskaya-m@yandex.ru

Resistance Management Around the Globe

ELECTROPHORETIC CONFIRMATION OF INSECTICIDE RESISTANCE IN BOLLWORMS FIELD STRAINS IN EGYPT

ABSTRACT

Electrophoretic bioassay analysis of proteins showed that protein bands were varied and there were differences among proteins of susceptible and field strains of pink bollworm (PBW) ***Pectinophora gossypiella***(Saund.) in their intensity and their molecular weights. These findings could interpret the susceptibility difference between the field and susceptible strains. Differences in protein bands between male moths or larvae of Bohira and Alexandria field strains in their intensity and their molecular weights is probably due to the history of insecticides application in the two regions. This is because Bohira Governorate has the most cultivated cotton area all over the country, so it receives the most applications of insecticides against both bollworm (pink bollworm and spiny pink bollworm) and cotton leafworm instead of the other economic pests. This area receives the heaviest insecticide applications and the continuous use of it throughout all of the season. On the other hand, insecticides are rarely used against bollworm and cotton leafworm in Alexandria Governorate. So, it is logical to get high levels of resistance against pink bollworm in Bohira strain compared to that of Alexandria strain. The obtained data from the biochemical studies are in good agreement with that of laboratory and field studies of the attracticide resistance monitoring technique and confirmed its results.

INTRODUCTION

Formulated pesticides are used in a large scale throughout the world as a major mean for pest management and control. Although pesticides provide numerous benefits in terms of increased agricultural production and improve its quality, their efficacy may not be as good because of the development of insecticide resistance in many pest species.

Resistance to one or more pesticides has been documented in more than 447 species of insects and mites (Roush and - McKenzie 1987). Pesticide resistance is an increasingly urgent worldwide problem. Resistance in vectors of human disease, particularly malaria-transmitting mosquitoes, is a serious threat to public health in many nations. Agricultural productivity is jeopardized because widespread resistance in crop and livestock pests. Serious resistance problems are also evident in pests of the urban environment, most notably cockroaches.

Resistance to insecticides is one of the most serious problems facing agriculture today. Many previous studies revealed the high resistance of pink bollworm to insecticides in the cotton fields. In Egypt, several pyrethroids and organophosphorus have been widely used against cotton pests. However, although pyrethroid and organophosphorus insecticides were the most efficient and widely used against bollworms, the onset of resistance development to these compounds in bollworms have been recently documented (Georghiou, 1983; Haynes et al., 1987; Knight and Hull, 1989; and Miller, 1990).

The major economic losses due to the pesticide resistance in the USA were: $1.5 billion (Pimentel, 2005). The price of insecticide resistance in lost yields and higher insect control costs is staggering - in some years more than $1 billion in cotton for the budworm/bollworm complex alone.

In IPM, pheromones are considered to be an essential component because they are used for detecting the economic threshold levels of pest populations and for mating disruption as a direct pest suppression measure. Pheromones of major pests have been found to be effective, economic and eco-friendly in agro-ecosystems in which cotton is cultivated (Tamhankar et al. 2000).

For resistance management tactics to be effective, resistance must be detected in its early stage (Rouch & Miller 1986) and early detection necessitates using one or more techniques that being accurate, easy, rapid and inexpensive, which would aid production, consultants and extension personal in making informed decisions on adequate control measures (Mink & Boethel 1992). Firstly, the traditional approach uses complete dose-response tests with 4-5 doses that produce 10-90% mortality. Resistance is expressed in terms of the ratio of LD50 or LC50 of the resistant strain to that of the susceptible strain. Alternatively, an approach called the discriminating or diagnostic dose test was used where one dose is often investigated and the mortality of the susceptible test strains is compared (Pasquier and Charmillot, 2003).

Another approach is the attracticide method which was developed in summer of 1985 as a rapid test to determined resistance in pink bollworm adult in cotton fields. The attracticide method was full implementation in 1986 and 1987 as an effective method to monitor insecticide resistance to pink bollworm to a wide range of insecticides (Miller, 1986 and Haynes et al, 1986 and 1987).

Detection of changes in response is needed, especially in the early stages of resistance development. Monitoring insecticide resistance in field population moth is in great importance in resistance management programs (Tabashnik and Cushing, 1987).

In Egypt, very few dose mortality studies using attracticide resistance monitoring technique were carried out [(Albeltagy et al.1996,Albeltagy et al.2001a, Albeltagy et al.2010, Albeltagy 2012a and 2012 b, and Albeltagy et al. 2012a an 2012b), also (Albeltagy et al. 1993, Albeltagy et al. 2001b

and Albeltagy et al. 2001c) carried out biochemical studies to assay insecticide resistance in bollworms field strains].

MATERIALS AND METHODS

1. Biochemical assays:-

1.1. Electrophoretic studies:

1.1.1. Electrophoretic detection of protein by sodium dodecyl sulphate, polyacrylamide gel electrophoresis (SDS-PAGE):

The method of Laemmili (1970) was adopted to use in the present study. Approximately 1g freeze dry of bodies and larvae from the susceptible-laboratory and resistant-field strains for pink bollworm was ground in a mortar and pestle in liquid nitrogen. Crushing continued until the sample completely homogenized. The crushed samples were transferred to 1 ml Eppendorf tube brought to 200 µl with extraction buffer (50 m Mtris-HCl buffer, pH 6.8, glycerol 10 % w/v, ascorbic acid 0.1%, cycteine hydrochloride 0.1 w/v). Centrifugation, 18,000 rpm for about 30 min was carried out to remove debris. The protein content in supernatant was estimated according to the method of Bradford (1976) by using bovine serum albumin as a standard protein. Protein content was adjusted to 2 mg / ml per sample.

1.1.1.1. Stock solutions:

For separating gel buffer (1.5 M tris-HCl, pH 8.8): Absolutely 18.17 g of tris-base was dissolved in 50 ml deionized water, pH was adjusted to be 8.8 using concentrated HCl. The final volume was made up to 100 ml. with distilled water. To prepare staking gel buffer (0.5 M tris-HCl, pH 6.8): Tris-base (6.05 g) was dissolved in 50 ml deionized water, pH was adjusted to 6.8 with concentrated HCl. The final volume was made up to 100 ml. with distilled water.

The following solutions were used for preparing sodium dodecyl sulphate solution (10 % w/v SDS): Sodium dodecyl sulphate (2.5 g) was dissolved in 25 ml deionized water. Ammonium per sulphate solution (1.5% w/v APS): Ammonium per sulphate (APS) 0.15 g was dissolved in 10 ml deionized water and kept at 4ºC. The solution is unstable and must be made just before use. To prepare electrophoresis buffer (pH8.3-8.5): The tank buffer consists of 3 g tris-base, 14.4 g glycine and 1 g sodium dodecyl sulphate dissolved in 1000 ml deionized water. For preparing monomer solution (Acrylamide stock solution): Acrylamide (29.2 g) and tris, N.N-methylene bis acrylamide (Bis) 0.8 g were dissolved in a final volume of 100 ml of deionized water, any insoluble materials were removed by filtration through Whatman filter paper No.1.

1.1.1.2. Preparation of gels:

Preparation of gels was made as described by Laemmili (1970). To prepare gradient gel: Gel mixture for polyacrylamide gel electrophoresis 11 % (SDS-PAGE) is prepared as shown in **table (1).**

Table (1): Chemicals used for electrophoretical studies.

#	Chemical	Volume
	1- Gel mixture for polyacrylamide gel electrophoresis 11 % (SDS-PAGE)	
1	Acrylamide solution 30%	*10 ml*
2	1.5 M tris-HCl (pH 8.8)	*7.5 ml*
3	10 % (w/v) SDS (sodium dodecyl sulphate)	0.3 ml
4	Deionized water	11 ml
5	1.5 % (w/v) APS (Ammonium per sulphate)	1.5 ml
6	TEMED (N,N,N,N-tetra methylene diamine)	15 µl
	2- For staking gel:	
1	Acrylamide solution 30%	3.5 ml
2	0.5 M tris-HCl (pH 6.8)	7.5 ml
3	10 %(w/v) SDS (sodium dodecyl sulphate)	0.3 ml
4	Deionized water	17.8 ml
5	1.5 % (w/v) APS	1.5 ml
6	TEMED	30 µl

1.1.1.3. Preparation of samples:

Sodium dodecyl sulphate (SDS) was added to the sample at a rate of 4 mg SDS/1 mg protein, then 50 µl 2-mercaptoethanol were applied to each 950 ml of the sample, then the mixture was heated at 100º C in water bath for 3-5 min. To Pouring the separating and staking gel: The resolving gel was poured between glass sandwich (San Francisco CA, USA, Model XPO77 Hoefer) and gently covered with 1 cm of water. Polymerization started within 25-30 min after pouring the stacking gel was then poured and allowed for polymerization after about 30 min. For loading of the samples: twenty microliters of this crude protein solution were applied to the wells of the stacking gel. The samples were covered with electrode buffer. Few drops of bromophenol blue (4 mg/100 ml deionized water) were added to the electrode buffer (tracking dye). Electrophoresis was performed in a vertical slab mold 16 x (Hoefer Scientific Instruments, San Francisco, CA, USA). Electrophoresis was carried out at 30 milliamper (m.A.) at 10º C for 3 hours. The silver staining method for protein described by Sammons *et al.* (1981) was used. This method of staining is sensitive and detects as little as 2 ng of protein in a single band staining procedure is shown in **table (2).**

Table (2): Silver staining method for proteins (Sammons *et al,* 1981).

Step.	Staining procedure	Time
1	Fix gels in 50 % ethanol containing 10 % acetic acid.	3x 1h
2	First wash in 50 % aqueous ethanol containing 10% acetic acid.	2hrs
3	Two washes in 25% aqueous ethanol containing 10% acetic acid.	2x 1h
4	Two washes in 10% aqueous ethanol containing 0.5% acetic acid.	2x 1h
5	Equilibrate gel in a degassed aqueous solution of silver nitrate (1.9 g/ l) the volume of solution being about 3 times as the volume of gel.	2hrs
6	Rinse briefly in degassed water.	
7	Place in reducing bath consisting of 0.75 M NaOH 87 mg/l of NaOH and 15 ml of 37% formaldehyde which is added to the solution immediately before use. The volume used should be 5.5 times the gel volume.	10 min
8	Place in colour enhancing solution (5.5 times gel volume) consisting of 7.5 g/l sodium nitrate in water.	1h
9	Transfer into fresh sodium carbonate (7.5 g/ l water).	1h
10	Transfer into sodium carbonate (7.5 g/ l).	1h

1.1.2 Electrophoresis analysis

The intensities of the bands on each gel were then scanned with a computing densitometer at absorbance of 633 nm and results were graphically depicted with Image Phoretic (IAD) Quantifier Software (Phoretix International, London). The peak heights, R_f, and intensity were used as a relative comparison of band intensity between zygomorphs on the same gel. The bands intensity was compared to the seven different protein markers (6, 14, 20, 29, 45, 66 and 79 KDa) used to the electrophoretic analysis.

Results and Discussions

Approximately 1 g freeze dry of bodies and larvae from the susceptible-laboratory and resistant-field strains for pink bollworm was ground in a mortar and pestle in liquid nitrogen. Crushing continued until the sample completely homogenized. Centrifugation, 18,000 rpm for about 30 min was carried out to remove debris. The protein content in supernatant was estimated according to the method of Bradford (1976) by using bovine serum albumin as a standard protein.

The general protein patterns demonstrated by polyacrylamide gel electrophoresis (PAGE) of both pink bollworm (PBW) larvae and male moths of susceptible and field strains are shown in **Figs (1, 2 and 3)** and recorded in **tables (3 and 4).**

1. Polyacrylamide gel electrophoresis of PBW male moths protein

The presence of four protein patterns of the susceptible strain, El-Bohira, Alexandria male moth field strains and the standard marker protein was illustrated **Figs (2 a, b, c and d)**. Total 36 bands of individual separated protein with different molecular weights and different protein amount percentages were detected and tabulated in **table (3).**

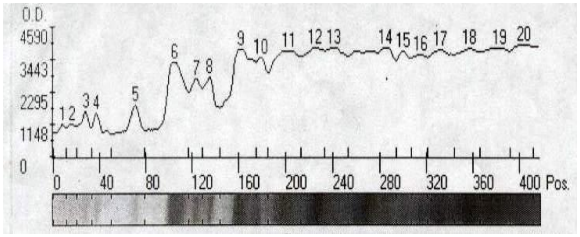

Fig. (2 a): Bohira field male moths

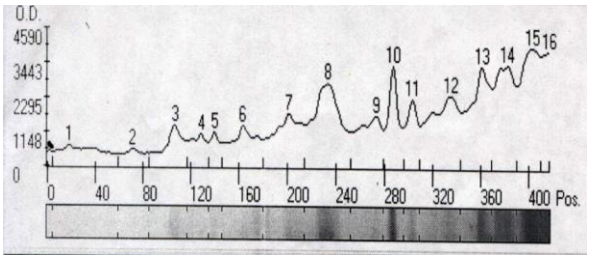

Fig. (2 b): Alexandria field male moths

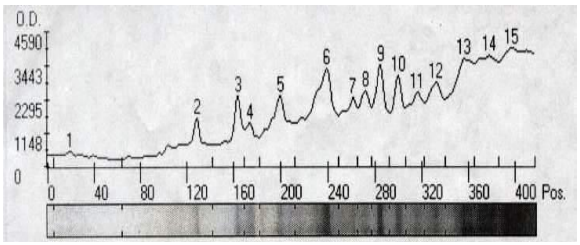

Fig. (2 c): Susceptible male moths

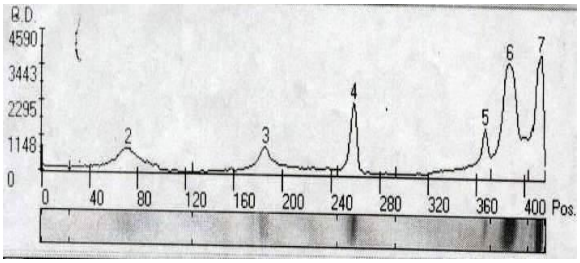

Fig. (2 d): Protein Marker

Figure (2): Spectrophotometric scanning of electrophoretic patterns in homogenate of pink bollworm male moths collected from susceptible laboratory and field strains.

1.1. Gel electrophoresis of PBW susceptible strain male moths.

Electrophoretic protein pattern of the susceptible strain male moths was showed in **Fig (2 c).** This protein pattern showed clear differences in protein bands and their intensities which related to the protein amount percentages. Generally, fifteen protein bands were detected (shown in table 4). The obtained data pointed out to the found of four obvious bands with protein amount percentages: 16.26, 11.08, 10.49 and 10.41 and their matching molecular weights were: 11, 33, 21 and 17 KDa, respectively.

1.2. Gel electrophoresis of Bohira PBW field strain male moths.

Twenty protein bands including different molecular weights and different intensities were showed in **Fig. (2a)**. The twenty protein bands were detected and included in **table (3)** with their corresponding protein amount percentages.

Table (3): Electrophoretic pattern of protein bands in homogenates of susceptible strain, Bohira and Alexandria (male moth) field strains and the standard marker protein.

Band No	M W (KDa)	Susceptible Strain		Bohira Strain		Alexandria Strain		Protein Marker	
		P/A	Amt %	P/A	Amt %	P/A	Amt %	P/A	Amt %
1	5	A	-	A	-	P	4.15	A	-
2	6	A	-	A	-	A	-	P	17.44
3	9	A	-	P	6.51	P	11.22	A	-
4	11	P	16.26	A	-	A	-	A	-
5	14	A	-	A	-	A	-	P	30.99
6	15	A	-	P	8.23	P	10.21	A	-
7	17	P	10.41	A	-	A	-	A	-
8	20	A	-	A	-	P	10.15	P	12.61
9	21	P	10.49	P	6.86	A	-	A	-
10	23	P	6.22	P	7.05	P	10.29	A	-
11	24	P	4.93	P	4.17	A	-	A	-
12	25	A	-	A	-	P	3.68	A	-
13	26	P	4.53	P	3.89	A	-	A	-
14	27	P	5.23	P	12.65	P	5.42	A	-
15	28	P	3.76	A	-	P	6.02	A	-
16	29	P	4.36	A	-	A	-	P	10.01
17	33	P	11.08	P	6.25	A	-	A	-
18	35	A	-	A	-	P	11.59	A	-
19	36	A	-	P	6.22	A	-	A	-
20	41	A	-	P	8.29	A	-	A	-
21	42	P	6.64	A	-	P	7.20	A	-
22	45	A	-	A	-	A	-	P	14.01
23	46	A	-	P	2.80	A	-	A	-
24	47	P	2.23	A	-	A	-	A	-
25	49	P	4.29	P	7.70	P	5.30	A	-
26	53	A	-	A	-	P	1.63	A	-
27	54	A	-	P	2.96	A	-	A	-
28	55	P	6.54	A	-	P	2.61	A	-
29	56	A	-	P	2.57	A	-	A	-
30	60	A	-	P	6.31	P	3.95	A	-
31	66	A	-	P	2.81	P	1.93	P	13.29
32	72	A	-	P	1.57	A	-	A	-
33	74	A	-	P	1.40	A	-	A	-
34	76	P	3.00	P	0.84	P	4.65	A	-
35	78	A	-	P	0.92	A	-	A	-
36	79	A	-	A	-	A	-	P	1.65

The obtained data showed that, there is one obvious band with molecular weights 27 KDa and its matching protein amount percentage was 12.65%, while there were eleven bands with moderate protein amount percentages between 3.89 and 8.29 and eight bands with faint protein amount percentages between 0.84 and 2.96.

1.3. Gel electrophoresis of Alexandria PBW field strain male moths.

Sixteen protein bands which were presented in the electrophoretic protein of Alexandria PBW field strain male moths illustrated in **Fig. (2 b)** and included in **table (3).** Moreover, differences between the intensities of these bands were noticed. Five bands were highly pronounced, eight bands had a moderate intensity and the other remaining three bands were faint.

2. Polyacrylamide gel electrophoresis of PBW protein larvae.

Four protein patterns of the susceptible strain, El-Bohira, Alexandria larval field strains and the standard marker protein were illustrated in **Figs. (3 a, b, c and d)**. Total 38 bands of individual separated protein with different molecular weights and different protein amount percentage were detected and tabulated in **table (4).**

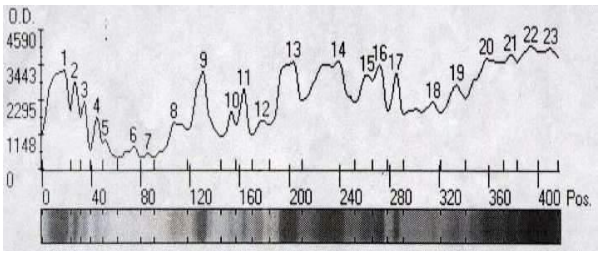

Fig (3 a): Bohira larvae

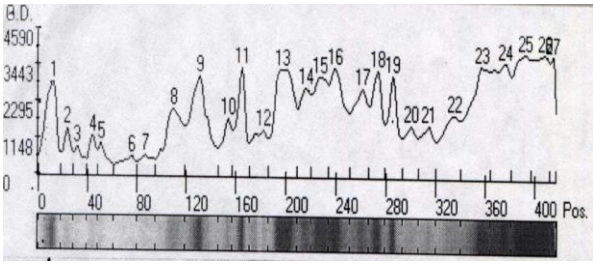

Fig (3 b): Alexandria larvae

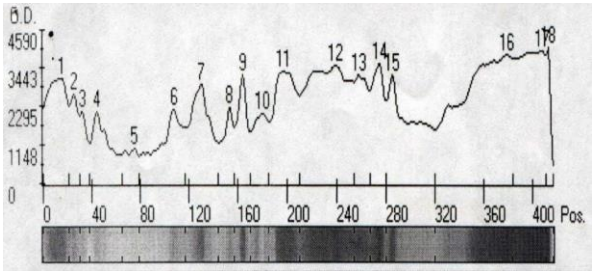

Fig (3 c): Susceptible larvae

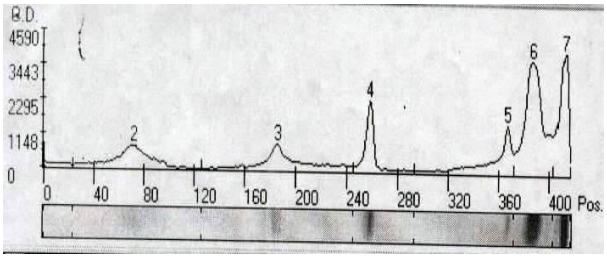

Fig (3 d): Protein Marker

Figure (3): Spectrophotometric scanning of electrophoretic patterns in homogenate of pink bollworm larvae collected from susceptible laboratory and field strains.

Table (4): Electrophoretic pattern of protein bands in homogenates of susceptible strain, Bohira and Alexandria PBW larval field strains and the standard marker protein.

Band No	M W (KDa)	Susceptible Strain		Bohira Strain		Alexandria Strain		Protein Marker	
		P/A	Amt %	P/A	Amt %	P/A	Amt %	P/A	Amt %
1	6	P	1.79	A	-	P	2.18	P	17.44
2	7	A	-	P	6.52	A	-	A	-
3	8	P	10.21	A	-	P	6.80	A	-
4	12	A	-	P	6.87	P	7.02	A	-
5	14	A	-	A	-	A	-	P	30.99
6	16	P	18.57	A	-	A	-	A	-
7	17	A	-	P	4.21	P	6.97	A	-
8	20	A	-	A	-	A	-	P	12.61
9	21	A	-	P	10.49	P	7.85	A	-
10	23	A	-	P	5.03	P	3.92	A	-
11	24	A	-	P	6.26	P	2.38	A	-
12	26	A	-	A	-	P	2.20	A	-
13	27	P	8.07	P	3.35	P	3.49	A	-
14	28	P	3.99	P	3.81	P	4.06	A	-
15	29	P	3.92	P	4.03	P	4.92	P	10.01
16	33	P	13.79	P	13.05	P	5.61	A	-
17	36	A	-	A	-	P	4.86	A	-
18	38	A	-	A	-	P	3.31	A	-
19	41	A	-	P	7.54	A	-	A	-
20	43	P	7.00	A	-	P	7.27	A	-
21	45	A	-	A	-	A	-	P	14.01
22	46	P	2.76	P	2.13	P	2.31	A	-
23	49	P	2.92	P	2.56	P	3.44	A	-
24	51	P	2.10	P	1.82	P	1.75	A	-
25	55	P	5.57	P	5.65	P	6.25	A	-
26	59	P	4.99	A	-	P	4.01	A	-
27	60	A	-	P	2.98	A	-	A	-
28	63	A	-	P	0.53	P	0.95	A	-
29	65	P	1.31	A	-	P	1.04	A	-
30	66	A	-	P	1.13	A	-	P	13.29
31	70	A	-	P	0.88	P	1.03	A	-
32	71	P	3.60	P	1.29	P	0.99	A	-
33	73	P	1.43	P	1.41	A	-	A	-
34	74	A	-	P	2.04	P	0.90	A	-
35	75	P	2.13	A	-	P	1.22	A	-
36	76	P	5.86	P	6.43	A	-	A	-
37	77	A	-	A	-	P	3.91	A	-
38	79	A	-	A	-	A	-	P	1.65

2.1. Gel electrophoresis of PBW susceptible strain larvae:-

Electrophoretic protein pattern of the susceptible strain larvae was illustrated in **Fig. (3c).** This protein pattern showed clear differences in protein bands in their intensities which related to the protein amount percentage. Generally, eighteen protein bands were detected (shown in table 4). The obtained data pointed out the presence of three obvious bands with protein amount percentages: 10.21, 13.79 and 18.57 and their matching molecular weights were: 8, 33 and 16 KDa, respectively.

2.2. Gel electrophoresis of Bohira PBW field strain larvae:-

Twenty three protein bands including different molecular weights and different intensities were illustrated in **Fig. (3a).** The twenty three protein bands were detected and shown in **table (4).** The recorded data showed that, there are two bands were obvious with molecular weights 21 and 33 KDa and their matching protein amount percentages were: 10.49 and 13.05, while there were eleven bands with moderate protein amount percentages between 3.35 and 6.87, and ten bands have faint protein amount percentages between 0.53 and 2.98.

2.3. Gel electrophoresis of Alexandria PBW field strain larvae:-

Twenty seven protein bands which were presented in the electrophoretic protein of Alexandria PBW field strain larvae were illustrated in **Fig. (3b)** and included in **table (4).** Moreover, differences among the intensities of these bands were noticed. Two bands were highly pronounced, eleven bands have a moderate intensity and the other remained ten bands were faint.

The recorded data in **table (5)** showed that, the total bands and molecular weight values of protein patterns in male moth and larvae of susceptible and field strains. The total bands which were detected in all strains (susceptible and field strains) were 48 bands. The male moths and larvae were similar in some bands and different in other bands.

Table (5): Electrophoretic pattern of protein bands in homogenates of susceptible strain, Bohira and Alexandria PBW male moths and larvae field strains.

Band No	MW KDa	Moths						Larvae					
		Bohira Strain		Alex strain		Susce strain		Bohira strain		Alex strain		Susce strain	
		P/A	Amt %	P/A	Amt %	P/A	Amt %	P/A	Amt %	P/A	Amt %	P/A	Amt %
1	5	A	-	P	4.15	A	-	A	-	A	-	A	-
2	6	A	-	A	-	A	-	A	-	P	2.18	P	1.79
3	7	A	-	A	-	A	-	P	6.52	A	-	A	-
4	8	A	-	A	-	A	-	A	-	P	6.80	P	10.2
5	9	P	6.51	P	11.22	A	-	A	-	A	-	A	-
6	11	A	-	A	-	P	16.26	A	-	A	-	A	-
7	12	A	-	A	-	A	-	P	6.87	P	7.02	A	-
8	15	P	8.23	P	10.21	A	-	A	-	A	-	A	-
9	16	A	-	A	-	A	-	A	-	A	-	P	18.96
10	17	A	-	A	-	P	10.41	P	4.21	P	6.97	A	-
11	20	A	-	P	10.15	A	-	A	-	A	-	A	-
12	21	P	6.86	A	-	P	10.49	P	10.49	P	7.85	A	-
13	23	P	7.05	P	10.29	P	6.22	P	5.03	P	3.92	A	-
14	24	P	4.17	A	-	P	4.93	P	6.26	P	2.38	A	-
15	25	A	-	P	3.68	A	-	A	-	A	-	A	-
16	26	P	3.89	A	-	P	4.53	A	-	P	2.20	A	-
17	27	P	12.65	P	5.42	P	5.2	P	3.35	P	3.49	P	8.07
18	28	A	-	P	6.02	P	3.76	P	3.81	P	4.06	P	3.99
19	29	A	-	A	-	P	4.36	P	4.03	P	4.92	P	3.92
20	33	P	6.25	A	-	P	11.01	P	13.05	P	5.61	P	13.79
21	35	A	-	P	11.59	A	-	A	-	A	-	A	-
22	36	P	6.22	A	-	A	-	A	-	P	4.86	A	-
23	38	A	-	A	-	A	-	A	-	P	3.31	A	-
24	41	P	8.29	A	-	A	-	P	7.54	A	-	A	-
25	42	A	-	P	7.20	P	6.64	A	-	A	-	A	-
26	43	A	-	A	-	A	-	A	-	P	7.27	P	7.00
27	46	P	2.80	A	-	A	-	P	2.13	P	2.31	P	2.76
28	47	A	-	A	-	P	2.23	A	-	A	-	A	-
29	49	P	7.70	P	5.30	P	4.29	P	2.56	P	3.44	P	2.92
30	51	A	-	A	-	A	-	P	1.82	P	1.75	P	2.10
31	53	A	-	P	1.63	A	-	A	-	A	-	A	-
32	54	P	2.96	A	-	A	-	A	-	A	-	A	-
33	55	A	-	P	2.61	P	6.54	P	5.65	P	6.25	P	5.57
34	56	P	2.57	A	-	A	-	A	-	A	-	A	
35	59	A	-	A	-	A	-	A	-	P	4.01	P	4.99
36	60	P	6.31	P	3.95	A	-	P	2.98	A	-	A	-
37	63	A	-	A	-	A	-	P	0.53	P	0.95	A	-
38	65	A	-	A	-	A	-	A	-	P	1.04	P	1.31
39	66	P	2.81	P	1.93	A	-	P	1.13	A	-	A	-
40	70	A	-	A	-	A	-	P	0.88	P	1.03	A	-
41	71	A	-	A	-	A	-	P	1.29	P	0.99	P	3.60
42	72	P	1.57	A	-	A	-	A	-	A	-	A	-
43	73	A	-	A	-	A	-	P	1.41	A	-	P	1.43
44	74	P	1.40	A	-	A	-	P	2.04	P	0.90	A	-
45	75	A	-	A	-	A	-	A	-	P	1.22	P	2.13
46	76	P	0.84	P	4.65	P	3.0	P	6.43	A	-	P	5.86
47	77	A	-	A	-	A	-	A	-	P	3.91	A	-
48	78	P	0.92	A	-	-		A	-	A	-	A	-

For protein patterns found in male moths and larvae in Alexandria Governorate, Alexandria University, Faculty of Agriculture Farm, (Table 51), the recorded data showed that, the presence of four major protein bands with MW 49, 27, 28, and 23 KDa were presented in the male moths and larvae patterns field strains and their intensities were higher (5.30, 5.42, 6.02, and 10.29%) in the male moths (5.30, 5.42, 6.02, and 10.29%) in the male moths of field strain than in the larvae field strain (3.44, 3.49, 4.06 and 3.92%). Eleven bands were found in the male moths field strain pattern with MW 5, 9, 15, 20, 25, 35, 42, 53, 60, 66 and 76 KDa are not detected in larvae field pattern, while other bands were present in the larvae pattern field strain at the MW of 6, 8, 12, 17, 21, 24, 26, 29, 33, 36, 38, 43, 46, 51, 59, 63, 65, 70, 71, 74, 75, and 77 KDa and are not detected in male moths field pattern. These data showed that, the patterns clear differences in protein bands between larvae and male moths of Alexandria field strains. The results showed that, the patterns distinguished differences in protein bands between male moths and larvae of PBW in their protein amount percentage as well as their molecular weights.

Electrophoretic bioassay analysis of proteins showed that protein bands were varied and there were differences among proteins of susceptible and field strains of PBW both in their intensity and their molecular weights. These findings could interpret the susceptibility difference between the field and susceptible strains. Differences in protein bands between male moths or larvae of Bohira and Alexandria field strains in their intensity and their molecular weights could be due to the history of insecticides application in the two regions, that is because of Bohira Governorate has the most cultivated cotton area all over the country, so it receives the most applications of insecticides against both bollworm (pink bollworm and spiny pink bollworm) and cotton leafworm, instead of the other economic pests. This area receives the heaviest insecticide applications and the continuous use of it throughout the entire season. On the other hand, insecticides are rarely used against bollworm and cotton leafworm in Alexandria Governorate. So, it is logical to get high levels of resistance against pink bollworm in Bohira strain compared to that of Alexandria strain. The data obtained from the biochemical studies are in good agreement with that of laboratory and field studies of the attracticide resistance monitoring technique.

This study strengthens the hypothesis that the mechanism associated with insecticides resistance found in PBW strains includes an increase of protein concentration or the presence of new proteins probably as results of gene amplification. These findings are in a good agreement with the findings of many authors who used electrophoretic bioassay analysis to detect the biochemical differences between susceptible and field strains of many insect species. Albeltagy*et al* (1993) revealed that, there are different bands of proteins of PBW that varied in intensity and molecular weights which could be interpreting the susceptibility differences between field and laboratory strains. The electrophoresis gels of resistant *Helicoverpa punctigera* showed bands that were not present in susceptible strain (Gunning *et al* 1997). Similar reports showed the presence of insecticides resistant in diamondback moth (Sun *et al* 1995) and green bugs. *Schizaphis graminum*(Siegfried *et al* 1997).Owusu*et al* (1996) showd that, the resistant strain of cotton aphid *Aphis gossypii* possessed 8 major bands, while the susceptible strain possessed only 3 bands are probably enzymes associated with dichlorovos resistance.

REFERENCES

Albeltagy, A.M. (2012a) : Pheromone Traps for the assessment of Insecticides Efficiency and Monitoring Insecticides resistance in Insect Field Strains . Proc. Of Minia Int. Conf. for Aric.And

Irrig. In the Nile Basin Countries, 26th – 29 th March 2012, El-Minia, Egypt, :213 – 222 .

Albeltagy, A. M. (2012b) : Insecticide resistance : The global problem of insect management. Abstract, ESA's Entomology Conference, November 11- 14, Knoxville, TN, USA.

Albeltagy, A.M. ; M. S. Shawir ; K.A. Osman ; M.M. Abo El-Saad and M. A. Mourad (1993). Biochemical technique for measuring susceptibility of pink bollworm, *Pectinophora gossypiella* to certain insecticides. Alex. J. Agric. Res.; 38 (3): 393-411.

Albeltagy, A.M. ; M.M. El-mourshedy and A.K. Mourad (1996). Pheromone traps for monitoring insecticide tolerance in three different lepidopterous insects: an effective tool for insecticide resistance management. Egypt.J. Agric. Res.; 74 (1): 29-37.

Albeltagy, A. M. ; H. S. Radwan ; Z. A. El-Bermawy ; M. E. Nassar ; A. G. Yousef and M. M. Shekeban (2001a). Attracticide resistance monitoring technique for assaying insecticide resistance in pink bollworm male moths, *Pectinophora gossypiella*(S) field populations, Egypt. J. Agric. Res.; 79 (3): 949-963.

Albeltagy, A.M., H.S. Radwan, G.E. Abo El-Ghar, I.M. Ammar, A.A. Eisa, M.M. Shekiban and M.M. El bassiony (2001b) . Biochemical impact of pyrethroid resistance management in bollworms field populations with Gossyplure disruption technique .1- in relation to total protein electrophoretic analysis. J. Adv. Agric. Res. , 6(4) : 1029 – 1041 .

Albeltagy, A.M., H.S. Radwan, G.E. Abo El-Ghar, I.M. Ammar, A.A. Eisa, M.M. Shekiban and M.M. El bassiony (2001c) . Biochemical impact of pyrethroid resistance management in bollworms field populations with Gossyplure disruption technique .2- in relation to AchE and total esterases activity determination. J. Adv. Agric. Res. , 6(4) : 1043 – 1055 .

Albeltagy, A.M.; M.M.K. Shekeban; M.M. Abo El-Amaym; S.M.I. Kassem; A.H. Mancee and S.A. El-Arami (2010) . Monitoring for Pyrethroid resistance in pink bollworm Pectinophora gossypiella (Saunders) field strains . Pest Management Newsletter , 19(2) : 46-52 .

Albeltagy, A.M.; M.M.K. Shekeban; M.M. Abo El-Amaym; S.M.I. Kassem; A.H. Mancee and S.A. El-Arami (2012a) : Assaying Insecticidal Efficacy of O.P.s against Pink Bollworm Male Moth Field Strains Using the Attracticide Efficacy Monitoring Technique. Pest Management Newsletter , 21(2) : 7-13 .

Albeltagy, A.M.; M.M.K. Shekeban; M.M. Abo El-Amaym; S.M.I. Kassem; A.H. Mancee and S.A. El-Arami (2012b) : Efficacy of Pyrethroids against Pink Bollworm Male Moth Field Strains Uisng the Attracide Efficacy Monitoring Technique. Pest Management Newsletter, 21(2) : 13-20 .

Bradford, M.M. (1976). A rapid and sensitive method for the quantitative of microgram quantities of protein utilizing the principal of protein dye binding. Anal. Biochem.; 72: 248-254.

Georghiou, G.P. (1983). Management of resistance in arthropods P.P. 769-792. In G.P. Georghiou and T. Satto (eds), pest resistance to pesticides. Plenum, New York.

Gunning, R.V. ; G.D. Moores and A.L. Devonshire (1997). Esterases and fenvalerate resistance in a field population of *Helicoverpa punctigera*in Australia.Pestic. Biochem.Physoil.; 58: 155-162.

Haynes, K.F. ; T.A. Miller ; R.T.Staten ; W.G. Li and T.C. Baker (1986).Monitoring insecticide resistance with insect pheromone. Experientia; 42:1293-1295.

Haynes, K.F. ; T.A. Miller ; R.T. Staten ; W.G. Li and T.C.Baker (1987). Pheromone traps for monitoring insecticide resistance in the pink bollworm moth (Lepidoptera :Gelechiidae) : New tool for resistance management. Environ.Entomol.; 16:84-89.

Knight, A.L. and L.A. Hull (1989). Use of sex pheromone traps to monitorazinphos-methyl resistance in tufted apple bud moth (Lepidoptera: Tortricidae). J. Econ. Entomol.; 82 (4): 1019-1026.

Laemmili, U.K. (1970). Cleavage of structural proteins during the assembly of the head of Bacteriophage T4. Nature, 227: 680-685.

Miller, T.A. (1986). Status of resistance in the cotton insect complex. Beltwide cotton production research conf.; 162-165.

Miller, T.A. (1990). Resistance monitoring of pink bollworm California Department of Food andAgriculture (CDFA), pink bollworm final report 1990; 146-147.

Mink, J.S. and D.J. Boethel (1992). Development of a diagnostic technique for monitoring permethrin resistance in soybean looper larvae (Lepidoptera : Noctuidae). J. Econ. Entomol.; 85(4):10565-1062.

Owusu, E.O. ; M. Horiike and C. Hirano (1996). Polyacrylamide gel electrophoretic assessments of esterases in cotton aphid (Homoptera: Aphididae) resistance to dichlorvos. J. Econ. Entomol. ; 89 (2): 302-306.

Pasquier, D. and P.J. Charmillot (2003). Effectiveness of twelve insecticides applied topically to diapausing larvae of the codling moth, *Cydiapomonella* L. Pest. Manag. Sci.; 60 (3):305–308.

Pimentel, D. (2005). Environmental and economic costs of the application of pesticides primarily in the United States.Environ. Develop. and Sustain.; 7 (2): 229-252

Roush, R.T. and G.L. Miller (1986). Considerations for design of insecticide. J. Econ. Entomol.; 87 (2): 184-192.

Roush, R. T. and J. A. McKenzie (1987). Ecological genetics of insecticide and acaricide resistance.Ann.Rev. Entomol.; 32: 361-380.

Sammons, D.W. ; Adams, L.D. and Nishizawa, E.E. (1981). Ultra-sensitive silver based color staining of polypeptides in polyacrylemide gels. Electrophoresis.; 2: 135.

Sanderson, J.P. and R.T. Roush (1992). Monitoring insecticide resistance in green house whitefly with yellow sticky cards. J. Econ. Entomol., 85(3):634-642.

Schouest, L.P. Jr. and T.A. Miller (1988). Factors influencing pyrethroid toxicity in pink bollworm (Lepidoptera :Gelechiidae) : Implications for resistance management. J. Econ. Entomol.; 81(2):431-436.

Siegfried, B.D. ; J.J. Swanson and A.L. Devonshire (1997). Immunological detection of greenbug *Schizaphisgra minum*esterases associated with resistance to organophosphate insecticides. Pestic. Biochem. Physiol.; 57:165-170.

Sun, Y. ; Y. Chen and Y. Ying (1995). Insecticide resistance in diamondback moth in South China.Pestic. Sci.; 43:355-357.

Tabashnik, B.E. and N.L. Cushing (1987). Leaf residues topical bioassay for assessing insecticide resistance in the diamondback moth. FAO plant Prot. Bull.; 35:11-14.

Abdallah M. Albeltagy
Bollworms Research Dep., Plant protection Res. Institute
Giza, Egypt
Albeltagy515@gmail.com

REGULARITIES IN DISTRIBUTION OF INSECTICIDE RESISTANCE IN POPULATIONS OF COLORADO POTATO BEETLE IN THE SOUTH URAL.

INTRODUCTION

Colorado potato beetle has substantial intraspecific polymorphism (Ushatinskaya, 1981; Fasulati, 2002). This allows it to adapt to anthropogenic influences. Resistant to almost all currently used insecticides developed in populations in much of its range (Benkovskaya, et al., 2008).

Colorado beetle invaded South Urals in the period from 1976 to 1979 (Migranov, 1994). Pest control was implemented with organochlorine insecticides (gamma-HCH, polychlorocamphene, Dilorom, despirol etc.) and organophosphates (trichlorfon, volaton, dibromo, ftalofos, phosalone, etc.). In the 80 years of pest control agents was carried out from the class of synthetic pyrethroids. Beetles originally settled in the territory of Bashkortostan were susceptible to insecticides, and one to two treatments per season was enough to curb the pest population (Amirkhanov, 1995; Amirkhanov, et al. 1986). Resistance to pyrethroids in the pest began to emerge by 1996. Later it was shown in the South Ural that long-term self-perpetuating process resulted in individuals with high resistance (Leontieva et al., 2002).

The aim of our work: Assessment of the current state of the Colorado potato beetle resistance to a number of insecticides in the South Ural and estimation distribution of resistance to different classes of insecticides for several years.

Keywords: Colorodo potato beetle, resistance to insecticides, regularities in distribution, South Ural.

MATERIALS AND METHODS

Objects of investigation were overwintering adults of the Colorado potato beetle Leptinotarsa decemlineata Say (Coleoptera, Chrysomelidae) in 2005 - 2012. Individuals were collected by hand from the crops in the Southern Urals (Bashkortostan). We used the preparations of several classes of compounds - organophosphate (Actellic®, the active ingredient - pirimiphos-methyl, EC 500 g / l), pyrethroid (Decis®, the active ingredient - deltamethrin, EC 2.5 g / l), neonicotinoid (Actara®, the active ingredient - thiamethoxam, WDG 250 g / kg and Mospilan, the active ingredient - acetamiprid, WP 200 g / kg), nereistoxin (Bancol, the active ingredient - bensultap WP 500g/kg) as well as a new class of phenylpyrazole (Regent®, the active ingredient - fipronil, WDG 800 g / kg)(PPDB, citing date: 04.04.2013).

We treated topically with insecticide by the working solution in a dose of 1 ml / specimen (Novozhilov, 2006). The solution is applied by a micro-syringe brand MSH-1 in the ventral region of the prothorax.

We used established diagnostic concentration (DC) of selected preparations (twice the SK95) to identify resistant individual Colorado potato beetles. We counted the number of survivors at day 10. Proportion of survivors after treatment with a diagnostic dose of

insecticide displayed a genotype frequency of resistance.

We used the Mantel test in Passage 2 (Rosenberg, 2011) to study the patterns of spatial distribution of resistant genotypes. The genetic distance between populations was considered by the share of stable species. Distance calculated from the Euclidean metric (geometric distance).

RESULTS AND DISCUSSION

The results of toxicological experiments for 2005 - 2012 showed proportion of resistant individuals is highly variable in some years. According to data presented in table 1, we can see that resistance to organophosphorus insecticides in Colorado potato beetle has spread everywhere. A portion of resistant individuals reached 90% in the observed period, but was 21% in 2010. At the same time we saw a dramatic increase in the percentage of individuals resistant to pyrethroids at 61%, which continues to rise, reaching 80% in 2011. Resistance to other insecticides also varies widely, but we saw that for neonicotinoid it does not exceed that of 2009 (49%). We also saw high rates of resistance to nereistoksinam, 80% in 2009. Average fraction of genotypes resistant to fipronil had already reached 28% in 2009, although the using of phenylpyrazole in potato protection started recently in 2003. Fluctuations in the proportion resistant individuals supposedly can be explained by the entry of resistant individuals in the long diapause and the dependence of the efficiency action of preparations on the temperature and weather conditions of the summer season.

Table 1: Survival of adult Colorado potato beetle in the South Ural, adjusted for control after the use of insecticides in the diagnostic concentrations for 2005 - 2012. (estimation on third day after treatment).

Year	Actellic	Decis	Actara	Regent	Bancol
2005	0.82±0.05	0.45±0.05	0.19±0.05	-	-
2006	0.83±0.03	-	0.13±0.04	0.01±0.02	-
2007	0.90±0.03	0.59±0.02	0.03±0.02	0.02±0.02	0.18±0.03
2008	0.88±0.04	0.24±0.07	0.30±0.06	0.08±0.04	0.78±0.04
2009	0.63±0.08	0.22±0.06	0.49±0.09	0.28±0.07	0.80±0.08
2010	0.21±0.10	0.61±0.11	0.26±0.15	0.13±0.07	-
2011	0.72±0.08	0.87±0.04	0.14±0.05	0.14±0.07	0.71±0.07
2012	0.84±0.04	0.31±0.05	0.38±0.05	0.05±0.01	0.35±0.06

We calculated the correlation of geographic distance and the distribution of proportion resistant individuals to all classes of insecticides in populations of Colorado potato beetle (Table 2). The absence of significant correlations suggests that genetic factors insecticide resistance does not apply to mass populations of Colorado potato beetle as a result of migration.

Table 2: Coorelations of geographic distance and the distribution of proportion individuals resistant to all classes of insecticides in populations of Colorado potato beetle (Mantel test).

Year	correlations with distance	Left-sided significance	Right-sided significance
2005	-0.16	0.06	0.93
2006	-0.24	0.22	0.77
2007	0.11	0.81	0.19
2008	-0.09	0.31	0.68
2009	-0.20	0.19	0.80
2010	-0.24	0.05	0.94
2011	-0.03	0.43	0.56
2012	-0.23	0.02	0.99

We considered that the resistance to insecticides in the local population is formed for the most part independently by frequent repeated treatments with insecticides. To confirm this hypothesis, we calculated the correlation of geographical distances and distribution of resistant individual proportion for each class of insecticides (Table 3). We used the combined data for the three years to improve the accuracy of the possible correlations. Significant correlations are absent in all cases regardless of the percentage resistant to each insecticide. This means that resistance to insecticides developed independently in each population.

Table 3: Correlations of geographic distance and the distribution of proportion resistant individuals for each class of insecticides for 2010 - 2012. (Mantel test).

Insecticides	correlations with distance	Left-sided significance	Right-sided significance
Regent	-0.09	0.08	0.91
Actara	-0.10	0.03	0.96
Actellic	0.02	0.60	0.30
Decis	-0.03	0.10	0.80

CONCLUSION

Insecticide resistance remains high in the Colorado potato beetle populations in the South Urals, but varies from year to year. Resistance to new insecticides is formed at high speed. Spread of resistance to insecticides is not due to migration, but permanent treatments with the same insecticide local populations of Colorado potato beetle.

REFERENCES

Amirkhanov D.V. (1995). Toxicological substantiation of improvement of chemical potato protection from Colorado Potato Beetle in Southern Ural. Doct. Dissert. Abstr. Saint-Petersburg: VIZR, 48 p. (in Russian)

Amirkhanov D.V., Abramova T.L., Benkovskaya G.V. (1986). Ecological aspects of new classes of insecticides using for Colorado potato beetle control // Ecological aspects of biocenothic gomeosthasis. Ufa, P. 140 – 149. (in Russian)

Belenkiy M.B. (1963). Elements of the Quantitative Assessment of the Pharmacological Effects. Leningrad, p.67 (in Russian)

Benkovskaya G.V., Leontieva T.L, Udalov M.B. (2008). Colorado beetle resistance to insecticides in South Urals Agrochemistry, No. 8, page 55-59 (in Russian)

Fasulati, S.R., (2002). Territorial spread and adaptation of Colorado potato beetle in the northern regions of potato growing. In: Ekologicheskie aspekty intensifikatsii s.h. proizvodstva. Mat. mezhdunar. nauch. – prakt. konf. Penza, P. 205-207. (in Russian)

Leontieva T.L., Benkovskaya G.V., Nikolenko A.G. (2002). The loss of pyrethroid effectivity in Leptinotarsa decemlineata Say control in Bashkortostan // The modern state of pests, phytopathogens and weeds resistance problem in Russia and bordering countries at the border-line of XXI century. Materials of IX Symposium (20-22 dec., 2002, Sankt-Petersbourgh: VIZR. P. 33 – 34. (in Russian)

Migranov M. G. (1994). Pyrethroids: domestic analogs and their toxicology. Ufa, 101 p. (in Russian)

Rosenberg M.S., Anderson C.D. (2011). PASSaGE: Pattern Analysis, Spatial Statistics and Geographic Exegesis. Version 2 // Methods in Ecology and Evolution. Vol. 2. P. 229–232.

Novozhilov K.V. (ed.) (2006). Technology and evaluation of pesticides side effects (by the example of resistance to insecticides in Colorado potato beetle overcoming). Sanct-Peterburg. 52 pp. (in Russian).

PPDB: Pesticide Properties Database http://sitem.herts.ac.uk/aeru/footprint/en/index.htm

Ushatinskaya, R.S., ed. (1981). Colorado Potato Beetle. Moscow: Nauka, 377 p. (in Russian).

Kitaev K.A., Penkin L.N., Surina E.V. and Benkovskaya G.V.
Institute of biochemistry and genetics of Russian Academy of Science, Russia.
cordek(at) ya.ru

Insecticide resistance status in *Anopheles spp* in Blue Nile State (December 2012 – May 2013)

ABSTRACT

During 2012-2013, a network for the monitoring of insecticide resistance was set up in the Blue Nile state to assess the insecticide resistance status of the major malaria vectors in Damazin - Rosaries - Geissan. Bioassays were performed on adult mosquitoes using the standard WHO susceptibility test with diagnostic concentrations of permethrin 0.75%, deltamethrin 0.05%, bendiocarb 1% and DDT 4% to determine if pesticide resistance had been developing in local mosquito populations. After using procedures developed by WHO, the results collected from areas serviced by our adulticide program were presented. After three months of intense insecticide resistance monitoring a clear picture of insecticide resistance status of malaria vectors was achieved. In Geissan, Damazin and Rosaries insecticide resistance in the malaria vectors *An. arabiensis* was almost present. According to the WHO criteria for characterizing insecticide susceptibility; in the present study the *An. arabiensis* was found to be resistant to DDT 4% in two areas, Damazin 78.4% and Rosaries 81.6%, and found to be tolerant in Geissan 94.4%. The status of pyrethroid (deltamethrin and permethrin) tested, the exposed *An. arabiensis* from two study area (Damazin and Rosaries) show tolerance, while in Geissan it found to be susceptible the mortality rate is 98%. A unique baseline data on insecticide resistance is now available in the Blue Nile State in three localities, which enable to follow trends in susceptibility status in the whole state and which will serve as basis for further resistance management .

INTRODUCTION

Malaria is a preventable and treatable mosquito-borne disease, whose main victims are children under five years of age in Africa. Caused, by the genus Plasmodium, of five species of parasites (*P. falciparum, P. vivax, P. ovale, P. malariae and P. knowlesi*), that affect humans. Malaria due to *P. falciparum* is the most deadly form and it predominates in Africa; *P. vivax* is less dangerous but more widespread, and the other three species are found much less frequently. Malaria parasites are transmitted to humans by the bite of infected female mosquitoes of more than 30 Anopheline species. Globally, an estimated 3.3 billion people were at risk of malaria in 2011, with populations living in sub Saharan Africa having the highest risk of acquiring malaria: approximately 80% of cases and 90% of deaths are estimated occur in the WHO African Region, with children less than five years of age and pregnant women most severely affected (WHO, 2012). Malaria is a major public health problem in Sudan. The disease

affects almost all the people in the country, with a variable degree of endemicity (Malik *et al.*, 2006).

Pesticides have been the cornerstone of vector-borne disease control for the past 50 years; however, use of chemicals on a vast and increasing scale has led to the widespread development of resistance as a result of selection for certain genes. The number of insecticide-resistant arthropods of public health importance rose from 2 in 1946 to 150 in 1980 and 198 in 1990. Some species have become resistant to multiple insecticides, making their control by chemical methods extremely difficult and expensive. Among the vectors of public health importance, mosquitoes only ones in which resistance does present a problem for control in the Sudan as a major vectors of malaria in various geographical areas, at El Gezira State and other agricultural areas, poses a certain threat to the malaria control strategies, and in central Sudan, *An. arabiensis* has been reported resistant to several insecticides BHC, DDT, and malathion, while in Whit Nile state resistance was found in four areas to DDT, malathion, and lambdacyhcolothrin (WHO, 1992).

Ma1aria in Sudan is considered as a 1eading cause of morbidity and morta1ity with 2,365,779 cases and 2076 deaths recorded in 2010 (**WHO**, World Malaria Report 2011). The Blue Nile State is one of the malaria-endemic areas in Sudan, and the proportion of the high prevalence of the disease especially during the autumn. For the year 2012, the total malaria cases (both confirmed and clinically diagnosed cases) reached 21% and the total malaria deaths (Both confirmed and clinically diagnosed cases) reached 17 % (NMCP 2013).

Anopheles arabiensis Patton is currently the only known malaria vector mosquito in northern and central Sudan (Abdalla *et al.*, 2007).

Vector control is an effective way of reducing malaria transmission. The main vector control methods include the use of insecticide-treated bed nets (ITNs) and indoor residual spraying (IRS). Both interventions rely on the continuing susceptibility of Anopheles to a limited number of insecticides. However, insecticide resistance, in particular pyrethroid-DDT cross-resistance, is a challenge facing malaria vector control in Africa because pyrethroids represent the only class of insecticides approved for treating bed nets and DDT is commonly used for IRS. (Martha *et al.*, 2009). The use of insecticides is the main strategy for controlling malaria vectors in Sudan through indoor residual house spraying and, more recently, the use of insecticide-treated bed nets (ITNs). Control methods, however, have been handicapped by the development and spread of vector resistance to insecticides, a growing problem in many African countries (Abdalla *et al.*, 2007).

Resistance monitoring should be an integral part of vector control programs. The susceptibility of vectors should be ascertained before selection of an insecticide and to provide baseline data for further resistance monitoring. Surveillance throughout a program will allow early detection, so that resistance management strategies can be implemented, or, in the case of late detection, evidence of control failure can justify replacement of the pesticide. Resistance can be monitored easily by using the standard WHO test kits.

There is inadequate knowledge regarding the susceptibility status of *Anopheles spp* in Blue Nile State. The present study will provide valuable data to plan more effective vector control interventions. Therefore the study is design to monitor insecticide susceptibility and detect the development of insecticide-resistant populations in Blue Nile State, through the investigation of insecticide resistance status of *Anopheles spp* as part of the overall mapping of insecticide resistance in the Sudan. Identification of the prevalent species of *Anopheles* in the area during the study period and recommendations regarding insecticides resistance management plan if resistance detected by the end of study, will be assessed using these specific objectives:

- Identify the malaria vector in the study area.
- Determine insecticide susceptibi1ity status of *Anopheles spp* to insecticides, Organochlorines (4% DDT), Pyrethroids (Deltamethrin o.05% and Permethrin 0.75%), and Carbamates (0.1% Bendiocarb) using WHO (1998) susceptibility test protocol.
- Estimate knockdown time to kill 50% and 95% of exposed mosquitoes (KDT_{50} and KDT_{95}) for DDT, deltamethrin, permethrin and bendiocarb.

MATERIALS AND METHODS

3-1.Study area:

The study was carried out in Blue Nile state in three localities (Damazin - Rosaries - Geissan). The Blue Nile state is located southeast of Sudan and has borders with two countries, South Sudan to the south and Ethiopia to the east. Also it borders with two states, Sennar state in the north and White Nile in west. It lies between latitude 11° 24' 47" N, Longitude: 34° 02' 25" E**.** An area of 45,000 km **(plate 1).**The humidity is 79% in august, 25% in April, temperature 35º C in May and 25º C in January, with medium rainfall that is approximately 650mm. Administratively, the Blue Nile state is divided into six localities (Damazin -

Eltadamon - Rosaries - Geissan - Bawo - Kurmuk). With (692) neighborhoods and villages, the total population is about 1,193,293 people. The sources of drinking water for public use come from the wells, pumps, Nile water, tanks & other means. The ground is naturally flat and low, composed of clay and soil that is cracked and sandy in addition to mountain chains which creates a favorable environment for the breeding of disease vectors. Malaria is one of the greatest health problems in the state and the most economic activity in the area is fishing, farming and small scale livestock.

A network for the monitoring of insecticide resistance was set up in the Blue Nile state to assess the insecticide resistance status of the major malaria vectors in Damazin - Rosaries - Geissan. Bioassays were performed on adult mosquitoes using the standard WHO susceptibility test with diagnostic concentrations of permethrin 0.75%, deltamethrin 0.05%, bendiocarb 1% and DDT 4%.

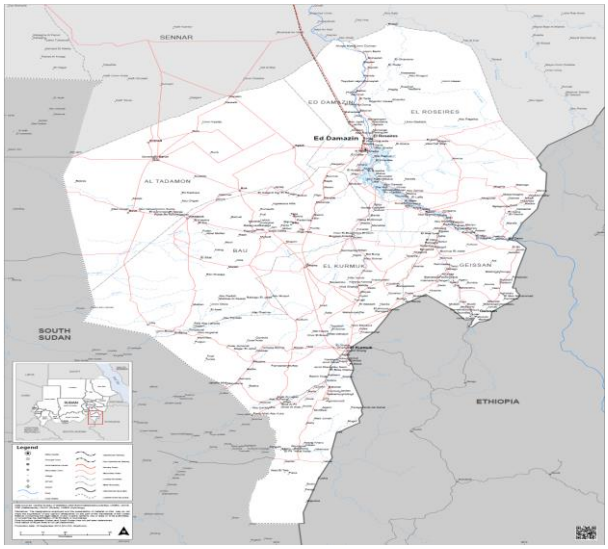

Plate (1) map

3-2. Study design: -
This is a Cross-sectional study conducted to test the susceptibility status of malaria vector in selected study area.

Methodology:
Mosquito Collection, Identification and Rearing
Anopheline larvae were collected from all the available breeding sites in the study area using standard larval collection kits including: dippers screened netting, plastic pipettes, and, iron dishes, all the equipment must be pooled together in plastic buckets. Then the collected larvae must be cleaned, isolated from predators in the field and transferred in the same day of collection to the insectary or rearing room in BNMCP Blue Nile state.

Insectary (rearing room) larvae were transferred into a number of rearing trays. During the rearing period, the larvae were fed on rice powder. Then pupae were collected daily using broad-mouthed pipettes, placed in plastic cups containing clean tab- water which are then put inside adult cages. When adults emerged they were transferred to cages with fine meshes (156 mesh/ inch) to rest, few number of emerged adults were selected randomly for morphological identification using morphological features according to the key for identification of common Adult Anopheline in Sudan and Gillis and Coetzee, (1987). One hundred twenty five adult per test were used in most cases, and 25 adults per test were used as control.

Insecticides used:
In this present study we chose certain chemicals used in the public health sector and recommended from WHO as in table 1.

Table (1): Insecticides Used in Susceptibility/Resistance Tests**:**

Class	Insecticides	Concentration %	Date of expiry
Organochlorine	DDT	4 %	July 2015
Pyrethroids	Permethrin	0.75%	February 2013
	Deltamethrin	0.05%	July 2013
Carbamates	Bendiocarb	0.1 %	June 2015

The Kits set tubes for testing susceptibility of adult mosquitoes
The WHO tube test kits (plate, 2) consists of two plastic tubes (125 mm in length, 44 mm in dia.), with each tube fitted at one end with a 16-mesh screen. One tube (exposure tube) is marked with a red dot, the other (holding tube) with a green dot. The holding tube is screwed to a slide unit with a 20 mm hole into which an aspirator will fit for introducing mosquitoes into the holding tube. The exposure tube is then screwed to the other side of the slide unit. Sliding the partition in this unit opens an aperture between the tubes so that the mosquitoes can be gently blown into the exposure tube to start the treatment and then blown back to the holding tube after the timed exposure (generally one hr). The filter-papers are held in position against the walls of the tubes by four spring wire clips: two steel clips for attaching the plain paper to the walls of the holding tube and two copper clips for attaching the insecticidal paper inside the exposure tube (WHO, 2006).

Insecticide Susceptibility/Resistance Tests
Two to five -day-old female mosquitoes non-blood fed were tested with different insecticides for their susceptibility, using the WHO standard protocol (WHO, 1998) under optimum conditions (temperature

26—29 °C and 70—80% R.H.). The test kits and the impregnated papers were obtained from the WHO Eastern Mediterranean Regional Office (EMRO) in Cairo, Egypt. Mosquitoes were exposed for 60 minutes in tubes places in vertical position. During exposure the number of mosquitoes knocked down was recorded after 10, 15, 20, 30, 40, 50 and 60 min. After exposure, mosquitoes were kept under observation for 24 h, 10% sugar solution was made available to survivors and mortality was read after this 24 h period. Each test had four to five replicates (due to unavailability of collected larvae) and on papers impregnated with insecticides and a control tube with oil-treated paper.

The bioassay results were corrected using the Abbott formula when the control mortality was between 5 and 20% (Abbott, 1925). The bioassay results were summarized in three resistance classes as defined by WHO (1) susceptible when mortality was 98% or higher, (2) possible resistant when mortality was between 97 and 80%, and (3) resistant when the mortality was lower than 80%.

Dead and surviving mosquitoes were stored separately in clearly apendroph tubes 1.5 ml containing silica gel and labeled. All mosquitoes surviving the exposure to insecticide during the bioassay and at least an equal number of mosquitoes killed in the bioassay will be subjected to PCR test later on in BNNICD to confirm resistance.

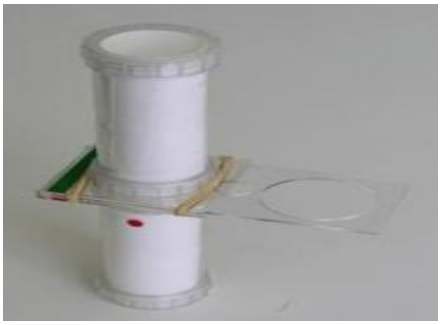

Plate (2) tube test kits

Data Analysis

Data was entered into a computer database and subjected to SPSS software and statistical analysis to determine KdT_{50}, KdT_{95}.

RESULTS

Insecticide resistance was an important factor responsible for outbreaks of malaria vectors. In the Sudan, insecticides for vector control have been adopted since the 1950's. The sustainability of the gains achieved in malaria control in Sudan is seriously threatened by the resistance of malaria vectors to pyrethroids and other chemical groups on different parts of the country. In 2012-2013 the present study surveyed the status of insecticides resistance, carried out in Blue Nile State to DDT, permethrin, deltamethrin, and bendiocarb insecticides in three populations collected from breeding sites in three localities (Geissan -Damazin and Rosaries),

Species Composition

Mosquitoes were collected from the three sites from one type of breeding which is broken pipe. A few numbers of emerged adults were identified morphologically as *An. arabiensis* according to Gillies and Coetzee, (1987) key.

Results of Susceptibility Tests

The total number of all tested specimens was 1425 *An. arabiensis* females, 57 replicates were used, each replicate contains 25 adult when bioassying for public health insecticides (Table 1). The numbers of knockdown mosquitoes were counted during the exposure time (one hr), and the overall mortality rates were recorded for each insecticide after 24 hr. Analysis was conducted using the SPSS software program and Probit analysis.

The WHO criteria for determining resistance/susceptibility were applied: 98—100% mortality indicates susceptibility; <80% mortality suggests resistance; and 80—97% mortality requires confirmation of resistance (WHO, 1998).

The results of the present study are presented in tables and figures. The results of insecticides resistance bioassay (Table 3) shows that *An. arabiensis* status of resistance can be divided into three categories. First, resistant to DDT 4% in two areas Damazin mortality rate was (78.4% ± 8) and Rosaries mortality rate was (81.6% ± 4). Secondly, insects proved to be tolerant to DDT 4% in one area Geissan mortality rate was (94 % ± 4). The result reflects low mortalities of *An. arabiensis* due to DDT, implicating wide distribution of resistance to this insecticide. While the *An. arabiensis* population showed tolerance to deltamethrin 0.05% in two areas, Damazin and Rosaries, the mortality rates were (92.8 % ± 9) and (96% ± 6) respectively, in spite of the fact that it showed susceptibility in Geissan to deltamethrin, with a mortality rate of (98% ± 2). The same happened to permethrin 0.75%, the anopheline population in Geissan, Damazin and Rosaries showed tolerance. The mortality rate in three localities were (93.04% ± 5), (91.67 % ± 6), and (95.2% ± 4), respectively. Thirdly, susceptibility is also significantly high to bendiocarb 1%, in Geissan. Damazin and Rosaries mortality rates for the three localities were (98% ± 2), (100%% ± 0) and (99.2 % ± 2), respectively.

Table (3): Bioassay results of KdT50 and KdT95 within 1 hr and % mortality after 24 hr for *An. arabiensis* collected from three areas in 2012 - 2013.

No	Insecticides	Area	No Replicates	%Mortality after 24 h	KdT_{50} minute (95% CL)	KdT_{95} minute (95% CL)	Test significance
1	Deltamethrin	Geissan	100 (4)	98 ± 2	20.62 (18.92 – 21.76)	53.17 47.57 – 60.86)	X^2 = 2.246 df = 3 P= 0.81
		Damazin	125 (5)	92.8 ± 9	25.90 (21.79 – 27.77)	67.87 (51.09 – 80.07)	X^2 = 1.858 df = 4 P = 0.05
		Rosaries	125 (5)	96 ± 6	29.79 (27.07 – 30.09)	67.45 (59.59 – 72.89)	X^2= 1.858 df= 4 P = 0.87
2	Bendiocarb	Geissan	100 (4)	98± 2	39.68 (34.23 – 43.49)	98.53 (69.99- 121.66)	X^2 = 11.22 df= 3 P = 0.05
		Damazin	125 (5)	100 ± 0	21.75 (18.46 - 3496)	42.85 (56.89- 289.42)	X^2 = 10.457 df = 4 P = 0.06
		Rosaries	125 (5)	9.2 ± 2	28.94 (23.84 – 29.63)	69.79 (62.49 – 99.01)	X^2= 8.64 df= P4 P = 0.13
3	Permethrin	Geissan	125 (5)	93.04 ± 5	15.74 (10.39 – 16.49)	96.73 (93.29 -271.09)	X^2= 3.625 df = 4 P = 0.61
		Damazin	125 (5)	91.67 ± 6	35.60 (32.04 – 37.00)	117.63 (99.30 – 145.73)	X^2 = 2.277 df = 5 P = 0.81
		Rosaries	125 (5)	95.2 ± 4	19.95 (17.77 – 21.38)	90.36 (73.14 – 110.63)	X^2 =3.658 df = 5 P =0.6
4	DDT	Geissan	100 (4)	94 ± 4	42.84 (39.49 – 39.23)	101.05 (82.14 – 110.34)	X^2= 5.986 df= 5 P=31
		Damazin	125 (5)	78.4 ± 8	52.77 (45.97- 55.22)	186.60 (135.81- 226.79)	X^2= 2.982 df= 5 P = 0.85
		Rosaries	125 (5)	81.6 ± 4	34.71 (32.52 – 43 .23)	200.64 (181.17- 439.67)	X^2=5.986 df=5 P= 0.31

This is the first large scale, cross-state survey of insecticide resistance in *Anopheles* species in the three localities in Blue Nile. Unique baseline data on insecticide resistance for the Blue Nile is now available, which enables the follow-up of trends in susceptibility status in the state and which will serve as the basis for further resistance management.

Knockdown Results:

Table (3) shows the time required for knocking down 50% (KDT_{50}) and 95% (KDT_{95}) of the exposed *An. arabiensis* females within one hour (60 mint.) to the insecticides tested. This was calculated using probit analysis model.

Large differences in insecticide knockdown status were observed among localities. For the low or non-knockdown group insecticides bendiocarb 0.1% (Figs.1, 2, and 3), the KDT_{50} values obtained at the three areas post exposure, in Geissan, Damazin and Rosaries were 39.68 minutes, 21.75 minutes and 28.94 minutes respectively. While the KDT_{95} value obtained at three localities were 98.53 minutes, 42.85 minutes and 69.79 minutes, respectively. The fastest $KDT_{50 \text{ and } 95}$ values were obtained at Damazin (KDT_{50} = 21.75 minutes, KDT_{95}= 42.85 minutes), while the most delayed one was obtained at Geissan (KDT_{50} = 39.68 minutes, KDT_{95} =98.53 minutes).

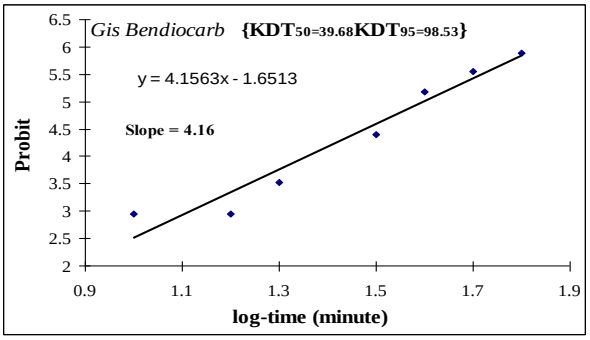

Figure (1): *Kd*T response (minutes) of *An. Arabiensis*; to Bendiocarb in Geissan.

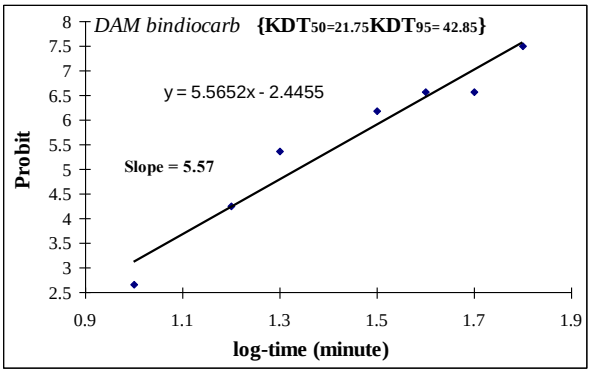

Figure (2): *Kd*T response (minutes) of *An. Arabiensis*; to Bendiocarb in Damazin.

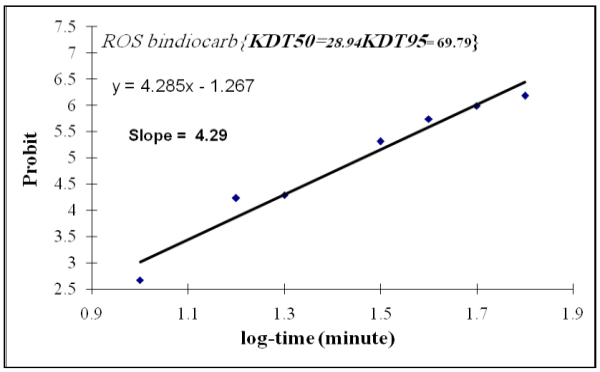

Figure (3): *Kd*T response (minutes) of *An. Arabiensis*; to Bendiocarb in Rosaries.

For the knockdown group insecticides (deltamethrin, permethrin and DDT) (Figs., 4, 5, 6, 7, 8, 9, 10, 11, and 12), the KDT_{50} values obtained for deltamethrin 0.05% at the three areas Geissan, Damazin and Rosaries were 20.62 minutes, 25.90 minutes and 29.79 minutes respectively. While the permethrin75% KDT_{50} values were 15.74 minutes, 53.06 minutes and 19.95 minutes and DDT4% values were 42.84 minutes, 52.77 minutes and 29.30 minutes respectively. The fastest KDT_{50} is permethrin 0.75%, except in Damazin, then followed by deltamethrin 0.05% and DDT4%.

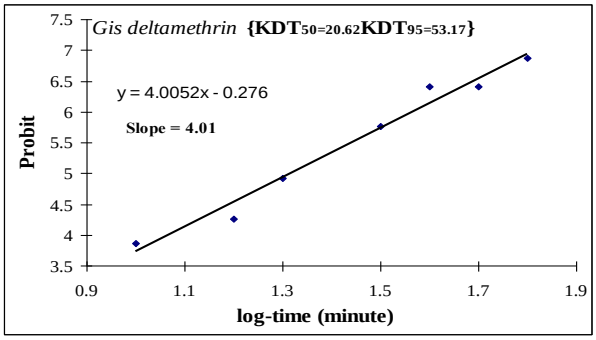

Figure (4): *Kd*T response (minutes) of *An. Arabiensis*; to Deltamethrin in Geissan.

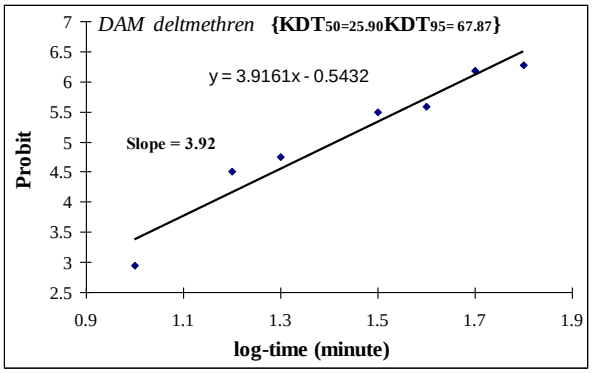

Figure (5): *Kd*T response (minutes) of *An. Arabiensis*; to Deltamethrin in Damazin.

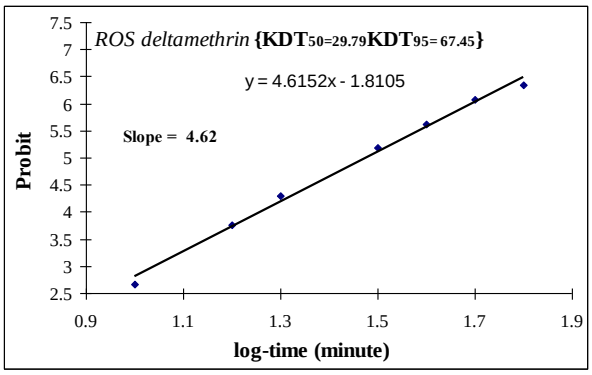

Figure (6): *Kd*T response (minutes) of *An. Arabiensis*; to Deltamethrin in Rosaries.

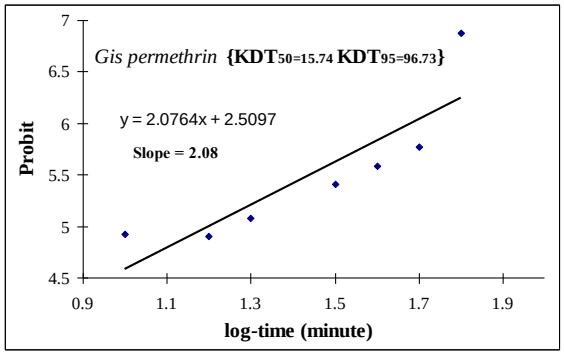

Figure (7): *Kd*T response (minutes) of *An. Arabiensis*; to Permethrin in Geissan.

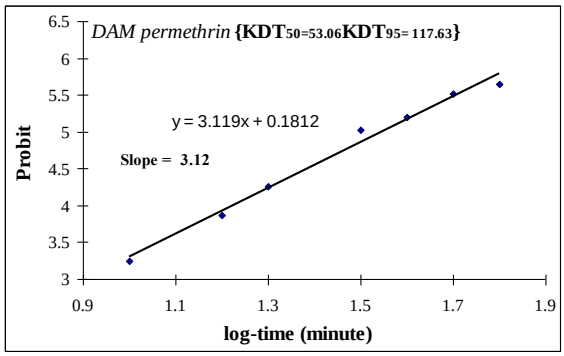

Figure (8): *Kd*T response (minutes) of *An. Arabiensis*; to Permethrin in Damazin.

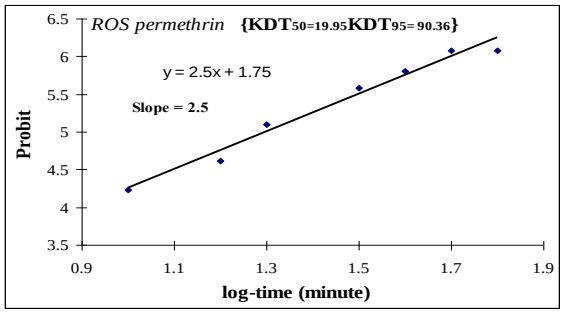

Figure (9): *Kd*T response (minutes) of *An. Arabiensis*; to Permethrin in Rosaries.

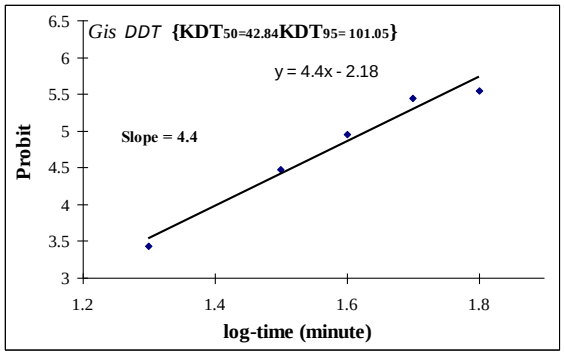

Figure (10): *Kd*T response (minutes) of *An. Arabiensis*; to DDT in Geissan.

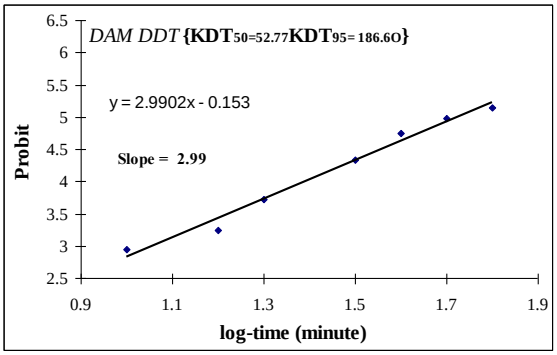

Figure (11): *Kd*T response (minutes) of *An. Arabiensis*; to DDT in Damazin.

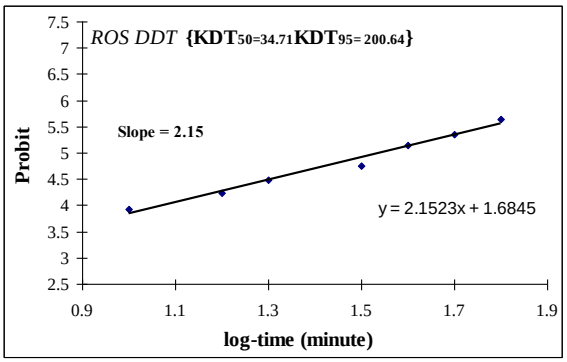

Figure (12): *Kd*T response (minutes) of *An. Arabiensis*; to DDT in Rosaries.

The fastest knockdown time for permethrin 0.75% was obtained at Geissan (KDT_{50} = 15.74 minutes), while the most delayed one was obtained at Damazin (KDT_{50} = 53.06 minutes). Regarding deltamethrin 0.05%, the fastest knockdown time was obtained at Geissan (KDT_{50} =20.62 minutes), while the most delayed one was obtained at Rosaries (KDT_{50} = 29.79 minutes). In case of DDT the fastest knockdown time was obtained at Rosaries (KDT_{50} = 34.71 minutes), while the most delayed one was obtained at Damazin (KDT_{50} =52.77 minutes).

Regarding KDT_{95} the picture is different. The fastest knockdown time was obtained by deltamethrin in all areas (*i.e*. Geissan, Damazin and Rosaries, were 53.17 minutes, 67.87minuts and 67.45 minutes, respectively) (Figs. 4, 5, and 6). On the contrary, the permethrin and DDT, in Damazin for example were obtains KDT_{95} in 117.63 minutes and 186.6 respectively (Figs. 8 and 11). Which reflect that the population of *An. arabiensis* more susceptible to deltamethrin in Damazin locality.

DISCUSSION

The preliminary study in Blue Nile state at all six localities showed that *An. Arabiensis* represent 95.82% and 4.18% were *An. Funestus* (Abd Elrhem, 2011). In the present study according to morphological identification *An. Arabiensis* represent 100 % for all specimens of three studied areas.

The status of insecticides resistance to deltamethrin, bendiocarb, permethrin and DDT was investigated in *An. arabiensis* from three areas in Blue Nile State, central Sudan using WHO discriminating concentrations for the four insecticides during December 2012 - March 2013 (dry season).

In Africa, resistance to pyrethroid insecticide in malaria vector mosquitoes might become a major problem for malaria interventions because pyrethroids seem to be the mainstay of vector control strategies (WHO, 2000). According to the WHO criteria for characterizing insecticide susceptibility; in the present study the *An. arabiensis* was found to be resistant to DDT 4% in two areas Damazin 78.4% and Rosaries 81.6%, and found to be tolerant in Geissan 94.4%. According to a previous study conducted in three regions of the Blue Nile State in each of Damazin city and Rosaries city and Guenis to test the susceptibility of permethrin and DDT resulted that there is no evidence for full susceptibility to permethrin or DDT among the populations tested, while the populations of *An. arabiensis* from Damazin were found resistant to both permethrin and DDT (Himeidan *et al*, 20011). The first record for DDT resistance in Sudan was from El Gunaid sugar cane area in 1970 (Haridi, 1972b), and all previous studies mentioned in literature showed resistance to DDT in Sudan.

In the present study the status of pyrethroid (deltamethrin and permethrin) tested, the exposed *An. arabiensis* from two study area (Damazin and Rosaries) show tolerance, while in Geissan it found to be susceptible the mortality rate is 98%. Evidence for resistance to permethrin 0.75% in *An. arabiensis* was clearly found in Gazira, Damazin, Kosti, Sennar, Estran states, and Northern State (Merowe –Nouri, and Eldabba).

The main interventions for malaria vector control in Blue Nile State are LLTNs and space spray. Permethrin, the only insecticide used since the year 2001 until now, may have led *An. arabiensis* to be tolerant to this insecticide. In addition to the lack of good storage in Damazin locality and the use of obsolete and expired insecticide, all these factors have resulted in the emergence of tolerant vectors. These findings need further investigation.

Bendiocarb, on the other hand was the only insecticide to which *An. arabiensis* was susceptible in all three areas recording 98, 99.2, and 100% mortality. This is because it has not been used previously in the area.

This study did not seek to understand the spatial and species related differences in susceptibility status. These patterns are generated by a complex interaction between the population biology and genetics of the vector and the insecticide pressure presence in the ecosystem. The relative role of insecticide pressure from agriculture and vector control in the selection of insecticide resistance is difficult to assess. Historically, DDT has been used to control malaria in the Blue Nile State. Nowadays use of pyrethroid based vector control measures such as ITNs to contain malaria and up scaling of treated nets is going on in high transmission areas. Pressure from agricultural activities is likely to show large spatial variation given the differences in land use in the Blue Nile State. *An. Arabiensis*, highly tolerant to pyrethroids (permethrin and deltamethrin) breeds in the Blue Nile, where intense agriculture activities are deployed but he use of vector control is limited due to the very low malaria endemicity. The high DDT resistance observed in different *An. arabiensis* populations is puzzling and might indicate that DDT pressure is still available despite the fact that DDT is not used any more for the control of malaria in Blue Nile State. The patchy distribution of insecticide resistance of *An. arabiensis* in central Sudan is remarkable. These spatial differences are not due to geographical distribution of the sibling species, *An. arabiensis* with different insecticide susceptibility status, but are likely to be due to differences in insecticide pressure in the different sites.

This study has revealed that even in relatively well-resourced and logistically manageable places like Damazin, malaria elimination is going to be difficult to achieve with the current control measures. These resistances may happen because the lack of operational research in the area, the well application of the insecticides and the accurate using of techniques related with dosages and procedures.

ossible factors influencing the frequency of resistant individuals observed in the study area were discussed. The results of this study highlight the importance of standardized longitudinal insecticide resistance monitoring and the urgent need for studies to monitor the impact of this resistance on malaria vector control activities.

CONCLUSION

This is the first cross-state survey of insecticide resistance of malaria vector species in the Blue Nile. After three months of intense insecticide resistance monitoring a clear picture of insecticide resistance status of malaria vectors was achieved. In Geissan, Damazin and Rosaries insecticide resistance in the malaria vectors *An. arabiensis* was almost present.

In Blue Nile State though, resistance management is important because of the risk of migration of mosquitoes carrying resistance genes from non-endemic to endemic areas.

Moreover, trends in resistance status should be carefully monitored, mainly in malaria endemic, and the impact of existing vector control tools on resistant populations should be assessed.

Effective resistance management depends on early detection and monitoring of trends in resistance status, understanding the underlying mechanisms, and assessing the operational implications of the observed insecticide resistance, so that rational insecticide choice can be made.

A unique baseline data on insecticide resistance is now available in the Blue Nile State in three localities, which enable to following trends in susceptibility status in the whole state and which will serve as basis for further resistance management.

REFERENCES

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

Abdalla, S.I. Malik, E.M. Ali,K.M. (2007) The burden of malaria in Sudan: incidence, mortality and disability – adjusted life – years,*Malaria Journal* (6) 97

Gillies, M.T.; and Coetzee, M. (1987). A supplement to the *Anophelinae* of Africa South of the Sahara. Publications of the South African Institute of Medical Research, Johannesburg, No. 55.

Malik, E. M.; Mohamed, T.A.; Elmardi, K. A.; Mowien, M. M.; Elhassan, A. H.; Elamin, S. B.; Mannan, A. A.; and Ahmed, E. S. (2006). From chloroquine to artemisinin-based combination therapy: The Sudanese experience. *Malaria Journal*. (5): 65.

Martha, B.; Musa, J. and Taiwo, S. A. (2009). Status of insecticide susceptibility in *Anopheles gambiae s.l.* from Malaria surveillance sites in The Gambia *Malaria Journal* 8:187 doi:10.1186/1475-2875-8-187
http://www.malariajournal.com/content/8/1/187

NMCP (2013). National Strategic Plan for RBM 2007-2012 Khartoum.

WHO (2012) *World Malaria Report*, (WHO Global Malaria Program) pages 105

WHO (1992) Vector resistance to pesticides. Geneva, WHO Technical Report Series, No. 818.

WHO (2011) *World Malaria Report*, (WHO Global Malaria Program) pages 107

WHO (1998). Test procedure for insecticide resistance monitoring in malaria vectors, Bioefficacy and

persistence of insecticides on treated surfaces . WHO/ CDS/ CPC/ MAL/ 98.12, WHO, Geneva.

WHO (2006). Guidelines for testing mosquito adulticide for indoor residual spraying and treatment of mosquito net. WHO/CDS/NTD/ WHOPES/ GCDPP/2006.3.

Nidal Shams Eldeen Ahmed Ali[1] and Yousif Oman Hussein. Assad[2]
1.Blue Nile Institute for Communicable Disease
2. Department of Pesticides and Toxicology, Faculty of Agric. Sciences, University of Gezira, yousifassad@yahoo.com

PIPERONYL BUTOXIDE (PBO) SYNERGIZED PYRETHROIDS IN COTTON BOLLWORMS *PECTINOPHORA GOSSYPIELLA* (LEPIDOPTERA: GELECHIIDAE) AND *CRYPTOPHLEBIA LEUCOTRETA* (LEPIDOPTERA: TORTRICIDAE) FROM CÔTE D'IVOIRE, WEST AFRICA

ABSTRACT
The pink bollworm *Pectinophora gossypiella* Saunders and the false codling moth *Cryptophlebia leucotreta* (Meyrick) are two major cotton pests in Côte d'Ivoire, West Africa. However, unlike the cotton bollworm *Helicoverpa armigera* Hübner, which was shown to be resistant to pyrethroids, there is a lack of knowledge on the status of the susceptibility to pyrethroids in the two formers. Bollworms were collected from cotton fields and reared in laboratory. In order to establish their susceptibility to pyrethroids and the resistance mechanism, adults were treated by topical method with cypermethrin and deltamethrin alone and in combination with piperonyl butoxide (Pbo). With regard to single insecticides, the LD_{50} values in *P. gossypiella* and *C. leucotreta* were respectively, 505.98 µg / g *vs* 41.36 µg / g for cypermethrin and 36.94 µg / g *vs* 0.32 µg / g for deltamethrin. Data indicated that *P.gossypiella* was 12.22 to 115.43-fold more resistant than *C. leucotreta* according to pyrethroid insecticides. In addition, Pbo synergized both insecticides cypermethrin and deltamethrin in both bollworm species. In *P. gossypiella* and *C. leucotreta*, the synergistic ratios were respectively 992.15 and 590.85 for cypermethrin and 57.71 and 8.00 for deltamethrin. The study showed relative resistance to pyrethroid insecticides in both endocarpic species as the actual LD_{50} values in *P. gossypiella* and *C. leucotreta* were respectively 9235-16866.66 and 80-1060.5 fold higher than previous LD_{50} obtained with reference strains in 1987. Since the two pyrethroids were synergized by Pbo, the study suggests that a metabolic detoxification of monooxygenases such as P450s may be involved in the resistance mechanism. Therefore, the study recommends a pyrethroid resistance management strategy to tackle the two endocarpic species on cotton crops in Côte d'Ivoire.

Keywords: *Pectinophora gossypiella*, *Cryptophlebia leucotreta*, insecticides, Pbo, susceptibility, resistance mechanism, cotton, Côte d'Ivoire

INTRODUCTION

Cotton is one of the main crops grown in the northern regions of Côte d'Ivoire. It is attacked by various insect pests, mostly lepidopterous species (Vaissayre *& al*., 2000). Besides the bollworm *Helicoverpa amigera* Hübner, the Pink Bollworm *Pectinophora gossypiella* Saunders and the False Codling Moth *Cryptophlebia leucotreta* (Meyrick) are permanent insect pests damaging cotton bolls. These pests require regular application of insecticides. The use of pyrethroid insecticides for the last 30 years has evolved to a selection of resistant individuals in *H. armigera* (Ochou & *al*., 1998). With this regard, the current pest control strategy in Côte d'Ivoire is based on pyrethroid resistance management in *H. armigera*. To reduce the selection pressure due to pyrethroids during the cotton growing period, the strategy recommends a restriction period in which pyrethroids are replaced by alternative chemicals with different mode of action such as profenofos, indoxacarb, spinosad, etc. (Ochou & Martin, 2001). The pyrethroid-organophosphorus associations are allowed only after August 10th during the fruiting stage of cotton. With this respect, while this strategy has proved very effective on *H. armigera*, it does not allow an effective control of other important pests, such as *P. gossypiella* and *C. leucotreta*. These two cotton bollworms, behaving as endocarpic species, have shown for years a regular predominance in cotton fields in Côte d'Ivoire (Ochou, 1994). The increasing field populations of these bollworms for the last decades (Doffou, 2005) raise questions about their resistance status to insecticides currently used on cotton. Indeed, unlike the cotton bollworm *H. armigera*, there is a lack of knowledge on the status of susceptibility to insecticides in *P. gossypiella* and *C. leucotreta* in Côte d'Ivoire. Nevertheless, recent laboratory studies (Doffou & *al.*, 2011) showed clear evidence that the level of pyrethroid susceptibility in both insect species was much less than the baseline data obtained with cypermethrin and deltamethrin by Vaissayre in 1988. In fact, data suggested eventual pyrethroid resistance in these two endocarpic bollworm species which are similar in their feeding habits.

Accordingly, elucidating the insecticide susceptibility status and eventually the mechanism of insecticide resistance in *P. gossypiella* and *C. leucotreta* is a pre-requisite to understanding resistance problem and is essential for the current pest management strategy as stated by Castle *& al*., 1999. The use of synergistic chemicals such as Piperonly butoxide (Pbo) enables preliminary information about mechanism of resistance in the easiest and fastest ways (Raffa & Priester, 1985;

Scott, 1990). The present study was carried out in order to find out whether or not the Pbo synergizes pyrethroids in the two emerging cotton pests, *C. leucotreta* and *P. gossypiella*.

MATERIALS AND METHODS

Insects

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

Insecticides and synergist

Two technical grade of pyrethroid were used for bioassays purposes: cypermethrin 93.4 % and deltamethrin 98.2 %. The technical grade samples were graciously provided by Bayer CropScience, Côte d'Ivoire. The synergist Piperonyl butoxide (Pbo) 92 % SC was obtained from Envirochem, France.

Topical bioassays

The technical active ingredients are diluted in acetone. Five to six doses expressed in µg a.i /g insect have been applied for each insecticide tested. The range of doses used in laboratory bioassays was set to cause a range in mortality between 0% and 100% after 48-h of exposure to insecticides. The tests were carried out on one-day old first generation individual moths weighting between 10 and 20 grams, regardless of gender. Insects were kept in a state of sleep with an ether solvent as an anesthetic. Batches of thirty (30) insects were treated per insecticide and per dose. Insects were placed individually in vials of 40 ml and held at 25°C and 75% Humidity. Each test was replicated three times and included acetone or Pbo alone to treated controls. Mortality in the controls was less than 10 %. Pbo was applied at 10 a.i / g in *C. leucotreta* and at 10^2 a.i / g in *P. gossypiella* 1 hour before application of cypermethrin or deltamethrin at a 1:1 ratio. This dose of Pbo did not result any toxicity. The topical tests were performed with a Burkard Arnold Microapplicator Type LV 65, by applying 1 µl of insecticide solution directly on the thorax of individual moths held between the thumb and the forefinger. Mortality was recorded after 48 h, and insects were considered dead if they did not move after prodding or were unable to right themselves 5 s after being turned upside down.

Analysis of dose-response data

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

RESULTS

P.gossypiella appeared more resistant than *C. leucotreta*

The LD_{50} values for the specific toxicity of each product in *P.gossypiella* and *C. leucotreta* were respectively, 505.98 µg/g *vs* 41.36 µg/g for cypermethrin and 36.94 µg/g *vs* 0.32 µg/g for deltamethrin (Table 1). Deltamethrin appeared to be more toxic in *C. leucotreta* and *P.gossypiella* than cypermethrin. *P. gossypiella* appeared more resistant than *C. leucotreta;* the resistance ratios (RR) between the two species were 12.22-fold with cypermethrin and 115.43-fold with deltamethrin. All slopes of regression lines were near 1 (Table 1). The similar slope values of regression lines shown in both pests, suggests the same mode of action with respect to each product.

Table 1: Toxicity of cypermethrin and deltamethrin without and with Piperonyl butoxide (Pbo) to the *C. leucotreta and P. gossypiella*.

Strains	Insecticides	R	N	χ^2	df	Slope ±E S	LD_{50}±ET	IC_{50} (95%)	RR	SR
C. leucotreta	Cypermethrin	-	330	13.18	9	0.92± 0.01	**41.36±0.11**	23.94-69.84	-	-
	Cyperm.+Pbo	1:1	300	13.40	8	0.41± 0.05	**0.07±0.32**	$1.28\ 10^{-2}$-$2.95\ 10^{-2}$	-	**590.85**
	Deltamethrin	-	210	8.58	5	0.63± 0.09	**0.32±0.21**	0.10-0.80	-	-
	Deltam.+Pbo	1:1	240	4.91	6	0.57± 0.08	**0.04±0.24**	0.010-0.11	-	**8**
P. gossypiella	Cypermethrin	-	240	9.27	7	1.13± 0.12	**505.98 10^2±0.09**	$3.26\ 10^2$-$7.86\ 10^2$	**12.22**	-
	Cyperm.+Pbo	1:1	270	15.14	7	0.40± 0.11	**0.51±0.38**	$2.17\ 10^{-2}$-3.42	-	**992.15**
	Deltamethrin	-	240	13.57	6	0.57± 0.14	**36.94±0.36**	0.2-1.65	**115.43**	-
	Deltam.+Pbo	1:1	240	6.05	6	0.62± 0.08	**0.64±0.22**	0.2-1.65	-	**57.71**

N : number of adults tested **χ2** : chi2 **df** : degree of freedom **LD_{50}:** lethal doses for 50 % of adults **CI** : confidence interval at LD_{50} **$RR_{PBW/FCM}$:** resistance ratio of PBW/FCM at LD_{50}= LD_{50} trial of PBW / LD_{50} of FCM **SR**: Synergist ratio = LD_{50} without Pbo / LD_{50} with Pbo

Pbo synergism

Pbo synergized the toxicity of cypermethrin and deltamethrin in both bollworm species (Table 1). A very high level of synergism was found with cypermethrin. The synergistic ratios for cypermethrin

were 590.85 in *C. leucotreta* and 992.15 in *P.gossypiella*, while for deltamethrin, the ratios were 8 in *C. leucotreta* and 57.71 in *P.gossypiella*. Indeed, the use of Pbo has reduced significantly resistance with cypermethrin and deltamethrin. *C. leucotreta* and *P. gossypiella* have developed a resistance to cypermethrin and deltamethrin. This resistance was more pronounced with cypermethrin and in *P. gossypiella*.

DISCUSSION

The toxicity of cypermethrin and deltamethrin based on their LD_{50}, showed different susceptibility levels in each of the two endocarpic species. In addition, the parallelism observed in the regression lines for each insecticide confirms that each pyrethroid acts by the same mode of action in both pests. The LD_{50} showed that the deltamethrin was more effective than cypermethrin on both *C. leucotreta* and *P. gossypiella*. This high toxicity of deltamethrin as compared to cypermethrin could be explained by the fact that deltamethrin is not widely used for chemical treatments of cotton crops in Côte d'Ivoire. Therefore, pests in the field are less likely in contact with this product, making both pests more susceptible to this toxic, contrary to the widely used cypermethrin. These results on deltamethrin confirm the effectiveness of pyrethroid products on cotton bollworms.

The relative resistance of *P. gossypiella* as compared to *C. leucotreta* may be explained by the feeding habit of the two pests. In fact, *P. gossypiella* is specifically limited to the family Malvaceae, unlike *C. leucotreta*, a highly polyphagous species, having a large number of host plants. The former insect tied mostly to cotton crops has been likely submitted for years to regular insecticide treatments in cotton, acquiring therefore some relatively specific resistance, while the polyphagous feeding habit of the latter would enable the dilution of its resistance through diverse non treated host plants such as maize or carambola fruits.

Pbo synergized the toxicity of cypermethrin and deltamethrin in both bollworm species. These results suggest that resistance to pyrethroids in *C. leucotreta* and *P. gossypiella* should involve a metabolic detoxification of mono-oxygenases by Pbo. These results match with those of Martin (2003). According to this author, in Côte d'Ivoire and West Africa, pyrethroid resistance in *H. armigera* Hübner, is due to an increase of metabolism through an overproduction of oxidases. He also noted an almost thorough virtual elimination of the resistance by the Pbo and a high concentration of oxidases in resistant insects. This metabolic resistance to pyrethroids involving oxidases has been identified in other Lepidoptera worldwide including *Heliothis virescens* Fabricius (McCaffery & *al.*, 1991; Ottea & *al.*, 1995; Zhao & *al*. 1996; Martin & *al.*, 1997*), Choristoneura rosaceana* Harris (Amad & Hollingworth, 2004), *Plutella xylostella* Linnaeus (Liu & *al*., 1981), *Spodoptera litura* Fabricius (Armes & *al*., 1997*), H.armigera* (Ahmad & McCaffery, 1991; Kranthi & *al*., 2001), and in the whitefly *Bemisia tabaci* Gennadius (Dittrich & *al*., 1990).

However, PbO synergism is not necessarily indicative of the action of mono-oxygenases in the mechanism of resistance to pyrethroids (Kennaugh & *al*., 1993). Gunning (1998) showed that over 70 % of the activity of esterases associated with resistance to *H. armigera* to pyrethroids is apparently inhibited by Pbo. In India, Kranthi and *al*. (1997), observed in *H. armigera* that an increase of oxidases should correspond to a decrease of esterases and vice versa. In addition, Pbo mediated resistance in heliothine Lepidoptera has been reported due to overproduction of esterases or monooxygenases (McCaffery, 1998). In Côte d'Ivoire, a strain of *H. armigera* selected with deltamethrin BK99R9 showed not only a higher concentration of oxidases but a low esterase activity and an increased activity of GST may indicate a potential secondary mechanism compared to the susceptible strain BK77 (Martin 2003). Therefore, the involvement of oxidases in the resistance mechanism of *C. leucotreta* and *P. gossypiella* to pyrethroids in Côte d'Ivoire is likely but this does not exclude that of esterases and GST.

Furthemore, *C. leucotreta* and *P. gossypiella* are able to survive during the inter campaign through cottonseed seedlings not pulled out. This allows the future settlement and multiplication of the first major generation of adults appearing in cotton crops. These successive generations have therefore regularly undergone insecticide treatments by referring to the treatment of the cotton plant (Ochou & *al*., 1998). Pyrethroids are applied in combination with organophosphates from 1985 onwards. According to Roush and McKenzye (1987), insecticide treatments on the plots should exert a selective pressure that would almost always come to more than one mechanism of resistance in present insects. These different insects would then be likely to develop resistance to insecticides applied. Therefore, it is quite possible that *C. leucotreta* and *P. gossypiella* in Côte d'Ivoire possess more than one mechanism.

Moreover, in Cote d'Ivoire, Vaissayre (1988) showed that strains of *H. armigera*, *C. leucotreta* and *P. gossypiella* were comparable to a susceptible reference strain of Montpellier BK77, because LD_{50} values which were contiguous, other against cypermethrin and deltamethrin (Table 2). In 2011, *C. leucotreta* and *P. gossypiella* seem to develop resistance to these two toxics under the FR very high: 16,866.66 for *P.*

gossypiella and 1060.5 for *C. leucotreta* with cypermethrin and deltamethrin, the FR were 9235 in *P. gossypiella* and 80 *C. leucotreta* (Table 2). This confirms the resistance in both species endocarpic facing cypermethrin and deltamethrin. The low level of FR obtained with deltamethrin versus cypermethrin should be explained by the fact that in Côte d'Ivoire, deltamethrin is rather used in market-gardening and not in cotton crops, unlike cypermethrin.

Table 2: LD_{50} of cypermethrin and deltamethrin on susceptible reference strain *H. armigera* of Mtpellier and BK 1987, on strain of *C. leucotreta* and *P. gossypiella* of BK 1987 and Laboratory's strain 2011, with their RR, RF, an SR.

Insecticides	Strains		LD_{50}	$RR_{PBW/FCM}$	RF	SR
Cypermethrin	*H. armigera*	S. BK 1987	**0.551**	-	-	-
		susceptible reference strain Mtpellier	**0.299**	-		
	P. gossypiella	S. BK 1987	**0.030**	**12.22**	**16866.66**	-
		S. Labo 2011	506	-		**992.15**
	C. leucotreta	S. BK 1987	**0.039**	1.3	**1060,5**	-
		S. Labo 2011	41.36	-		**590.85**
Deltamethrin	*H. armigera*	S. BK 1987	**0.058**	-	-	-
		susceptible reference strain Mtpellier	**0.055**	-		
	P. gossypiella	S. BK 1987	**0.004**	1	**9235**	-
		S. Labo 2011	36.94	**115.43**		**57.71**
	C. leucotreta	S. BK 1987	**0.004**	1	**80**	-
		S. Labo 2011	0.32	-		**8**

LD_{50}: lethal doses for 50 % of adults **$RR_{PBW/FCM}$**: resistance ratio of PBW/FCM at LD_{50}= LD_{50} trial of PBW / LD_{50} of FCM **SR**: Synergist ratio = LD_{50} without Pbo / LD_{50} with Pbo **RF** : Resistance Factor (RF) =LD_{50} trial/LD_{50} of susceptible strain (SBK 1987 or susceptible reference strain Montpellier-France) **S. BK** : Strain of Bouaké-Côte d'Ivoire **S. Labo** : Laboratory's Strain-Côte d'Ivoire

SR also, measures the relative involvement of resistance-related mechanisms. A greater SR would indicate the degree that the metabolic mechanism detoxifies an insecticide. In this study, SR was high in both toxic. According to Kasai *& al.,* (1998) and Wu *& al*., (1998), the level of synergism of pyrethroids with Pbo is greater when P450s are responsible for conferring insecticide resistance. So we can say that P450s played an important role in the mechanism of detoxification of cypermethrin and deltamethrin in *C. leucotreta* and *P. gossypiella.*

CONCLUSIONS

This study showed that pyrethroids are effective in *P. gossypiella* and C. *leucotreta*. In addition, *P. gossypiella* is more resistant than *C. leucotreta* to pyrethroids and the addition of Pbo synergized the effect of the pyrethroid insecticides and increases its effectiveness in bollworm species. Therefore, it shall remain provisional in present study that Pbo suppressible resistance is due to monooxygenases unless confirmed biochemically.

ACKNOWLEDGEMENTS

This research was supported by grant from CNRA (Centre National de Recherche Agronomique) and FIRCA (Fonds Interprofessionnel pour la Recherche et le Conseil Agricoles) in Côte d'Ivoire. We acknowledge Bayer CropScience and Envirochem for insecticide samples provided.

REFERENCE

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

Ahmad, M. and McCaffery, A. R., 1991. Elucidation of detoxication mechanisms involved in resistance to insecticides in the third instar larvae of a field-selected strain of *Helicoverpa armigera* with the use of synergists. *Pestic. Biochem. Physiol.* 41 : 41–52.

Armes, N.J., Wightman, J. A., Jadhav, D. R. and Rao, G. V. R., 1997. Status of insecticide resistance in *Spodoptera litura* in Andhra Pradesh, India. *Pestic. Sci.* 50 : 240–248.

Castle, J. S., Prabhaker, N. and. Henneberry, T. J., 1999. Insecticide resistance its management in cotton insects. ICAC *Rev. Article on Cotton Production Res., No*. 5. 55 pp.

Dittrich, V., Ernst, G. E., Ruesch, O. and Solang, U. K., 1990. Resistance mechanisms in sweet potato whitefly (Homoptera: Aleyrodidae) populations from Sudan, Turkey, Guatemala and Nicaragua. *J. Econ. Entomol*., 83: 1165-1670.

Doffou, N. M., 2005. Gestion de la résistance de *Helicoverpa armigera* Hübner, 1808 (Lepidoptera : Noctuidae) aux pyréthrinoïdes en culture cotonnière de Côte-d'Ivoire de 1999 à 2004 : Impact sur les principaux ravageurs et implications agronomiques. *Mémoire de DEA. Université d'Abidjan Cocody.* Côte-d'Ivoire. 51p.

Doffou, N. M., 2011. Susceptibility of *Pectinophora gossypiella* (Lepidoptera: Gelechiidae) and *Cryptophlebia leucotreta* (Lepidoptera: Tortricidae) to insecticides used on cotton crops in Côte d'Ivoire, West Africa. Implications in insecticide resistance pest management strategies. *Resist. Pest Manag*. 20 (2) : 10-15.

Finney, D. J., 1971. Probit Analysis, third ed., Cambridge, United Kingdom: *Cambridge University Press*. 333p.

Gunning, R. V., 1998. Inhibition of resistance-related esterases by piperonyl butoxide in *Helicoverpa armigera* (Lepidoptera : Noctuidae) and *Aphis gossypii* (Hemiptera : Aphididae). In Piperonyl Butoxide, The insecticide synergist. *Edited by Denys Glynne Jones. Academic Press Ltd,ISBN 0-12-286975-3*., 13, 215-226.

Kasai, S., Weerashinghe, I. S. and Shono, T., 1998. P450 monooxygenases are an important mechanism of

permethrin resistance in *Culex quinquefasciatus* Say larvae. *Arch*. *Insect Biochem. Physiol.* 37: 47-56.

Kennaugh, L., Pearce, D., Daly, J.C. and Hobbes, A. A., 1993. A Pbo synergisable resistance to permethrin in *Helicoverpa armigera* which is not due to increased detoxification by cytochrome P450. *Pestic Biochem Physiol* 45:234–244.

Kranthi, K. R., Armes, N. J., Rao, N. G. V., Raj, S. and Sundaramurthy, V. T., 1997. Seasonaldynamics of metabolic mechanisms mediating pyrethroid resistance in *Helicoverpa armigera* in central India, *Pestic. Sci.*, 50, 91-98.

Kranthi, K. J., Jadhav, D., Wanjari, R., Kranthi, S. and Russell, D., 2001. Pyrethroid resistance and mechanisms of resistance in field strains of *Helicoverpa armigera* (Lepidoptera: Noctuidae). *J. Econ. Entomol*. 94:253–263.

Martin, T., 2003. La résistance aux insecticides de *Helicoverpa armigera* HÜBNER, 1808 (Lepidoptera : Noctuidae) en Afrique de l'Ouest : du mécanisme à la gestion. *Thèse Université Toulouse III*. France 81p.

Martin, S. H., Ottea, J. A., Leonard, B. R., Graves, J. B., Burris, E., Micinski, S. and Church G.E.,1997. Effects of selected synergists on insecticide toxicity in tobacco budworm (Lepidoptera: Noctuidae) in laboratory and field studies. *J. Econ. Entomol.* 90:723–731.

McCaffery, A. R., 1998. Resistance to insecticides in heliothine Lepidoptera: a global view. *Phil. Trans. R. Soc. Lond. B*. 353: 1735-50.

McCaffery, A. R., Gladwell, R. T., El-Nayir, H., Walker, C. H., Perry, J. N. and Miles, M. J., 1991. Mechanisms of resistance to pyrethroids in laboratory and field strains of *Heliothis virescens*. *Southwest Entomol.* 15:143–158.

Ochou, O. G., 1994. Decision making for cotton insect pest management in Côte d'Ivoire.*Ph D Thesis,Imperial College, University of London*, U.K., 306 p.

Ochou, O. G. et Martin, T., 2001. Une stratégie pour gérer la résistance de *Helicoverpa armigera* Hübner aux pyréthrinoïdes en Côte d'Ivoire : Une fenêtre en phase végétative. *Actes 3ème Réunion Bilan du PR-PRAO*, Yamoussoukro, Côte d'Ivoire, 3-6 avril 2001, p. 58-67.

Ochou, O. G., Martin, T. and Hala, N. F., 1998. Cotton insect pest problems and management strategies in Côte d'Ivoire, West Africa, *In World Cotton Research Conference N°2 Septembre 6-12, 1998 Athens*, Greece, 833-837.

Ottea, J. A. and Holloway, J. W., 1995. Target site resistance to pyrethroids in *Heliothis virescens* (F.) and *Helicoverpa zea* (Boddie). *Pestic. Biochem. Physiol*. 61: 155-167.

Raffa, K. and Priester, T. M., 1985. Synergists as research tools and control agents in agriculture. *J. Agric. Entomol*. 2: 27-45.

Roush, R. T. and McKenzie, J. A., 1987. Ecological genetics of insecticide resistance. *Annu. Rev. Entomol*. 32: 361-80.

Scott, J. G., 1990. Investigating mechanisms of insecticide resistance: Methods, strategies and pitfalls. In: Roush R.T., B.E. Tabashnik (Eds.), Pesticide Resistance in Arthropods Chapman and Hall New-York, pp. 39-57.

Vaissayre, M. et Cauquil, J., 2000. Principaux ravageurs et maladies du cotonnier en Afrique au Sud du Sahara. *Cirad-Cta*, 60 p.

Vaissayre, M., 1988. Détermination de la DL_{50} de trois pyréthrinoïdes sur les adultes de *Pectinophora gossypiella* Saunders et de *Cryptophlebia leucotreta* (Meyrick) pour l'établissement de données de bases. Note Technique. Département des Cultures Industrielles, Filière Coton. Institut des Savanes, Bouaké, Côte d'Ivoire, 47 p.

Wu, D., Scharf, M. E., Neal, J. J., Suiter, D. R. and Bennett, G. W., 1998. Mechanisms of fenvalerate resistance in the German cockroach, *Blatella germanica* (L.). *Pestic. Biochem. Physiol*. 61: 53-62.

Zhao, G, Rose, R. L., Hodgson, E., Roe, R. M., 1996. Biochemical mechanisms and diagnostic microassays for pyrethroid, carbamate, and organophosphate insecticide resistance/cross-resistance in the tobacco budworm, *Heliothis virescens*. *Pestic. Biochem. Physiol.* 56: 183–195.

Doffou N. M.[2], Ochou O. G.[1] and Kouassi P.[2]

[1]Cotton entomological laboratory, CNRA, 01 BP 1740 Abidjan 01, Côte d'Ivoire

[2] Zoology laboratory, University of Cocody, 22 BP 582 Abidjan 22, Côte d'Ivoire

Comparative screening of cotton bollworm, *Helicoverpa armigera* (Hübner) on different growth stages of Non-Bt and Bt-cotton hybrids in Raichur, Karnataka, India

ABSTRACT

The present investigation reveals that the incidence of *Helicoverpa armigera* eggs on both Bt and Non-Bt cotton hybrids spreaded across nearly three months which coincided with peak vegetative stage to boll maturation stage, however there was a slight shift in the peak egg incidence across the seasons and hybrids. On both Bt [MRC-6918 (BG-I)] and Non Bt [MRC-7918(Non-Bt)] cotton hybrids, the maximum egg incidence was observed during square formation stage. Similarly, larval population was also observed on non-Bt cotton from peak vegetative stage to boll maturation stage but with a

peak larval population was noticed during boll maturation stage. Whereas on Bt-cotton, a considerable larval population was recorded during boll maturation stage. This indicates the susceptibility of Bt cotton hybrids to bollworms during the later stages of crop growth. Hence recommended insect resistant management (IRM) strategies *viz*, refuge crops, gene pyramiding and sequential rotation of Bt cotton hybrids should be strictly followed to get rid of *Helicoverpa* menace.

INTRODUCTION

Cotton "The white gold" is an immensely important crop for sustainable economy of India which provides employment and sustenance to a large group of farming communities. While considering the threats to cotton production, the damage caused by insect pests, mainly bollworm complex, stands out, it comprises of American bollworm *Helicoverpa armigera*, Pink bollworm *Pectinophora gossypiella*, Spotted bollworm *Earias vitella* and Spiny bollworm *Earias insulana*. The bollworms were reported to cause 60 percent yield loss in cotton which amounts monitory loss to the tune of $300 million per annum. So farmers were forced to spray chemical insecticides to tackle the bollworms and thus cotton became the single most crops which consumed more than 50 percent of total insecticide used for plant protection purpose in India. This indiscriminate use of insecticides resulted in the development of resistance in the bollworms against the lethal insecticides and resurgence of minor sucking pests, thus ultimately curtailed the cotton production in India.

At present, India ranks first in global cotton area with 11.1 million hectares (mha) and second position in global cotton production with 5304 '000 tonnes (CICR, 2011). Much of this success owes itself to cultivation of transgenic Bt cotton which commenced in India since 26 March 2002. This transgenic Bt cotton plants are developed with the assistance of modern biotechnological tools like recombinant DNA technology and genetic engineering wherein the gene(s) from the soil bacterium *Bacillus thuringiensis* is introgressed into the genetic makeup of cotton plants to produce *Cry* toxin in the entire plant system which is detrimental to bollworms. But, the extensive cultivation of Bt cotton can impose continuous and intense selection pressure on bollworms leading to the latter's development of resistance to the toxin (Kumar, 2004). Hence a comparative screening of egg and larva of *Helicovrpa armigera* on different crop growth stages of Non-Bt and Bt cotton hybrids was carried out at UAS campus Raichur to assess the association between Bt and Non Bt cotton hybrids with respect to incidence of Cotton bollworm.

MATERIALS AND METHODS

The present study was carried out at cotton experimental plot of UAS campus Raichur, during the year 2009-10 to screen the population of *Helicoverpa armigera* on different growth stages of Bt and non-Bt cotton hybrids. The screening of egg and larval stages of *H. armigera* was carried out on Bt (MRC6918) and Non -Bt (MRC 7918) cotton hybrids sown on 18th August 2009. All the recommended package of practices was followed to raise a good crop except plant protection measure against bollworms.

The egg and larval population was recorded from different plant parts on 30 to 135 days old crop which coincided with important growth stages *viz*., peak vegetative stage, square formation, boll formation and boll maturation stage. From peak vegetative stage to square formation stage, the plant parts such as top three leaves and growing shoots were observed, whereas after square formation stage even the squares, flowers and bolls were observed for recording egg incidence. For larval incidence, leaves, squares, flowers and bolls were examined thoroughly from peak vegetative stage to boll maturation stage.

From each Bt and non-Bt field 50 plants were selected randomly to record egg and larval incidence at an interval of one week. The data obtained for each crop stages was pooled and mean was calculated. Differences among the different stages of Bt and non-Bt cotton hybrids with respect to *H. armigera* egg and larval incidence was tested for significance using the Pearson Chi-square test using statistical software (SPSS :Version 17).

RESULTS

The data on incidence of egg and larva of *H. armigera* on different stages of cotton during the year 2009-10 are presented in the Table 1. During peak vegetative stage, the mean number of eggs of *H. armigera* observed on non –Bt cotton was 49 eggs per 50 plants, whereas 65 eggs per 50 plants were recorded on Bt cotton. The incidences of eggs were reached a maximum of 104 and 138 eggs per 50 plants on non-Bt and Bt cotton respectively during the square formation stage. Thereafter the egg population was reduced to 82 and 129 eggs per 50 plants on non-Bt and Bt cotton during boll formation stage. However, during boll maturation stage egg population of 83 eggs per 50 plants was recorded on non-Bt cotton plants whereas, the incidence of eggs became half (64 eggs per 50 plants) on Bt cotton hybrids.

Table: 1- Incidence of egg and larval population of *H. armigera* on different growth stages of **Non Bt and Bt cotton hybrids**

Helicoverpa / *Cotton*	Days after sowing (DAS)	1. Egg			2. Larva		
		Non-Bt (MRC 7918)	Bt (MRC 6918)	Total	Non-Bt (MRC 7918)	Bt (MRC 6918)	Total
1. Peak vegetative stage	30-50	49	65	**114**	12	0	**12**
2. Square formation	50-70	104	138	**242**	40	0	**40**
3. Boll formation	70-85	82	129	**211**	65	0	**65**
4. Boll maturation	85-135	63	64	**127**	88	46	**134**
	Total	**298**	**396**	**694**	**205**	**46**	**251**
Pearson Chi-Square (χ^2) Value		11.564**			49.177**		

The larval incidence of *H. armigera* on non-Bt cotton was observed from peak vegetative stage (12 larvae per 50 plants) which gradually increased to 40 larvae per 50 plants during square formation stage, which was further increased to 65 larvae per 50 plants during boll formation stage and reached a maximum of 88 larva per 50 plants during boll maturation stage. However, no larval population was recorded on Bt cotton plants during peak vegetative, square formation and boll formation stage. Whereas, a considerable larval population (46 larvae per 50 plants) was recorded on Bt cotton hybrids during the boll maturation stage.

DISCUSSION

The incidence of *H. armigera* eggs on both Bt and Non-Bt cotton hybrids spreaded across nearly three months which coincided with peak vegetative stage to boll maturation stage. However, there was a slight shift in the peak egg incidence across the seasons and hybrids. On both Bt and Non Bt cotton hybrids, the maximum egg incidence was observed during square formation stage. These findings are in accordance with report of Kengegowda (2003) and Manju (2006) who observed more eggs on Bt and Non- Bt cotton hybrids between late vegetative stage to square formation stage. Thereafter the egg population was reduced in boll formation and boll maturation stage. The mean egg incidence on Bt-cotton hybrids was more than that on non-Bt cotton hybrids, these results are in close agreement with the report of Kengegowda (2003), Patil *et al*. (2004) and Manju (2006) who also reported almost similar levels of egg population on both Bt and non-Bt cotton plants.

The larval population was observed on non-Bt cotton from peak vegetative stage (12 larva per 50 plants) with a maximum during boll maturation stage (88 larva per 50 plants). Whereas in Bt-cotton, a considerable larval population (46 larva per 50 plants) was recorded on MRC-6918 (BG-I) during boll maturation stage. The present results are in accordance with Udikeri *et al*. (2002) who also reported the incidence of *Helicoverpa* larvae (0.45 per plant) in MECH-184 (BG-I). Patil *et al*. (2004) reported the larval population in MECH-184 Bt (0.22 larvae per plant) whereas, it was 0.28 larvae per plant in NCS-145 hybrid cotton. Prasad *et al*. (2009) also reported incidence of *H. armigera* on Bt (MECH-162 Bt (8.8%)), (RCH-2Bt (7.53%)) and non Bt cotton hybrids under unprotected condition. Similarly, Bambawale *et al*. (2004) reported lowest larval population in Bt-cotton (0.03 per plant) compared to 0.05 and 0.09 larvae per plant in conventional cotton IPM and non IPM plots, respectively.

The Pearson Chi-Square analysis indicated that there was an association between Bt and Non-Bt cotton hybrids (χ^2 = 11.564**) with respect to *H. armigera* egg incidence. The adults of *H. armigera* do not differentiate Bt and Non-Bt cotton during oviposition and it treat both versions of cotton in the same manner. Similarly, there was an association between *H. armigera* larval populations on both Bt ant Non-Bt cotton (χ^2 = 49.177**). Since Non-Bt cotton do not possess genes for insect resistance, the *H. armigera* larvae are able to complete life cycle on it. However, Bt cotton are designed with inbuilt resistance mechanism against *H. armigera*, so it cannot be able to develop on Bt cotton. In the present study a considerable larval population (46 larvae per 50 plants) was recorded on Bt cotton hybrids during the boll maturation stage. This indicates the reduced efficacy of insecticidal gene during later crop growth stages of Bt cotton, succumbs to the attack of *H. armigera* (Kranthi *et al*., 2005). Hence recommended insect resistant management (IRM) strategies *viz*, refuge crops, gene pyramiding and sequential rotation of Bt cotton hybrids should be strictly followed to get rid of *Helicoverpa* menace.

REFERENCES

Bambawale, O. M., Singh, A., Sharma, O. P., Bhosle, B. B., Kavekor, R. C., Dhandapani, A. Kanwar, V., Tanwar, R. K., Rathod, K. S., Patange, N. R. and Pawar, V. M. (2004). Performance of *Bt*-cotton (MECH-162) under integrated pest management in farmers' participatory field trial in Nanded district, Central India. *Curr. Sci.*, 86:1628-1633.

CICR [Central Institute for Cotton Research]. (2011). *Vision 2030*. Central Institute for Cotton Research, Nagpur, India. 56p.

Kengegowda, N. (2003). Studies on the population dynamics and screening of Bt cotton hybrids against insect pests. M.Sc. Thesis submitted to UAS Dharwad.

Kranthi, K.R., Naidu, S., Dhawad, C.S., Tatwawadi, A., Mate, K., Patil, E., Bharose, A. A., Behere, G.T., Wadaskar, R. M. and Kranthi, S. (2005) -

Temporal and intra-plant variability of Cry1Ac expression in Bt-cotton and its influence on the survival of the cotton bollworm, *Helicoverpa armigera* (Hübner) (Noctuidae: Lepidoptera). *Current Science*. 89: 291-298.

Kumar, P. A. (2004). Cautious use of Bt genes in transgenic crops. *Curr. Sci.*, 632-633.

Manju, A. C. (2006). Abundance of pests and their natural enemies on Bt and Non Bt cotton. M.Sc. Thesis submitted to UAS Dharwad.

Patil, B.V., Bheemanna, M., Hanchinal, S. G. and Kengegowda, N. (2004). Developing IPM module for Bt cotton under irrigated ecosystem. Paper presented at *the International Symposium on Strategies for Sustainable Cotton Production. A Global Vision*, 3- Crop protection, University of Agricultural Sciences, Dharwad, Karnataka, 23-25 November, 2004, pp.152-154.

Prasad, N. V. V. S. D., Rao, M. and Rao, M. N. (2009). Performance of Bt cotton and non-Bt cotton hybrids against pest complex under unprotected condition. *Journal of Biopesticides*, **2**: 107-110.

Rai, M., Acharya, S. S., Virmani, S. M. and Aggrawal, P. K. (2009). Status of Indian Agriculture. National Academy of Agricultural Sciences, New Delhi

Udikeri, S. S., Patil, S. B., Hegde, R. N., Kulkarni, V. N. and Patil, S. S. (2002). Performance of Bt cotton genotypes under unprotected condition. In: *National Seminar on Bt Cotton Scenario with Special Reference to India*, held at Univ. Agric. Sci., Dharwad, Karnataka, 23rd May, 2002, pp. 88-89.

RANJITH, M. T. [1*], PRABHURAJ, A[2]. AND AYYOOB, K. C.[3]
[1,2]Department of Agricultural Entomology, College of Agriculture-Raichur, UAS Raichur
[3]Department of Agricultural Statistics, College of Agriculture- GKVK, UAS Banglore
*Email address: ranjith.mt16@gmail.com

MANAGEMENT AND CONTROL OF LEAFCUTTER ANT (*Atta mexicana* Smith) IN BERRIE CROPS IN ZAPOLTITIC, JALISCO, MEXICO.

A research project was developed about the management and control of local leafcutter Ant (*Atta mexicana* Smith) in production systems of berries in 3-producing farms in the area of Zapoltitic, Jal. Mexico.

The formulation and presentation of this project emerged from the obvious need of having management and control methods for the local ant that were more effective, economic and less polluting.

The local leafcutter ant, in natural forests, play important roles as accelerating the cycling of bioelements, aeration of the soil, spreading of seeds, promotion of new bud growth on the trees, their landfill waste serve as habitat for some species. However, when the natural vegetation is removed to establish subsistence and semi-commercial crops, an overflowed increase of the numbers of colonies and individuals occurs that compete advantageously with the man.

The knowledge of the biology, ecology and habits of the leafcutter ant is an indispensable component for the design and implementation of programs in the region seeking the management and efficient control, with an emphasis on the species of ants that are of particular importance for the damage caused to the farmers. We also present recommendations about the management and control of the insect taking into account the unique characteristics of most of the territory and the Pacific in general.

The genera *Atta* and *Acromyrmex* are the most advanced and the most recent. They are distributed in the American continent between the 33rd north and south latitudes. From the South of the United States down to Argentina, and from sea level up to 2,000 to 3,000 meters.

Their existence has been mentioned since very ancient times, for example in the Popul Vuh, the mythical creation of the Central America Maya's civilization (300-900 BC). But the reason for the cutting of leaves by ants was unknown for a long time. In 1874, Thomas Belt, naturalist and mining engineer, wrote about the special use ants make of the leaves which was the preparation of a substrate in which they cultivate tiny fungus species on which they feed. This discovery marked the start of the scientific study of this mutual symbiosis between an Ant and a fungus.

The distribution and abundance of the leafcutter ant (*Atta; Acromyrmex*) is associated with the change in terrestrial habitats, since it is often found in territories that have been deprived of the natural vegetation for the establishment of crops.

The objectives of this study were to: determine the species of the dominant ant in the cultivars of BlackBerry (*Rubus fruticosus*), raspberry (*Rubus idaeus*) and Blueberry (*Vaccinium erythrocarpum*) in the area of Ciudad Guzman, Jalisco, Mexico; to determine the acceptance of the product for the control of these species and to determine the advantages for the use of the products against competitors.

The assay was conducted in three farms known as Fresnos, Guayabos and Los Ocotes, which had the three crops mentioned above and it was developed during the year of 2012.

The treatments were 3 products with 3 replicates each and a witness being these: PATRON (Imidacloprid), SEIGE (Hidrametilnona) and TROMPA (Abamectin); all under the dose of 10 grams/30 ants.
For this assay applications of 10 Grams were put on the ant foraging routes in the early hours of the start of the activity and the size of the experimental unit was adequated to the size of the nests of the ants having a minimum distance of 50 meters between a nest and the other.

24 hours prior to the first application a monitoring was conducted to take into account the number of individuals passing through the foraging line and knowing the initial populations of ants. The first application made was of 10 grams of the product for each 30 average ants placed in a fixed point at a distance of 1.5 meters from the inlet of the nest, then each 1.5 meters in at least three application points.

The variables evaluated were the identification of the species present, the population's dynamics, and palatability of the product and percentage of control.

As for the population's dynamics the normal process was the count of individuals who passed through a specific point of the foraging line in a 5 minute period.
The residuality of control was evaluated at 24 Hours, 10, 20 and 30 days after the application of the products.
The level of irritation with the different treatments evaluated was determined, and notation of the quality of the formulation and mixing during application was taken.

After analyzing thoroughly the different samples of ants collected in the three farms in question, we arrived to a positive identification of the ant that is:

Kingdom: Animalia
Phylum: Arthropoda
Class: Insecta.
Order: Hymenoptera.
Family: Formicidae
Subfamily: Myrmicinae
Tribe: Attini
Género: Atta
Specie: mexicana (Smith)
Coumun name: Hormiga arriera, leafcutter ant

Images of the ants recollected for identification.

The assay began on March 21, 2012 with the sampling of ants passing in the location of nests laid down in the farms Ocote cuate, Los Guayabos and Los Fresnos under the following factors:
The locations of the farms are:

Farms	Altitude above sea level	GPS Location
LOS OCOTES	1,355 mts	N 19° 35' 31.0" W 105° 26' 13.6"
LOS GUAYABOS	1,415 mts	N 19° 37' 14" W 103° 26' 57.5"
LOS FRESNOS	1,255 mts	N 19° 37' 20.5" W 103° 22' 35.7"

PALATABILITY OBSERVATIONS:
At the time of installation the ant did not present a clear preference by any of the products taking the three immediately of the plates. With the exception of those present at the farm Ocotes where a clear preference was observed for the products SEIGE and TROMPA taking them immediately after they were put on the dishes, but did not show any interest for PATRON.

ANALYSIS OF MEANS OF RECORDED INDIVIDUALS:
According to the analysis of means retrieved through transformation by logarithms to decrease the range of error, it is observed that the best product was TROMPA in Fresno farm followed by SEIGE in Guayabos and Ocotes Farms respectively. Subsequent use of TROMPA in Guayabos and Ocotes again respectively.

However according to the 95% significance level TROMPA proved to be the best product showing a

non-significant difference between SEIGE and TROMPA in Ocotes and Guayabos farms.

Based on the prior the application of TROMPA is recommended at this time in three ranches for Ant control.

The next step to continue the test is the dose adjustment on the ranches by areas according to mapping as well as the analysis of climatological data for the preparation of an annual control program as well as the rotation of the product.

It is noteworthy that the 20-day trial was conclusive for which it was recommended to pass to the next stage of the test starting cabinet works immediately as well as bi-weekly visits which the first toke place on May 4 in the morning and during the course of the day.

COMMERCIAL APPLICATION STAGE.

After having identified the species in question and the product to be used, it gave the task of implementing the commercial application to see the behavior of the product in field and the possible changes that might done.

It was noted that the product TROMPA worked to perfection during the stage of evaluation than lasted three months from May to July, in full rainy season where it was even observed that this caused no alteration in the effectiveness or the product's palatability.

As it was obvious the first 15 days showed a slow process, but after that a complete control on the farms Los Guayabos and Fresnos was observed where ant attacks were suppressed and only appeared on two occasions being controlled immediately.

In the case of the farm Ocotes new colonies were still appearing at different points, but they were immediately controlled and the apparent reason was due to the location of new nests in each inspection but which were immediately controlled by placing product.

OBSERVATIONS AND RECOMENDATIONS

It was noted that the problems of the leafcutter ant (*Atta mexicana* Smith) is controllable in berry farms if the next guidelines are followed:

1. You should not lower the guard in terms of use of personnel for review of the field, since during the 4 months of the test it was noted that staff was withdrawn for other activities, and immediately ant population would increment its presence.
2. You should not miss product for the control since on two occasions we observed that there was no product in storage, and immediately ant population incremented its presence.
3. The product TROMPA must continue during the months of March to September which is the critical time of presence of these ants. Then the product should be changed to SEIGE during the months from October to February in which it is estimated the second generation will be smaller and thus creation of resistance will be prevented.
4. Also the product may change at any time the ants start to show lack of palatability to the product TROMPA.
5. It was established that the best conditions for the presence of the ant according to historical weather data is in the months from May to October, after which the presence was reduced drastically. Here the problem was moisture since we were working with perennial crops and therefore due to irrigation there was always existing moisture. However the low in ambient temperatures helped in good control and the doses were able to be downloaded by half with no affection.

BIBLIOGRAPHIC REFERENCES

CURRIE, C.R.; SCOTT, J.A.; SUMMERBELL, R.C.; MALLOCH, D. 1999. Fungus-growing ants use antibiotic-producing bacteria to control garden parasites. Nature 398: 701-704. USA

ESCOBAR, R.; NEITA, J.C.; MENA, G.2001. Caracterización de colonias de hormiga arriera en cuatro municipios del Depto. del Chocó. Revista Institucional No 14 (13-20). Universidad Tecnológica del Chocó. Quibdó

ESCOBAR, R. et al. 2002a. Hormigas cortadoras de la Tribu Attini en sistemas productivos del Departamento del Chocó. Revista Institucional No 15 (35-41). Universidad Tecnológica del Chocó. Quibdó

ESCOBAR, R. et al. 2002b. Deshechos de *Atta colombica G*. y su uso como bioabono en el Departamento del Chocó. revista Institucional No 15. Universidad Tecnológica del Chocó (en preparación).

Evan Solomon, Scott. 2007. Biogeography and evolution of widespread leaf cutting ants, A*tta* spp. (Formicidae, Attini). Tesis doctorado. University of Texas. 106 Pags.

HART, A.G.; RATNIEKS, L.W. 2002. Waste management in the leaf-cutting ant *Atta colombica*. Behav. Ecol 13: 224-231

HÖLDOBLER, B.; WILSON, E.O., 1996. Viaje a las hormigas. Una historia de exploración científica. Crítica. Barcelona 270p.

MORGAN, RANDY C. 1991. Natural history and captive management of leaf-cutting ants in the genus A*tta* (Hymenoptera: formicidae). AAZPA 1991 ANNUAL CONFERENCE PROCEEDINGS: pp. 321-328

SÁNCHEZ PEÑA, SERGIO RENÉ. 2005. Essays on Organismal Aspects of the Fungus-Growing Ant Symbiosis: Ecology, Experimental Symbiont Switches and Fitness of *Atta*, and a New Theory on the Origin of Ant Fungiculture. Tesis doctorado. University of Texas. 170 Pags.

Santillan Santana, J.[1], Martínez Ramirez, J.L.[1], Duran Martínez, C.M.[1], Rendón Salcido, L.A.[1], Aceves Núñez, V.A.[1], Santillan Loza, J.J.[2], Rojas Peña, S.A.[2]

[1] Profesor investigador del Departamento de Producción Agrícola del Centro Universitario de Ciencias Biológicas y Agropecuarios de la Universidad de Guadalajara.

[2] Alumno de la carrera de Ingeniero Agrónomo del Centro Universitario de Ciencias Biológicas y Agropecuarios de la Universidad de Guadalajara.

BIOEFFICACY OF INSECTICIDES TO *AMSACTA LACTINAE* (CRAMER) (LEPIDOPTERA: ARCTIIDAE) IN FIELD POPULATION OF TAMILNADU

ABSTRACT

Amsacta lactinae is an important pest in oil seed crops in India. It causes the several damages to productivity of groundnut and guar crops. Conventional insecticides like pyrethroid, organophosphate, neonicotinoids, botanicals and newer chemistry such as diamide and avermectins classes are used for controlling many agricultural pests. In the present study we investigate the toxicity of ten insecticides belonging to different groups against third instar larvae of *Amsacta lactinae.* Based on the LD_{50}, LD_{90} value the result shows that emamectin benzoate was the most toxic insecticide (LD_{50}: 0.13892g a.i/ha), followed by imidacloprid (LD_{50}: 3.69534g a.i/ha) cypermethrin (LC_{50}: 5.0852g a.i/ha). The data of the present study will be helpful for effective control of this insect in oilseed crops.

INTRODUCTION

Amsacta lactinae, commonly known as ragi hairy caterpillar (Cramer, 1777), is a voracious defoliator that destroys many crops like sorghum, castor, maize, groundnut, etc. They migrate in groups from field to field feeding voraciously on the crops they come across on their way giving them grazed appearance (NBAII, 2013). Currently pyrethroids and organophosphate insecticides have been the primary choice of pest control method. Every year, several tons of pesticides are used to control hairy caterpillar in Karnataka, Tamil Nadu and Andhra Pradesh in Southern India. In spite of this, the pest recurs unabated each year causing considerable damage to *Sesamum*, red gram; cotton, cowpea and castor in addition to groundnut (Veenakumari *et al.*, 2007). Many conventional insecticides are being used to manage these pests due to which many folds of resistance was reported in pests like *S. litura* (Prasad *et al*., 2008), *Spodoptera exigua* (Wang *et al.*, 2002), *H. armigera* (Kranthi *et al*., 2002). The pyrethroids and organophosphorus combination insecticides were found to be effective against the *H. armigera* (Martin *et al*., 2003). Similar study on emamectin benzoate showed high mortality effect on larva of *Tuta absoluta* (Gacemi and Guenaoui, 2012). Effective control of *A. lactinae* can be done by evaluating the toxicity of various groups of chemical insecticides, in order to identify the effective one. In the present study toxicity of ten insecticides against *Amsacta lactinae* was assessed to find out the best molecule for the insect control.

MATERIALS AND METHODS

Insects

Amsacta lactinae (Lepidoptera: Arctiidae) population was collected from groundnut field in Salem District, Tamilnadu, India. Larvae were brought to the laboratory and maintained at 25°C with 75% relative humidity. Larvae were fed on groundnut leaves. Larval bioassay was performed on third instar larvae.

Chemicals

The active ingredients, trade name and manufacturers of the chemical pesticides used in this study are: Imidacloprid (17.8% SL, Bayer), Cypermethrin (10% EC, SIDCO), Emamectin (dispersible granule WG, Syngenta, Turkey), Neem (1% EC, Parry) and Flubendiamide, (480 SC, Bayer) λ- Cyhalothrin (25% EC), Deltamethrin (11% EC), Profenofos (50% EC), Dichlorvos (76% EC) and Monocrotophos (36% SL).

Bioassay

Topical bioassay was conducted to determine the toxicity of the pesticide to *Amsacta lactinae.* For the topical bioassay, 3rd instar larvae were treated with different chemical insecticides with more than five concentrations per insecticide. Chemicals were diluted with deionized water to prepare the required dosage. Emamectin granules were dissolved in deionized water, from the dilution, 2µl of chemicals delivered at the ventral side of the larvae using micropipette; larvae treated with deionized water served as a control. Groundnut leaves were provided as the food source. Three replicates per treatment and ten larvae per

replicate were taken. The mortality was determined 24 hours post treatment.

Statistical Analysis

Data from bioassay were corrected using Abbott's formula (Abbotts, 1925). The mortality data were subjected to probit analysis, using the SPSS software (version 13.00). The LD_{50} and LD_{90} values of the insecticides and the slopes of dose-mortality were generated.

RESULTS AND DISCUSSION

Among the insecticides tested in topical bioassay, emamectin (LD_{50}: 0.13892g a.i/ha) showed most toxicity followed by imidacloprid (LD_{50}: 3.69534g a.i/ha) and cypermethrin (LD_{50}: 3.69534g a.i/ha) (Table 1). Emamectin benzoate is a semi synthetic derivative of natural products and is quite effective against a number of lepidopteran insect pests including *S. exigua* and *S. litura* (Ahmad *et al*., 2006). It acts as an antagonist of neurotransmitter (GABA and glutamate) gated chloride channels in the insect nervous system resulting in strong chloride ion influx into the cell followed by disruption of nerve impulses, paralysis and finally death (Nauen, 2006). Emamectin targets various lepidopteran pests of field crops and vegetables (Ishaaya *et al*., 2002; Gupta *et al.,* 2004; Murugaraj *et al.,* 2006). Saeed *et al.,* (2012) reported that cypermethrin was most effective for controlling field population of *S. exigua*. Next to the imidacloprid shows high efficacy to *A. lactinae* (3.69534g a.i/ha), studies on efficacy of imidacloprid less reported against agriculturally important pest. However in contrast to this work, our previous study reported less effective of imidacloprid on *Spilosoma obliqua* (Muthusamy *et al.,* 2011), followed by imidacloprid and cypermethrin shows high efficacy. Effectiveness of imidacloprid and cypermethrin has been well established in insects like *Spodoptera litura* (Kumar and Srivastava, 2009); *Bactrocera dorsalis* (Lin *et al.,* 2013). From this study it can clearly indicate that emamectin was the effective one for controlling *A. lactinae* among insecticides tested. This data provides basic information regarding the choice of pesticide which is to be used for effective management of *A. lactinae*.

Table: 1 Toxicological response of *A. lactinae* to different insecticides of different chemical classes: imidacloprid, cypermethrin, emamectin, flubendiamide, neem, λ-cyhalothrin, deltamethrin, profenofos, dichlorvos, and monocrotophos.

Insecticide	LD_{50} g a.i/ha	LD_{90} g a.i/ha	Intercept	Slope	SE	χ^2 value	Significance value
Imidacloprid	3.69534	193.377	-0.42326	0.74562	0.25463	0.01105	P=0.99
λ-cyhalothrin	7.66319	26.8501	-2.08143	2.35346	0.78291	8.49409	P=0.03
Flubendiamide	36.1008	234.446	-2.45658	1.57724	0.43450	2.46048	P=0.48
Cypermethrin	5.0852	121.714	-0.65645	0.92934	0.24076	7.04931	P=0.07
Emamectin	0.13892	1.00340	1.27934	1.49245	0.28243	0.78613	P=0.85
Neem	80.4731	725.758	-2.55688	1.34174	0.31104	3.68198	P=0.29
Deltamethrin	6.81180	25.6589	-1.85401	2.22500	0.54372	7.07447	P=0.06
Profenofos	6.87702	193.392	-0.74060	0.88441	0.35313	19.8498	P=0.001
Dichlorvos	134.234	2203.36	-2.24400	1.05458	0.36489	1.66757	P=0.64
Monocrotophos	62.4154	210.199	-4.36295	2.43022	0.92132	11.4669	P=0.001

REFERENCES

Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*, 18: 265-267.

Gacemi, A & Guenaoui, Y., 2012. Efficacy of Emamectin Benzoate on Tuta absoluta Meyrick (Lepidoptera: Gelechiidae) Infesting a Protected Tomato Crop in Algeria. *Acaemic. Journal of Entomology*, 5 (1): 37-40.

Gupta, G.P., Rani, S., Birah, A & Raghuraman, M., 2004. Relative toxicity of certain new insecticides against *Spodoptera litura* (Fab.) *Journal of Pesticide Research*, 16: 45-47.

Ishaaya, I., Kontsedalov, S., Horowitz, A.R., 2002. Emamectin, a novel insecticide for controlling field crop pests. *Pest Management Science*, 58: 1091-1095.

Kranthi, K.R., Jadhav, D.R., Kranthi, S., Wanjari, R.R., Ali, S.S & Russell, D. 2002. Insecticide resistance in five major insect pests of cotton in India. *Crop Protection*, 21: 449-460.

Kranthi, K.R., Jadhav, D.R., Wanjari, R.R., Ali, S.S., Russel, D., 2002. Insecticide resistance in five major insect pests of cotton in India. *Crop Protection*, 21: 449-460.

Kumar, Y & Srivastava, R.P., 2009. Status of bio-efficacy of synthetic pyrethroids against tobacco caterpillar, *Spodoptera litura* (Fabricius). *Journal of Entomological Research*, 33 (3): 235-23.

Lin, Y., Jin, T., Zeng, L & Lu, Y., 2013. Insecticide Toxicity to Oriental Fruit Fly *Bactrocera dorsalis* (Diptera: Tephritidae) is Influenced by Environmental Factors. *Journal of Economic Entomol*ogy, 106(1): 353-359.

Murugaraj, P., Nachiappan, R.M. & Selvanarayanan, V., 2006. Efficacy of Emamectin benzoate (Proclaim 05 SG) against tomato fruit borer, *Helicoverpa armigera* (Hubner). *Pestology*, 30: 11-16.

Muthusamy, R., Karthi, S and Shivakumar M.S., 2011. Baseline susceptibility of five classes of insecticides on Bihar hairy caterpillar *Spilosoma obliqua* (Walk.) (Lepidoptera: Arctiidae). *Resistant Pest Management Newsletter*, 21 (1): 11-13.

National Bureau of Agriculturally Important Insects, Bangalore, India 2013. http://www.nbaii.res.in/insectpests/Amsacta-lactinea.php,

Nauen, 2006. Insecticide Mode of Action; return to the ryanodine receptor. *Pest Managemnet Science*, 62: 690-692.

Prasad, D.K., Madhumathi, T., Arjunarao, P, & Srinivasarao, V. 2008. Status of OP and Pyrethroid resistance in *Spodoptera litura* (Fab) on cotton. *Journal of Pesticide Research*, 20 (1):103-106.

Saeed, Q., Mushtaq, A. Saleem & Ahmad, M., 2012. Toxicity of Some Commonly Used Synthetic Insecticides against *Spodoptera exigua* (Fab) (Lepidoptera: Noctuidae). *Pakistan Journal of Zoology*, 44(5): 1197-1201.

Veenakumari, K., Rabindra, R.J., Srinivasa Naik, C.D & Shubha, M.R., 2007. In situ field level mass production of *Amsacta albistriga* (Lepidoptera:Arctiidae) nucleopolyhedrovirus in a groundnut ecosystem in South India. *International Journal of Tropical Insect Science*, 27: (1): 48–52.

Wang, K.Y., Jiang, X.Y, Yi, M.Q, Chen, B.K & Xia, X.M. 2002. Insecticide resistance of *Spodoptera exigua*. *Acta – Phytophylacica – Sinica*, 29(3): 229-234.

Muthusamy R, Karthi S and M.S. Shivakumar*
Molecular Entomology Lab
Department of Biotechnology
Periyar University
Salem- 636 011
Tamil Nadu, India
Corresponding Author*: skentomol@gmail.com

Final Announcements

Solutions Workshop: Resistance Management for Consultants/Advisors at NAICC Annual Meeting, New Orleans, LA
by Jim Steffel, LABServices

Speakers from Agricultural Science Societies, Crop Protection Industry Resistance Action Committees (RAC's), USDA, EPA and Consultants/Advisors will discuss topics influencing pest resistance and resistance management. Presentations will be brief and followed by blocks of time dedicated to discussions by consultants and advisors in attendance. Bring your experience, ideas and frustrations in dealing with resistance to share with the group and become part of the solution.

Join your peers in New Orleans just prior to the Annual Meeting on **Wednesday, January 29, 2014, from 8:00 a.m. – Noon** at the Sheraton New Orleans, for an interactive session of vital interest that will address effective resistance management from the crop consultant's perspective, including:

Introduction: A multidisciplinary overview of weed, fungicide and insecticide resistance problems.

Overview: Interactive, problem-solving discussions of managing resistant weeds, insects and diseases in viable crop production programs. Crop consultants and other professionals will share differing perspectives, potential solutions and barriers to implementing resistance management strategies at the grower level. Topics will include availability of the products needed implement resistance management, compatibility with of strategies with IPM and conventional production practices. Discussions of the potential for technology to assist consultants and advisors implement management strategies and the need for area wide strategies if resistance management is to be effective. Additional topics and will be outlined in the workshop program.

Strategies and Solutions: Working collaboratively with groups like WSSA, Industry RAC's, USDA and EPA, professionals will develop workable strategies for resistance management in the wide variety of crops, pests and productions systems encountered at the grower level.

Program details and registration are available now at naicc.org. CPCC and CCA CEUs will be available.

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

rpmnews@msu.edu, or

Newsletter Coordinator
Resistant Pest Management Newsletter
B-11 Center for Integrated Plant Systems
Michigan State University
East Lansing, MI 48824-1311
USA

Please visit us online today at**http://whalonlab.msu.edu/Newsletter/index.html**