Resistant Pest Management Newsletter

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Table of Contents

Resistance Management from around the Globe

Western bean cutworm management in the Texas Panhandle

Western bean cutworm (*Striacosta albicosta* (Smith)) and southwestern corn borer (Diatraea grandiosella Dyar) are the two major lepidopteran pests of corn grown in the northwest Texas Panhandle. Recently, these two insects have become difficult to manage. Treatment estimates from crop consultants in the Panhandle have been as high as 40% or approximately 116,000 acres of corn in the four northwest Texas counties.

Due to an ongoing tolerance issue in this area of spider mites to the broad spectrum pesticide, bifenthrin, producers and crop consultants need an effective alternative to pyrethroids to manage lepidopteran pests in corn (Table 1).

Table 1: Spider mite response to bifenthrin in the northwest Texas Panhandle

County	LC_{50}[1] Range	TR(NM)[2]	TR(Low)[3]
Dallam	0.40-10.96	70.5-548	14.5-27.4
Sherman	1.97-31.23	98.5-1561.5	4.5-15.9
Hartley	0.05-150.30	159.5-7512.5	65.0-3005.0
Moore	0.57-23.44	130.5-1172.0	7.2-41.1

[1] LC_{50}'s expressed in ppm
[2] New Mexico outgroup not historically exposed to bifenthrin LC_{50} = 0.02 ppm; TR(NM)-tolerance ratio based on New Mexico sample
[3] TR(Low)-tolerance ratio based on lowest LC_{50} within the county
Tolerance Ratio= LC_{50} from individual field/ LC_{50} of NM sample or lowest LC_{50} within the county

Flubendiamide, or Belt, is a new broad-spectrum lepidoptera control product that disrupts cellular calcium balance. An advantage to this new insecticide is that it offers a new mode of action. Therefore, there should be no cross-resistance to insecticides from other chemistries. Flubendiamide must be ingested to act as it has minimal contact or ovicidal activity. This insecticide is fast acting. It causes feeding to cease and paralysis to occur within minutes.

We conducted this study to determine if flubendiamide is a viable alternative to the pyrethroids that have historically been used to control both lepidopteran and spider mite pests.

Materials and Methods

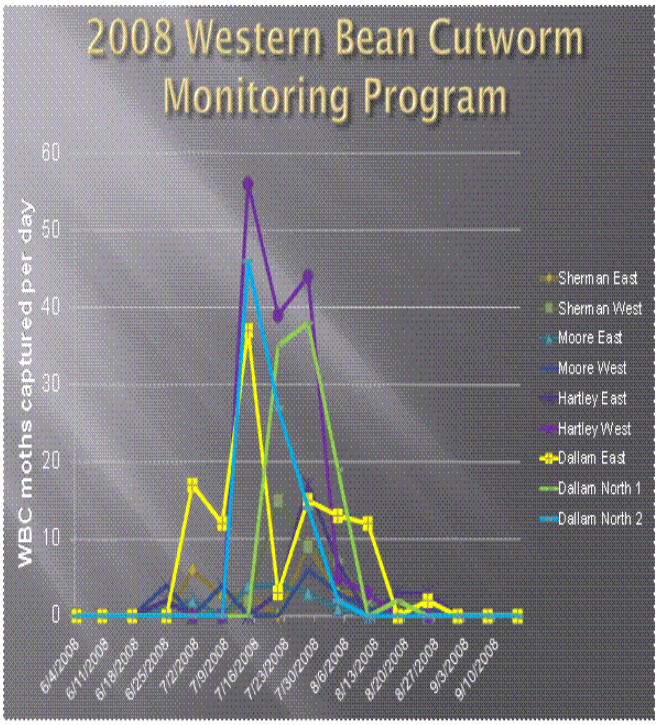
Western bean cutworm moths were monitored using a pheromone trap placed on the perimeter of 9 non-Herculex corn fields grown in the 4 county regions. The traps were monitored weekly and used to determine the flight phenology of western bean cutworm moths.

Using a randomized complete block 3 different insecticides and 2 different surfactants labeled for Lepidoptera management were compared in one of the 9 fields (Dallam North 1) to determine the most effective management tool. The treatments consisted of an untreated control, cyfluthrin with non-ionic surfactant, flubendiamide with non-ionic surfactant, flubendiamide with concentrated crop oil and methoxyfenozide with concentrated crop oil. Each treatment was applied to four – 40 feet long rows and was replicated 4 times. Applications were timed to coincide with 95% crop tassel and occurred July 27, 2008. Five primary corn ears in each of the two center rows of each treatment for a total of 40 ears per treatment were sampled 14 days post application. All lepidopteran larvae present were identified and counted.

Results and Discussion

Flight activity of western bean cutworm was greater in the western region (Dallam and Hartley counties) of the area monitored (Figure 1.). The monitoring program showed that flight activity peaked in three fields at least 7 days before the crop reached 95% tassel. Extended flight activity by this pest is creating a management problem similar to that being experienced with southwestern corn borer. However, flight activity of the two pests does not occur at the same time rendering management more difficult and expensive.

Figure 1: Western Bean Cutworm Flight Activity in the Northwest Texas Panhandle



Unlike corn earworm, Helicoverpa zea (Boddie), western bean cutworm is not cannibalistic and often found in multiple numbers in an individual ear. The percent ears with multiple western bean cutworm larvae are reported in Table 2. Multiple western bean cutworm larvae may result in up to 40% yield loss. This level of yield reduction could be approximately 60 bushels in this corn growing region of Texas.

Table 2: Percent of Ears with Multiple Western Bean Cutworm larvae

Treatment	Average Percent Ears with Multiple Larvae
Untreated	12.5%
Cyfluthrin+ nis	7.5%
flubendiamide + nis	10.0%
flubendiamide + cco	10.0%
methoxyfenozide	2.5%

Nis - non-ionic surfactant
cco - concentrated crop oil

No significant difference was detected between the five different treatments (Table 3.). Therefore, making an insecticide application at this time was not an economically good management decision. Two reasons may account for the insecticide application not being effective in managing western bean cutworm. First, the application may have been timed too late to be effective. Second, more than one application may be needed to effectively manage this pest with an extended flight activity pattern.

Table 3: Percent of Ears with at Least One Western Bean Cutworm Larva

Treatment	Rate	Western Bean Cutworm
Untreated		35.0% a
cyfluthrin w/nis	2.2 oz	37.5% a
flubendiamide + nis	3 oz	45.9% a
flubendiamide + cco	3 oz	48.7% a
methoxyfenozide +cco	6 oz	34.2% a

nis - non-ionic surfactant
cco - concentrated crop oil
No significant difference between treatments (P-value=0.59,F statistic=0.70,df 4,189 SAS Pro GLM)

Much more research is needed to develop a more effective management protocol for this pest. We will increase use of bucket style pheromone traps to try to assist with determining a more effective management protocol.

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Jerry Michels, Texas AgriLife Research
and Bonnie Pendleton, West Texas A&M University

Modelling establishment and spread potential of *Trogoderma granarium* Everts: Australian concerns for a surveillance program

The Khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae), is one of the world's most feared stored grain and cereal pests (Banks 1977); it has been cited as one of the 100 worst invasive pest species in the world (Lowe et al. 2004). Khapra beetle, like many other stored-product pests, is spread by commerce and trade and to a lesser extent – by human movement. Its significance lies not only in the economic impact but also the trade restrictions a country is facing, should the Khapra beetle become established. Therefore it is a regulated quarantine pest in most countries (Banks 1977).

T. granarium has a high capacity for population growth. Infestation increases of 1.7 to 2.4 times per week have been reported, but under optimal conditions (33-37°C) populations of *T. granarium* can even increase by 12.5 times per month (Karnavar 1973, French and Venette 2005). According to Stibick (2007) and Aldryhim and Adam (1992), *T. granarium* usually has 4 - 5 generations per year, but this figure can reach 12 under optimal conditions. Females lay an average of 50 to 100 eggs, which are loosely scattered in host material (Harris 2006, Szito 2007). Larvae are able to hide in cracks and crevices of shipping containers, bulk cargo holds and packing material. They are capable of entering an inactive state, diapause, for extended periods which enables them to be transported undetected (Burges 1962, Pasek 1998).

It has never been observed to fly and may be unable to do so. The natural means of dispersal is by young larvae being carried by wind, and by older larvae crawling (Lindgren et al. 1955). This is a slow process, which mainly allows for dispersal over short distances. If this was the only mechanism of dispersal, the insect would probably not pose a risk to Australia. However, it is the long distance, human-aided movement that pose a risk to Khapra beetle free countries, particularly because the insects can spread through growing international trade (Harris 2006).

According to Bailey (1958), Hinton's (1945) book referred to a single record - based on Durrant's (1921) publication – which led the scientific community to accept that *T. granarium* Everts. was established in Australia. Bailey (1958) established that all of the suspected occurrences have been associated with shipping. Since then, specialists have looked at countless *Trogoderma* specimens of Australian origin, (specifically because of the recent establishment of the warehouse beetle, *T. variable* Ballion), and did not find a single specimen of *T. granarium*. Unfortunately, Australia is still being erroneously listed in publications such as Stibick (2007) as a Khapra beetle country.

Though *T. granarium* has never established in Australia; nevertheless, an accidental introduction occurred in a suburban house in Perth, Western Australia in 2007, via household goods imported from the United Kingdom. The house was fully covered with plastic sheeting for fumigation using the application rate recommended by Australian Quarantine and Inspection Service for Khapra beetle fumigation (T9056), 80 g/m^3 methyl bromide for 48 h at 21ºC atmospheric pressure (see Emery et al. 2008). Following the fumigation a surveillance trapping program was put in place to monitor the site for Khapra beetle adults and larvae. Traps were placed in the treated residence and in neighbouring properties, the shipment container receival facility, the waste recycling plant and the grounds of the waste transfer company (see Emery et al. 2003). A total of 1193 trap inspections were run over 18 months and no Khapra beetles were detected. Trapping will continue until the end of May 2009**.** Australia will act decisively and effectively to stop any possible risks that could damage our international trade and the grain industry markets.

In addition to the ongoing threat, this risk will be exacerbated if a phosphine resistant strain of Khapra beetle comes to this country. Pasek (1998) and Stibick (2007) have both reported that Khapra beetle shows signs of resistance to some common pesticides including phosphine (hydrogen phosphide, PH_3); improper application may be a contributing factor to the development of this resistance. In 2007, Ahmedani et al. reported a phosphine resistant strain of *T. granarium* which exhibited resistance levels of 2.54 - 3.98 fold. It is general knowledge that phosphine resistance is present in at least 11 species of stored-product insect pest in 45 countries (Chaudhry 2000). Phosphine resistance can be easily selected from field populations of most stored product insects and, unfortunately, this suggests that the frequency of resistant gene(s) might be quite high and that multiple genes may be involved in the selection for the strong resistance, explained Chaudhry (2000). Furthermore, once developed, phosphine resistant insects show little loss of fitness and the resistance is stable. Economically speaking, it would be a disaster if a strain of *T. granarium* resistant to Phosphine was introduced into Australia where the grain industry relies almost exclusively on phosphine to provide the international and national markets with insect and chemical residue free grain (Rees and Banks 2008, Collins and Daglish 2001).

In view of the serious threat of this insect to Australia, a better understanding of the spread biology of Emergency Plant Pests (EPPs), such as Khapra beetle, is crucial for the development of detection,

eradication, and containment or control strategies. At the Cooperative Research Centre for National Plant Biosecurity, data on species behaviour and dispersal will be used to test a prototype real-time simulation model that will be spatially linked to the risk site of interest and would allow more timely predictions of spread of EPPs spread in urban/peri-urban/rural landscapes in order to predict their possible establishment and spread over these areas. We intent on using a two dimensional grid of interacting automata with each automaton, modelling a sub-population at a given location. The automaton interacts, capturing the dynamics of an EPPs mobility. First of all, we will require appropriate generic modeling framework and simulation technology which could be translate into an invasive pest-specific simulation habitat. This could be applied and allowed us the exploit the fact that many invasive pests could have common dispersal mechanism in relation to distance (natural dispersal and short/long dispersal) that are able to spread over the landscape, host population density and quality, insect population movement, vectors, rain, wind (dependent on strength and direction). A simulation model would need to considerer risk sites points in the transport chain, proximity of these risks sites to suitable host environments, density and patterns of host environments and the biology of the organism. This project will develop methods for designing optimal surveillance strategies that accounts quantitatively for these factors mentioned above.

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Esterase isozymes in Indian strains of diamondback moth, *Plutella xylostella* L. (Plutellidae: Lepidoptera)

ABSTRACT

We studied esterase isozyme profiles of six insecticide resistant field populations of diamondback moth (DBM), *Plutella xylostella* L. collected from major cruciferous growing regions of Karnataka, northern parts of India and a susceptible strain using NATIVE – PAGE (Polyacrylamide gel electrophoresis). Considerable variation on esterase isozymes of different strains of DBM was detected. A total of 7, 9, 9, 11, 10, 8 and 9 bands of varying intensities were observed in the susceptible, Bangalore, Hassan, Shimoga, Belgaum, Ludhiana and Delhi populations, respectively. On the basis of relative mobility, two distinguishable banding groups were observed and were independent of each other. These results emphasized a need for a location specific management practices for effective management of the pest.

Key words: Esterases, insecticide, resistant, diamondback moth (DBM), *Plutella xylostella*

INTRODUCTION

Many insect species are subjected to intense selection pressure. As a consequence, wide spread resistance threatens the success of pest control programs (Field and Willamson, 1998). There are several ways by which insects become resistant to insecticides. Increased esterase activity or esterase based resistance is one of the mechanisms exhibited in many insect species. This form of resistance was first implicated in the 1960's by the demonstration that all the resistant strains showed an increased activity of esterase to hydrolyze the model substrate, 1- naphthyl acetate (Needham and Sawicki, 1971). It was subsequently shown through electrophoretically (Devonshire, 1989). Biochemical evidence for the role of these esterases in resistance was also supported by selection experiments. For example, spraying *Myzus persicae* with pyrethroid insecticides selected strongly for aphids carrying esterase based resistance to organophosphates and cabamates and vice versa. Among insect species, caboxylesyrases of the aphid, *M. persicae* (Devonshire, 1977), *Culex pipens* (Georghiou and Pasterur, 1980), house fly (Oppennoorth, 1965), leaf hoppers (Ozaki and Kassai, 1970) and tobacco cutworm (Bull and Whiten, 1972) have been studied extensively because of their involvement in resistance to organophosphate, carbamate and pyrethriod insecticides. Enhanced esterase activities were also found in a Malathion and phenthoate resistant population of *Plutella xylostella* L. (Nuppon *et al.*, 1987). To support this, earlier Sun *et al.*, (1978) demonstrated qualitative differences in esterase activity between susceptible and methomyl resistant diamondback moth (DBM) strains. Similarly, recent studies also indicated that amplification of esterase genes was responsible for insecticide resistance in *Culex* mosquito (Mouches *et al*, 1986) and in green peach aphid (Field *et al.*, 1988; Field and Williamson, 1998.). However, most of the literature on esterase isozyme profile of DBM is from outside India and studies on isozyme profiles of Indian populations of DBM is limited. In the present study, the esterase isozyme profile of DBM populations collected from major crucifers growing regions of Karnataka and northern parts of India was studied using PAGE technique. Understanding of nature of resistance in DBM strains is necessary for developing effective management strategies.

MATERIALS AND METHODS

Test Insects

The field strains of DBM were collected from major cruciferous growing regions of Karnataka (Bangalore, Hassan, Shimoga and Belgaum) and northern India (Ludhiana and Delhi). The susceptible strain was procured from Rallis Research Centre, Bangalore where it was been maintained in the laboratory without exposure to insecticides for more than 268 generations.

Rearing of test insects

The cultures were reared separately in the laboratory on mustard seedlings by adopting the mass-rearing technique developed by Liu and Sun (1984) with suitable modifications. These strains were reread at 25 ± 1^0 C, 70% RH, and a photoperiod of 14:10 hrs (L: D) conditions.

Chemicals

All the chemicals and reagents used in the experiment were of analytical or reagent grade. Fast blue RR salt (staining agent) and 1 napthyl acetate (substrate) were obtained from Sigma Aldrich Company, USA. TEMED, acrylamide and bis-acrylamide were purchased from Hi-media and Merck companies. The rest of the chemicals were obtained from other companies.

Preparation of enzyme extract

The enzyme extract was prepared by homogenizing 2-3 days-old fourth instar DBM larvae in 0.1M sodium phosphate buffer (pH 7.0) in a pre-chilled hand operated pestle and mortar (100mg larvae in 10ml of buffer). The homogenate was centrifuged at 7500 rpm for 20 min at 4^0C. Supernatant was used for electrophoretic analysis

Electrophoresis

Native PAGE (Polyacralymide gel electrophoresis) was performed using a Bio-Rad vertical slab system with 7.5 per cent resolving/lower gel and 4.5 percent stacking/spacer gel following

procedure developed by Davis (1964). Electrophoresis was carried out at 100V for 10 minutes. Then voltage was increased to 200V and a maximum current of 2.5mA, and was continued for approximately 3 hours until the marker dye/tracker dye reached the bottom of the gel. Gels were stained for esterases activity according to Hunter and Markert (1957). The staining was done for 15 minutes at 37^0C with 100ml of 100mM ice cold sodium phosphate buffer (pH 7.0) containing 40mg staining agent, fast blue RR salt and 20 mg substrate, 1-naphathyl acetate (dissolved in acetone).

Isozyme analysis

Esterase bands of the seven samples were scored as present or absent. Only clear bands with resolution were scored in each sample, except for very faint and ghost bands. The relative frequency or mobility/ migrating distance was also measured for the sample to study the qualitative differences in the esterases.

RESULTS AND DISCUSSION

Study of esterase isozymes of different strains of DBM larvae showed considerable variation in isozyme profiles (Plate 1). A total of 7, 9, 9, 11, 10, 8 and 9 bands of varying intensities were observed in the susceptible, Bangalore, Hassan, Shimoga, Belgaum, Ludhiana and Delhi populations, respectively. Similarly, Maa *et al.*, (1990) had detected 17 esterase isozymes in larval homogenate of DBM using PAGE. Among them, few esterase bands were more prominent.

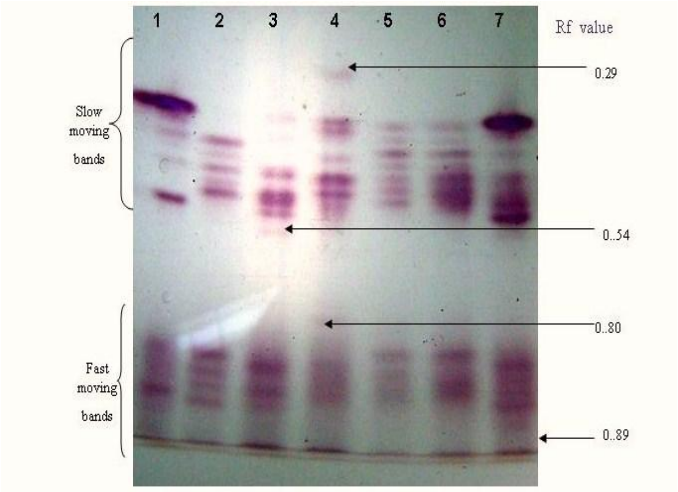

Plate 1: Esterase isozyme profiles in Indian strains of diamondback moth, *Plutella xylostella* L. (Lane1: Susceptible; Lane 2: Bangalore; Lane3: Hassan; Lane 4: Shimoga; Lane 5: Belgaum; Lane 6: Ludhiana; Lane 7: Delhi)

On the basis of relative frequency or mobility, esterase isozymes were classified into slow moving and fast moving bands with the relative mobility values ranging from 0.29 to 0.54 and 0.80 to 0.89, respectively. These banding groups were independent of each other. In the case of slow moving bands, majority of them were dark in most of the populations and very few isozyme bands were faint. The role of higher activity of slow moving esterase identified by PAGE in insecticide resistance in *P. xylostella* was earlier reported by Maa and Liao (2000). A fast moving band group was slightly faint in all the populations. These results imply a major resistance mechanism is likely to be associated different strains which had slow moving esterases.

Therefore, slow moving esterases are more important than the fast moving ones in view of resistance monitoring. The differences in isozyme profiles and relative frequency or mobility values clearly demonstrated the existence of qualitative differences in the esterases of different strains of DBM. This variation could be attributed to the differential selection pressure on the DBM in these locations and even the genetic variation that could exist in the populations which are widely separated. Similar results were obtained by Mohan and Gujar (2003) who observed variation in the esterase isozyme profiles of *P. xylostella* populations collected from three locations in India and attributed it to the differences in the use of insecticides in these locations. Similarly, significant intra-regional variation in isozymes of different populations has been reported in Taiwan (Maa *et al.*, 1990) and Pakistan (Rafiq, 2005). Similar results were also documented by Murai (1991) who also reported variation in esterase zymograms of four populations of DBM. The results of the present study clearly showed that populations of DBM differed in their esterase isozyme patterns and these results emphasized a need for a location specific management practices for effective management of the pest.

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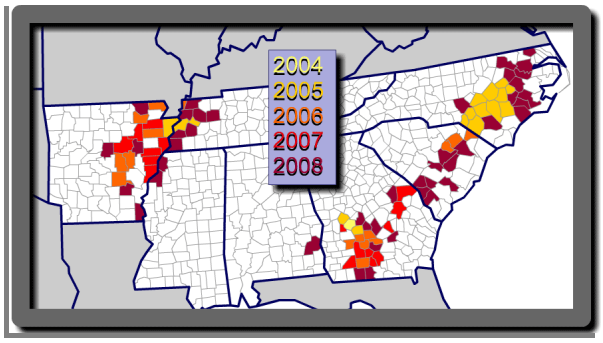
Glyphosate-Resistant Palmer Amaranth (*Amaranthus palmeri*) Spreads in the Southern United States (U.S.)

Glyphosate (*N*-phosphonomethyl glycine) is an exceptionally broad-spectrum herbicide that was first registered for use in 1974. Because of its lack of selectivity, glyphosate was originally used to control annual and perennial weeds in non-crop areas, in perennial crops such as vines, orchards, and plantations, for renovation of turf and timber, and as a pre-plant treatment in no-tillage systems. Applied only for such uses, no weeds were reported to have developed resistance to glyphosate for over twenty years. In 1996, soybean (*Glycine max*) cultivars transgenically modified for resistance to over-the-top treatment with glyphosate were commercialized. Subsequently, glyphosate was used in conjunction with transgenic, glyphosate-resistant soybean, canola (*Brassica napus*), cotton (*Gossypium hirsutum*), and corn (*Zea mays*) cultivars, and became the most widely-used herbicide in the world (Duke and Powles, 2008). In addition to its former uses, glyphosate has now been applied extensively and repeatedly in row-crops for control of annual weeds that have high rates of reproduction - thus greatly increasing the selection of weed populations for potential resistance. In the U.S., glyphosate is often used year after year on the same ground, even when crops are rotated, since the principal row-crop cultivars are all resistant to glyphosate. Since the introduction of transgenic glyphosate-resistant crops, fifteen weed species throughout the world have developed resistance to glyphosate

One of the most serious of the glyphosate-resistant weeds is Palmer amaranth because of its wide distribution through the southern and southwestern U.S., its rapid growth, its ability to compete with crops, and its very high reproductive potential (Klingaman and Oliver 1994; Rowland et al. 1999; Smith et al. 2000; Webster 2005; York et al. 2007). Glyphosate-resistant Palmer amaranth has emerged as a threat to economic weed control in cotton and soybean in the major row-crop growing areas of the southern U.S., particularly in the southeastern Coastal Plain and the north Delta region (Nichols et al. 2008). In this article, we update the distribution (Figure 1.) and the estimation of the area infested (Table 1.) with glyphosate-resistant Palmer amaranth populations, and we seek to alert the pest management community to the apparent rapid spread of this resistant weed.

For each county reported as having a glyphosate-resistant population, a field failure occurred, seed from the field were collected by state extension personnel, and F1 progeny were confirmed resistant in a greenhouse assay using multiple rates of glyphosate and compared to a susceptible local population as a reference (Figure 1.). In many cases, F2 progeny were tested as well. Last spring, we reported that 49 counties had at least one glyphosate resistant population (Nichols et al. 2008). At this time, using the same criteria, 93 counties have confirmed populations. Moreover, numerous other populations are pending confirmation, including populations in Florida and Louisiana that are in counties that are not contiguous with currently confirmed counties.

Figure 1: Counties with confirmed populations of glyphosate-resistant Palmer amaranth



Whereas the county data are documented, the area estimates are approximations (Table 1.). However, they are approximations made by experts who are directly dealing with the problem and supported in some states by surveys done by county extension agents. We do not purport that our estimates are precise, but they have been conscientiously made based on field observations in the affected areas. Moreover, since they have been made consistently by the same individuals using the same criterion, they are indicative of a trend. The categories 'present', 'moderate', and 'severe' (Table 1.) indicate that: 1) resistant populations have been observed; 2) weed management practices had to be changed and escapes were present; and 3) weed management was extremely difficult, many escapes occurred, and in some cases fields were abandoned, respectively.

Table 1: Estimated Hectares Infested with Glyphosate-Resistant Palmer Amaranth*

State	in Soybeans			in Cotton			Total**
	Present	Moderate	Severe	Present	Moderate	Severe	
Alabama		104			104		415
Arkansas	87,996	107,153	27,967	28,963	45,182	13,053	310,315
Georgia	23,155	3,473	5,789	42,048	5,557	9,262	129,668
Mississippi	11,121	14,609	7,305	5,484	7,082	3,541	49,142
North Carolina	8,472	8,472	8,472	15,733	15,733	15,733	72,614
South Carolina	35,000	4,500	6,000	15,000	8,000	3,700	72,200
Tennessee	13,693	6,225	830	10,374	8,298	2,074	41,494
Total							675,848

*Estimated by State Cooperative Extension Cotton, Soybean, and/or Weed Specialists (See Authors) with assistance from County Cooperative Extension Agents.
**Total Infested Hectares is Greater than the Total for Soybean and Cotton alone, because Infestations in Corn and Peanuts were also provided by Alabama and Georgia.

Expansion of area covered by the resistant populations may be occurring in several ways, by seed, pollen, spreading of crop residues, such as cotton gin trash, and the occurrence of new resistance events. Movement of seed may occur over short distances when plants shed their seed, within and between fields by transport of seed on equipment, or by long range transport on equipment going to remote locations, by field spreading of cotton gin trash, or by birds. Since Palmer amaranth is dioecious and wind-pollinated, movement of pollen has been the subject of on-going investigation and is considered a significant means of spread (Sosnoskie et al. 2007). Inheritance of resistance in the first identified population was described as dominant and conferred by a single-gene (Culpepper et al., 2006). The pattern of expansion to adjacent counties is consistent with aerial movement of the resistance gene by pollen; it is likely abetted by movement of seed. After the initial resistant population was found in Georgia, additional resistant populations shortly were described in Arkansas, North Carolina, and Tennessee in counties that were more than 500 kilometers distant from the Georgia field where the resistance was first reported (Norsworthy et al. 2008; Culpepper et al. 2008, Steckel et al. 2009). Although the evidence is indirect, such a pattern of incidence suggests that more than one resistance event may have occurred.

Anticipated Impacts

Palmer amaranth is a very robust annual that frequently grows to 2 or more meters in height. It is a C4 plant that grows very rapidly and is drought tolerant. Seed production is prolific. Palmer amaranth is highly competitive with cotton, soybean, and corn; significant yield losses are likely to occur if Palmer amaranth populations survive in the crop. Even before

being resistant to glyphosate, it was already considered one of the most difficult to control weeds in agronomic crops in the southern region (Webster, 2005).

Glyphosate, used with transgenic, glyphosate-resistant cultivars, is still the primary herbicide used for soybean and cotton weed control (http://usda.mannlib.cornell.edu/). In the affected areas, growers have been forced to use herbicides in addition to glyphosate in their weed management programs, thereby incurring input additional costs. Weed control in soybean still can be accomplished with use of protoporphyrinogen oxidase inhibiting herbicides, although excessive reliance solely on this mode of action will have an unfortunate but predictable result. In cotton, the seedling grows slower than do those of soybean and fewer modes of herbicide action are selective for the crop; thus weed control is more problematic in cotton in the affected areas. Overall weed management costs have increased for growers, and in certain of the most heavily infested counties in Georgia the use of primary tillage has increased, displacing conservation tillage hectarage which had expanded when glyphosate was highly effective. Given the rate of spread observed since the initial confirmation of resistance, it is probable that Palmer amaranth will be resistant to glyphosate throughout its range in the not too distant future. Such occurrences will likely result in crop yield and quality losses, increased herbicide costs, and the possibility of changing tillage and cropping systems.

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MONITORING THE RESISTANCE OF RED SPIDER MITE (*Oligonychus coffeae* Nietner) OF TEA TO COMMONLY USED ACARICIDES FROM THE DARJEELING FOOTHILLS AND PLAINS OF NORTH BENGAL, INDIA

ABSTRACT

Toxicity levels of five acaricides viz. ethion, dicofol, propargite, fenazaquin and fenpropathrin were determined in populations of the red spider mite (RSM), *Oligonychus coffeae*. Mite populations were obtained from tea plantations of Darjeeling foothills and their plains spreading over the Dooars (located between 26^0.16" to 27^0.0" N latitude and 88^0.4" to 89^0.53" E longitude) and Terai (25^o 57" to 26^0 36" N, Latitude and 89^0 54" to 88^0 47" longitude) regions of North Bengal, India. LC_{50} values were found to be high for ethion and dicofol (261.585, 625.689 and 309.437, 403.349 ppm); intermediate for propargite (46.246 and 97.100 ppm); and low for fenpropathrin and fenazaquin (2.785, 9.383 and 4.523, 6.765 ppm, respectively). It was further observed that red spider mite populations in the tea planting zone of Terai showed significantly less susceptibility to all five acaricides used, than the populations of the Dooars. Levels of susceptibility corresponded to the amounts of

acaricides used against the red spider mite in these two locations, possibly implying the response of mite population to the selection pressure exerted by the acaricides.

INTRODUCTION

Red spider mite, *Oligonychus coffeae* Nietner (Acarina: Tetranychidae) (RSM) is a major arthropod pest that attacks most tea cultivars of North-East India. Recently it has wrecked havoc in the Sub-Himalayan Terai and the Dooars regions located in the foothills of Darjeeling Himalaya and plains of North Bengal (Anonymous 1994; Mukhopadhyay and Roy 2009). In North East India, economic loss caused by the RSM may range from 50% to 75% of total crop yield (Subramanian 1995 and Gurusubramanian 2005). To check crop loss, several kinds of acaricides have been tried under chemical control strategy. The average annual consumption of insecticide and acaricide in the Dooars and Terai is 7.05 and 3.49 kg / lt / hectare respectively (Barbora and BIswas 1996). Among the synthetic pesticides, ethion and dicofol are recommended / preferred as acaricides. Recent information available indicates that the pesticides (insecticide and acaricide) consumption has increased in Terai as well as in the Dooars tea plantations, 85% of the acaricides being used between the month of January and June (Sannigrahi and Talukder 2003). In spite of the use of all types of synthetic pesticides, such as organochlorides, phosphates and synthetic pyrethroids, the red spider mite remains a serious problem of tea and a difficult pest to control. Many workers have reported that management of *O. coffeae* has become a challenge apparently due to higher tolerance (Das 1959; Banerjee 1968; Sahoo et al. 2003; Roy et al. 2008). The extensive use of acaricides such as, dicofol and ethion in tea for more than a decade has probably led to pesticide resistance in red spider mite (Sahoo et al. 2003). So, in other words the mite appears to have developed a greater tolerance to the above acaricide. Pesticide resistance in mite pest is known to develop very quickly because of its numerous generations every year and exposure to high frequency of pesticide spray applications (Cranham and Helle, 1985).

There are increasing restrictions for trading tea contaminated with pesticides in international markets and as well as for domestic markets due to health concern. These reasons, combined with the evolution of the red spider mite resistance to the chemicals and their costs, make it imperative to plan alternative pest management programs. Such programs should include regular assessment of the resistance of red spider mite populations to different acaricides. The principal objective of this work was to evaluate the resistance levels of the red spider mite populations from different segments of Sub-Himalayan tea growing area of North Bengal, (India) to the four most commonly used acaricides , by determining LC_{50} and LC_{95} values, their confidence limits, and dose-mortality slope values.

MATERIAL AND METHODS

This study was conducted from January 2006 to March 2008, in the main tea cultivating areas in Darjeeling foothills and their plains of North Bengal, India. The locations were the Dooars (located between 26^0.16" to 27^0.0" N latitude and 88^0.4" to 89^0.53" E longitude) in the district of Jalpaiguri and Terai (25^o 57" to 26^0 36" N, Latitude and 89^0 54" to 88^0 47" longitude), in the district of Darjeeling. These landscapes having unique phytogeographic climate and soil, harbor tea plantations often dissected by river courses, interspersed patches of forests, low hills, highways and agricultural lands.

Rearing of red spider mite

A culture of red spider mite was maintained at 25 ± 2^oC and 70-80% RH on a susceptible tea clone, TV1 by following detached leaf culture method of Helle and Sabelis (1985) with slight modifications at the Entomological Research Laboratory, Department of Zoology, North Bengal University, West Bengal, India.

Acaricides

The acaricides ethion (Ethion 50 EC: aliphatic organothiophosphate insecticides), dicofol (Colonel-S 18.5 EC: diphenyl aliphatics organochlorine), propargite (Omite 57 EC: organosulfurs), fenazaquin (Magister 10EC: quinazolines) and fenpropathrin (Meothrin 30 EC: 4^{th} generation pyrethroid ester) were obtained from respective manufacturers and used for the present study.

Estimation of relative toxicity

The log-dose-probit-mortality (LDPM) assays of aforesaid acaricides were used with concentrations of each test acaricides by diluting them in distilled water. Toxicity assays were conducted as per the standard 'Leaf Dipped Method" recommended by FAO (Method No. 10a, FAO, 1980). Mature tea leaves of TV 1 clones were collected from the experimental garden plots and brought to the laboratory. The leaves were washed thoroughly with distilled water and air-dried. Five tea leaves for each treatment were dipped for up to five seconds in the acaricides solutions to ensure complete wetting and then the treated tea leaves were kept under ceiling fans for 15 minutes to evaporate the emulsion before placing these dorsoventrally in the cultural Petri dishes. Cotton wool strips, 1 cm in width, soaked in tap water were laid around the margin of each treated leaf with half over the leaf and half over the cotton wool bed. A small piece of damp cotton wool was placed around the petiole of each leaf. A population (collected from the

Dooars and Terai regions) of at least 10 adult mites per leaf was released under the observation of a binocular microscope. It was necessary to ensure that there are no gaps between the leaves and cotton wool strips. Each treatment was replicated five times.

The adult red spider mites were exposed initially to concentrations of a wide range of acaricides, and on the basis of mortality a series of concentrations of a narrow range were selected to which the test mites were again exposed. The same procedure was repeated until mortality data in the range of 10-90% was recorded. Mortality was recorded 24 hours after treatment. The moribund mites were counted as dead. The data thus obtained were subjected to probit analysis for calculating regression equation and lethal concentration by Finney's method of probit analysis (Finney 1971).

RESULTS AND DISCUSSION

For most red spider mite populations, fenpropathrin and fenazaquin had the highest toxicity, while propargite was intermediate and ethion and dicofol were the least toxic (Table 1).

Table: 1. Toxicity of acaricides against red spider mite, *Oligonychus coffeae* in two locations of Darjeeling foothills and their plains, India

Acaricides	LC_{50} (ppm)	95% CR	LC_{95} (ppm)	Slope	SE
Ethion 50 EC	261.585	243.160 - 281.407	3600.468	1.454	0.003
Dicofol 18.5 EC	309.437	292.241 - 327.644	2033.922	2.024	0.002
Propargite 57 EC	46.246	44.301 - 48.573	263.054	2.193	0.002
Fenpropathrin 30 EC	2.785	2.582 - 3.004	32.860	1.544	0.003
Fenazaquin 10 EC	4.523	4.224 - 4.863	24.717	1.560	0.002
Ethion 50 EC	625.689	537.242 - 682.935	5715.722	1.723	0.004
Dicofol 18.5 EC	403.349	373.624 - 435.440	2585.635	2.051	0.004
Propargite 57 EC	97.100	93.228 - 101.133	268.285	3.074	0.002
Fenpropathrin 30 EC	9.383	8.935 - 9.853	30.373	3.24	0.003
Fenazaquin 10 EC	6.765	6.208 - 7.372	46.114	1.986	0.005

LC50: The acaricides concentration lethal to 50% of the mites; CR 95%: The confidence range at 95%; LC_{95}: The acaricides concentration lethal to 95% of the mites, Slope: the slope of each regression line and SE: The standard error

Among different chemicals tested on the Terai and the Dooars populations, ethion and dicofol showed LC_{50} values > 300 ppm and propargite showed intermediate LC_{50} value (i.e. 46.246 and 97.110 ppm) and other two acaricides viz. fenazaquin and fenpropathrin showed LC_{50} values < 10 ppm.

Based on the overlapping confidence ranges, it was determined that sensitivity to all acaricides was significantly different among the two populations evaluated. The Dooars mites were more sensitive and susceptible than those from Terai. The variation in the resistance level to ethion and dicofol was caused by the excessive use of this pesticide for more than 50 years (Barbora and Biswas 1996; Sannigrahi and Talukdar 2003), as these were the acaricides most recommended by monitoring agencies.

The present study showed that the LC_{50} value of RSM against ethion and dicofol were 261.585, 625.689 and 309.437, 403.349 ppm respectively in the Dooars and Terai region (Table 1). The recommended field dose for these acaricides in North Bengal was 2500 ppm. This means that a 2500 ppm concentration has the potential to kill off red spider mites in the fields to achieve desirable control. Although there are no generalized relationship between laboratory LCs and field application rates, researchers mostly follow the method of MED 95 for determination of field dosage. According to this method the field application rates of pesticides should at least be 20 fold or more of the LC_{50} value (determined through bioassay methods) to achieve satisfactory control of the pest in agriculture (Misra 1989). Following this simple logic, the expected effective dosages of these acaricides were worked out. The comparison of expected effective dosages of ethion and dicofol are to be 5231.7, 12513.8 ppm for ethion and 6188.4, 8066.9 ppm for dicofol in the Dooars and terai RSM populations respectively. Comparison of these doses with the present recommended dosage (2500 ppm) revealed a pronounced shift in the level of susceptibility of red spider mite against these acaricides in the North Bengal tea plantation. In case of ethion it was observed that 2.90-5.00 times more and for dicofol 2.46- 3.22 times more of the recommended dose of these acaricides would be required to achieve desirable control of the pest.

Considering the above fact and the result of the present study it may be surmised that the red spider mite can not be controlled with recommended field rates (doses) of ethion and dicofol. As such the LC values may be used as a good criterion to assess and compare the red spider mite resistance to ethion and dicofol in the tea fields of the Dooars and Terai region of North Bengal.

For combating and delaying the problem of resistance, therefore, either the authority must reassess the dose or the planters must change over their strategies in the light of above findings.

Based on the LC_{95} values of present study, fenazaquin and fenpropathrin were more toxic among the commonly available acaricides against red spider mite. The recommended field rates for these acaricides in North Bengal were 2500 ppm (for fenazaquin) and 500 ppm (for fenpropathrin). The expected calculated effective field dosages of these acaricides were worked out by using the laboratory data of the present study and the results showed that they were effective even at a lower dose than the recommended dose. These acaricides were highly effective against the target pest because these were newly introduced in tea. It might be mainly due to their unique modes of action and trans-

laminar movement and lack of cross-resistance with other commercially used pesticides (Ware and Whitacre 2004).

So, it is recommended that ethion and dicofol should no longer be used to control the red spider mite in any of the mentioned areas. The susceptibility of the two populations of red spider mite to propargite showed intermediate state. The field recommended dose of propargite was 2500 ppm but expected effective field dose which was calculated from the above mentioned hypothesis showed that it was at marginal level. Therefore, it is suggested that if propargite is used, precaution should be taken to avoid repetition of the resistance problem as encountered in case of ethion and dicofol applications.

Thus, for combating and delaying the problem of resistance either the authority must reassess the dose or the planters must change over their strategies in the light of above findings.

Changes in pest management tactics are prompted by environmental and human safety concerns, development of insect pest susceptibility changes, and increases in pesticide cost and availability. Thus, before spraying any chemicals, the tea planters must consider i) the impact of pesticides on non target organisms, human health, wild life habitat and environment and ii) adopt IPM strategies to reduce the pesticide load and produce residue free tea. Potential cultural practices for conserving and enhancing the natural enemies need to be integrated with our current crop management strategies for developing sustainable tea crop protection. Therefore, the following integrated resistant management practices must be followed for combating and delaying the problem of resistance, so that it does not assume unmanageable proportions:

- Unshaded conditions are favorable for red spider mite infestations so adequate shade status needs be maintained. In the Dooars and Terai a moderate shade status of 60% in tea plantation is preferable, since under such light condition least pests attack and best crop yield take place.
- Alternate hosts must be eliminated or kept at bay (viz. *Borreria hispida*, *Scoparia dulcis*, *Melochia corchorifolia***,** *Fussiala suffruticosa*, *Melastoma malabathricum*, *Polyantha* sp., *Scoparia dulcis*, coffee, jute, cotton caster, mulberry and many Jungle plants).
- Mites generally persist on old leaves during the cold weather which are responsible for attacks in the following growing season. Therefore, the type of pruning which removes more old leaves and side branches from bushes during the cold weather would likely reduce the possibility of attack. Unpruned sections must be monitored regularly during December-February and proper care should be taken to avoid the population build up.
- Red spiders prefer dusted leaf surface for egg laying. Moreover, larvae and nymphs are also protected from the attack of predators under dust cover. Protecting the roadside bushes from dust by growing hedges, such as *Phlogacanthus thrysiflorus* (titaphool) can be a good choice.
- To prevent migration of red spider mites, pluckers should be prevented from entering into un-infested areas from infested areas, and cattle trespassing inside the tea sections should be stopped.
- Underperformance of spraying equipments should be avoided.
- Spraying of ethion and dicofol in severely infested sections should be restricted as these have low killing efficacy.
- Selection and usage of chemicals, assurance of the quality, required spraying fluid, and trained man power for overall good coverage are obligatory for better management of RSM.
- Under dense plantation of tea bushes, care must be taken for good and uniform coverage of chemicals.
- Incompatible chemicals must be avoided in tank-mix formulations in severely affected sections.
- Sub- and supra- lethal doses must be avoided to minimize the chances of susceptibility change in red spider mite populations.
- Spraying during hot sunny days should be avoided as this degrades the chemical activity, and cause phytotoxicity. Hence early morning and late afternoon spraying is preferable.
- With prior knowledge of red spider mite infestation pattern, the infested bushes to be marked in the early stage and spot application of spray instead of blanket application is recommended to check the pests as well as to reduce the chemical load.
- Conservation and preservation of the natural enemies present in the natural tea ecosystem for their natural regulation is

encouraged by minimizing the load of chemicals.

The above-mentioned recommendations, if adopted, for management of Red spider mite population of the Dooars and Terai tea plantations are expected to provide a solution to the growing menace, that is often causing heavy losses of tea production. The suggestion, if implemented in letters and spirit, would possible reduced the load of chemical acaricides on the crops and also the environment, all the same usher in a region wide practice of integrated management of the pest, the necessity of the hour.

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Research in Resistance Management

Preliminary Effects of Insecticidal Control of Asian Citrus Psyllid and Combinations of Nutrients and Systemic Acquired Resistance Elicitors on Incidence of Greening Disease in Citrus

ABSTRACT

A combination of systemic acquire resistance elicitors, with macro- and micro-nutrients have been used, with apparent success, by at least one Florida citrus grower to mitigate expression of symptoms related to Huanglongbing (HLB) or greening disease. A large scale field experiment to test the effects of this combination with and without insecticidal control of the vector, *Diaphorina citri* (Homoptera: Psyllidae) was initiated in a young block of citrus trees in southwest Florida. Visual symptoms of HLB were initially reduced, although differences compared to untreated trees became negligible four months after the first Special Foliar Treatment (SFT) in early summer application, and two months after the second application. Insecticide applications suppressed populations of *D. citri* adults and virtually no movement of the psyllid among plots inside the orange block was being observed. However, expression of HLB symptoms in the plants was not affected by insecticides.

INTRODUCTION

Huanglongbing (HLB) or citrus greening disease is considered by many as the most important disease of sweet orange, mandarin and grapefruits worldwide (Schwarz and Bove, 1975). HLB is found

in over 40 citrus-producing countries in Asia, Africa, Oceania, and the Americas. The disease is associated with the presence of several genera of the bacterium *Candidatus* Liberibacter, of which only C. L. asiaticus is present in Florida where it is vectored by *Diaphorina citri* kuwayama (Bove, 2006).

Candidatus Liberibacter spp. are considered to be Gram-negative bacteria and restricted to the phloem of the plant (Garnier et al., 1984). The bacteria appears to reduce the distribution of nutrients through the phloem, and increase concentrations of starches in the leaves (Takushi et al., 2007). Foliar symptoms appear as micronutrient deficiencies and can sometimes be distinguished by a more characteristic blotchy mottling of older leaves that abscise early leading to dieback. Fruits are often misshapen, undersized, and green or remain green at the stylar end, green fruits, have aborted seeds and drop from the tree. Analysis of symptomatic leaves shows a higher potassium content and lower calcium, magnesium, and zinc (Da Graca, 1991). These low nutrient contents might be attributed to an obstruction of phloem function by the bacteria that would explain the resemblance between HLB and nutrient deficiency symptoms.

Systemic Acquired Resistance (SAR) is a plant response to the effect of pathogenic organisms or other stress factors induced by elicitors such as salicylic acid or jasmonic acid. Salicylic acid induces activation of pathogenesis-related genes (PR), which cascade into the production defensive metabolites or in the development of a hypersensitive reaction (Gaffney et al., 1993; Ryals et al., 1996). Salicylic acid is also known to induce antioxidants that could serve to attenuate pathogenesis directly (Huang et al., 2008).

At least one Florida citrus grower has been experimenting with nutrient/salicylic acid combinations to ameliorate symptoms of HLB with apparent success (Giles, 2009). Our objective was to evaluate separate effects of his formula and of insecticidal control of the vector on incidence and severity of the disease. The present report is intended to provide preliminary results of what will likely require more time and experiments under varying conditions to fully evaluate.

MATERIALS AND METHODS

A 12-acre parcel of young Valencia oranges located at 26° 29' N., 81° 23' W., in a large planting of oranges in Collier Co. in southwest Florida was selected. In this block HLB was first identified in 2005. The selected parcel showed a homogenous distribution of HLB symptoms and was divided into 16 plots organized in 4 replicates with 4 treatments set out in a randomized complete block design. The 4 treatments consisted of two factors, the SAR nutrient combination applied as a foliar spray, and insecticidal control, each at two levels (with and without).

Foliar treatment

A special foliar treatment (SFT) consisting of a combination of SAR inducer products including: Serenade Max ®WP (*Bacillus subtilis*) and Saver™ (salicylic acid source), macronutrients, and several micronutrients including zinc, manganese, molybdate, etc. (Giles, 2009). Treatments were sprayed using an airblast sprayer at 210 gallons per acre. Applications to the designed plots were conducted in 2008 on 19 March, 3 June, 8 September, and 16 October.

Insecticides

Foliar insecticide applications were made to the designated plots on 2 May (Danitol 2.4 EC (fenpropathrin) at 16 fl oz/acre [Valent USA, Walnut Creek, CA], and on 7 August Delegate WG (spinetoram) at 4 oz/acre [DOW AgroSciences, Indianapolis, IN]. In addition, two insecticides were used before the beginning of the experiments, Temik (aldicarb) [Bayer CropScience, Research Triangle Park, NC] applied to the soil at 20 lb/acre, and a dormant season spray of Danitol 2.4 EC (fenpropathrin) at 16 fl oz/acre during December 2007.

Sampling

Ten trees were randomly selected in the center row of each plot. To evaluate the population of adults of *D. citri* in the field, a white laminated field sheet divided into a 4/4 grid was held approximately one foot under a leafy branch. The branch was tapped three times and the number of *D. citri* adults that fell from the branch onto the sheet was counted. (Arevalo et al. 2009). This tapping process was repeated twice in each tree. This sampling system for psyllid adults has demonstrated to be efficient and reliable to estimate *D. citri* fluctuations in the field (Hall et al., 2007; Qureshi and Stansly, 2007).

Symptoms of HLB were evaluated on the same 10 trees used for the tap sample, and trees were designated as positive or negative based on the presence of leaves displaying blotchy mottle, dieback, shock bloom or other symptoms as mentioned above. The presence of one of more of these symptoms was enough to qualify a plant a positive.

RESULTS

Insecticide treatments significantly reduced the number of adult *D. citri* in the treated plots when compared with the untreated plots. Despite high populations of the psyllid in the treated plots, populations were very slow to re-infest treated plots even after several months following the insecticide applications (Fig. 3). Insecticides did not have an effect on the percentage of trees showing HLB symptoms (Fig 2).

Fig 1: Mean number of *D. citri* adults per tree (two tap samples) in plots treated or untreated with insecticides. Vertical lines represent the dates in which the insecticide treatments were sprayed

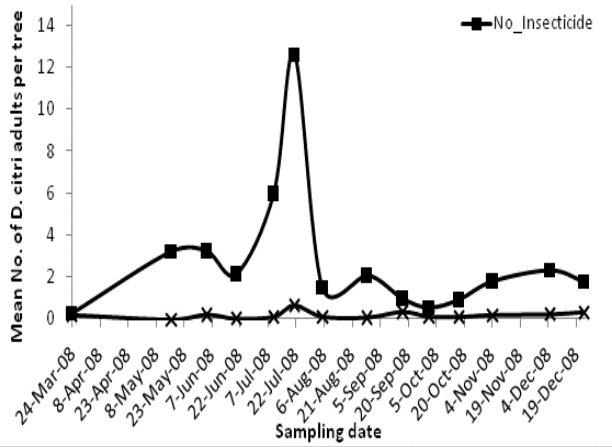
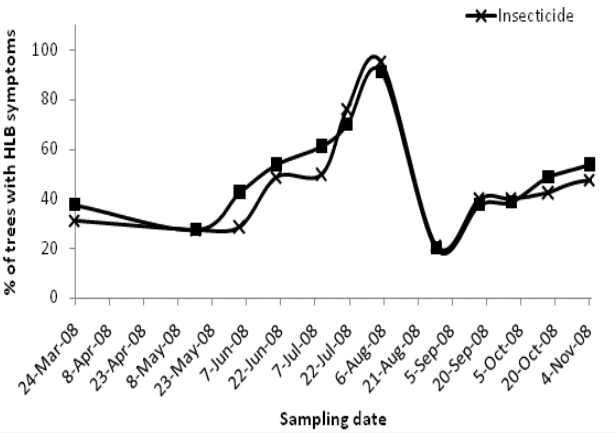


Fig 2. Effect of insecticide versus No insecticide applications on the percentage of trees presenting HLB symptoms. Vertical lines represent the dates for the Insecticide applications



A significant positive response was observed after the first (19 March, 2008) and second (3 June, 2008) applications of the SFT. Differences were observed from early May 2008 to late July 2008. Subsequently, the percentage of trees showing HLB symptoms did not differ between plots with STF and plots without the STF treatments (Fig. 3). Special foliar treatment did not have any effect on the mean number of adult psyllids observed (Fig. 4).

Fig 3. Percentage of trees observed with greening symptoms in plots with the special foliar treatment (SFT) versus the plots without SFT (No-SFT). Vertical lines represent the dates for the SFT application

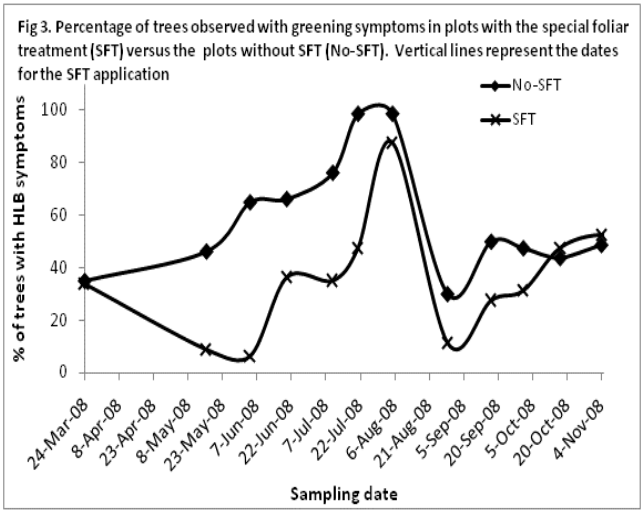
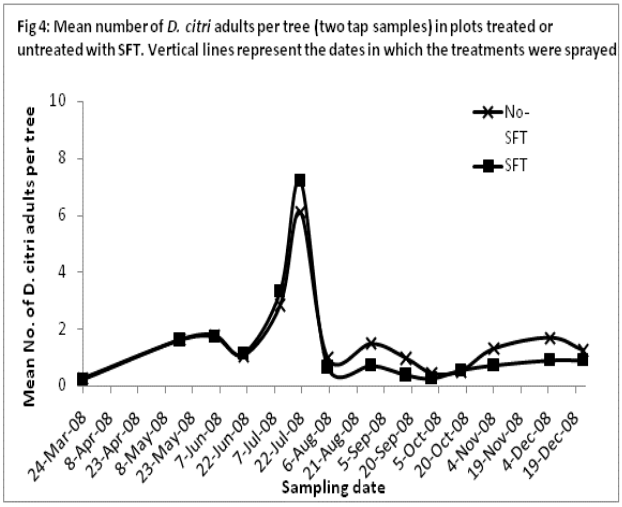


Fig 4: Mean number of *D. citri* adults per tree (two tap samples) in plots treated or untreated with SFT. Vertical lines represent the dates in which the treatments were sprayed



DISCUSSION

Plots that had insecticide applications presented a very slow re-infestation rate despite being adjacent to non-treated plots with high populations. Thus, the psyllid did not appear to be very vagile during the study period. Further studies of psyllid movement and dispersal are ongoing.

Results observed during the first year of the experiment indicated that the use of the SFT have a short effect on the presence or absence of HLB symptoms in the plants. This phenomenon could be explained by the temporary nature of the increase in gene expression of defense-related genes, as occurred in cucumber when *Trichoderma asperellum* is used to induce resistance these plants (Shoresh et al., 2005). Another explanation might include the high cost in fitness to the plant when producing SAR compounds (Cipollini et al., 2003). Hopefully, more light will be shed on these issues as this and other studies progress.

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Occurrence of resistance to the fungicide boscalid in isolates of Alternaria alternata pathogenic on pistachio

ABSTRACT

One hundred and twenty isolates of *Alternaria alternata* isolated during 2005 to 2007 from pistachio orchards with a history of Pristine® (pyraclostrobin + boscalid) applications and displaying high levels of resistance to boscalid fungicide (mean EC_{50} values > 100 µg / ml) were identified following mycelial growth tests. A cross-resistance study also revealed that the same isolates were resistant to carboxin, a known inhibitor of succinate dehydrogenase (SDH). To determine the genetic basis of boscalid resistance in *A. alternata* the entire iron sulfur gene (*AaSDHB*) was isolated from a fungicide-sensitive isolate. Comparison of AaSDHB full or partial sequences from sensitive and resistant isolates revealed that a highly conserved histidine residue (His) (codon CAC in sensitive isolates) was converted to either tyrosine (codon TAC, 53 mutants) or arginine (codon CGC, 27 mutants) at position 277. In forty other resistant isolates there was no mutation in the AaSDHB sequence, suggesting that resistance could be controlled by mutations at other loci. PCR-based assays were developed for the rapid identification of the mutants carrying the identified SDHB-mutations.

Key words: ***Alternaria alternata***, succinate dehydrogenase, carboxamides, mechanisms of resistance

INTRODUCTION

Alternaria late blight of pistachio caused by *Alternaria* spp. in the *alternata*, *tenuissima*, and *arborescens* species-groups is one of the most common fungal diseases of pistachio in California affecting both foliage and fruit (Pryor & Michailides, 2002). Control of the disease is a difficult task and mainly relies on multiple fungicide sprays. However, efficacy of fungicides is continuously challenged by the development of fungal resistant populations to the fungicides used against them. Boscalid is a new carboxamide fungicide, introduced in pistachio orchards in 2003 in a mixture with the quinone outside inhibitor (QoI) pyraclostrobin in the product Pristine®, to control the widespread resistance to QoI among populations of *Alternaria* spp. of California pistachio (Yong et al., 2007). Pristine® has been very effective in controlling Alternaria late blight, but reductions in its efficacy have been observed in some pistachio fields only two years after its registration and commercial use.

Carboxamide fungicides are single-site inhibitors and their target is the succinate ubiquinone reductase or succinate dehydrogenase (SDH) complex of the mitochondrial electron transport chain (Kuhn, 1984). This enzyme is composed of four subunits SDH A, B, C and D. Carboxamides inhibit the electron transfer by blocking the ubiquinone-binding sites, which are formed by amino acid residues from SDH B, C and D proteins. Point mutations in conserved regions of the genes *SDH B*, *C* and *D* have been shown to confer resistance to boscalid analog fungicides in some fungi (Broomfield and Hargreaves, 1992; Matsson et al., 1998; Matsson et al., 2001; Skinner et al., 1998).

In this study, we evaluated the sensitivity to boscalid of *A. alternata* isolates collected from 2005 to 2007 and investigated the cross resistance patterns in these isolates with carboxin, another member of the carboxamide group of fungicides. We also determined the molecular mechanism of resistance to boscalid by isolating the iron sulfur gene *SDH B* and studying the polymorphism of its sequence between *Alternaria alternata* boscalid-resistant and sensitive isolates.

MATERIALS AND METHODS

Strains and media

All the *Alternaria alternata* isolates used in this study were isolated from pistachio leaves, showing putative Alternaria infection (necrotic lesions). The collections were from the pistachio growing seasons from 2005 to 2007 from different pistachio orchards where boscalid had been used in mixture with pyraclostrobin (Pristine®) for two or three consecutive years in two or three spray applications per season. Conidial isolates were obtained according to the procedure described by Pryor and Michailides (2002). Single spore isolates were maintained on PDA at 4°C until use.

Fungicides used in the study were the pure (a.i. 98.4%) technical grade of boscalid (BASF Corporation, Research Triangle Park, NC) and the commercial formulation of carboxin (Vitavax 34 FF, Chemtura Corp, Middlebury, CT).

Technical grade of boscalid was dissolved in acetone to provide stock solutions containing 10 and 50 mg a.i. / ml. Autoclaved PDA was cooled to 50°C and appropriate volumes of the fungicide stock solutions were added into the liquid medium to obtain boscalid solutions. Mycelial plugs (5 mm-diameter) of each isolate were removed from the colony margins of actively growing 72-h-old colonies on PDA and placed upside down on the center of 30 Petri dishes containing the boscalid-amended media at the final concentrations of 0, 0.001, 0.005, 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 50, 100, 250 and 500 µg / ml. The concentration of acetone in all PDA media never exceeded 1 ml / l. Cultures were incubated at 24°C in the dark for 7 days. Radial growth of each isolate was measured (minus the diameter of inoculation plug) after 7 days by calculating the mean of two perpendicular colony diameters. The EC_{50} value (effective concentration that reduces the mycelial growth by 50%) of each isolate was calculated by regressing the relative inhibition of growth against the $\log_{10}$ fungicide concentration.

Determination of sensitivity to carboxin was carried out on *A. alternata* isolates which were either identified as boscalid–resistant or –sensitive. Measurements of sensitivity, in terms of EC_{50} values, were based in inhibition of mycelial growth. For this purpose autoclaved PDA was amended with 0, 0.1, 0.5, 1, 5, 10, 50, 100, 200, 300 and 500 µg / ml a.i. carboxin by adding appropriate volumes of aqueous fungicide stock solutions into the medium. The sensitivity determination procedure was similar to that previously described for boscalid.

DNA manipulations

Genomic DNA was extracted from mycelium of *A. alternata* using the FastDNA® kit (Qbiogene, Carlsbad, CA, USA) according to the manufacturer's instructions. A specific primer pair was designed by aligning conserved sequences spanning the cysteine-rich clusters associated with the S1 and S3 iron-sulfur redox centers of succinate dehydrogenase gene of *Alternaria brassicicola* (http://genome.wustl.edu/pub/organism/Fungi/Alternaria_brassicicola/assembly/Alternaria_brassicicola-1.0/) and *Stagonospora nodorum* (syn. *Phaeosphaeria nodorum*) (accession number SNOG_03351).

This primer set was used to amplify a putative *SDH B* gene fragment from *A. alternata* genomic DNA from the boscalid-sensitive strain

AaY16. PCR reactions were performed in a 50-µl volume containing 50 ng of DNA, 50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 0.2 mM of each dNTP (Promega, Madison, WI), 0.2 µM of each primer, and 1 unit of Pfu DNA polymerase (Stratagene, La Jolla, CA). PCR was carried out in a Mastercycler® (Eppendorf®, Hamburg) with an initial pre-heat for 3 min at 95°C, followed by 40 cycles of denaturation at 94°C for 40 s, annealing at 51°C for 50 s, extension at 72°C for 1 min, and terminated with a final extension at 72°C for 10 min. PCR products were separated by electrophoresis in 1.2% agarose gel in Tris-acetate (TAE) buffer. The PCR products from each isolate were purified from gel using the Geneclean® Kit (MP Biomedicals, LLC, Solon, Ohio), cloned into a pGEM-T Easy vector (Promega, Madison, WI) and subsequently sequenced. The 5' and 3' regions of *A. alternata Sdh B* gene (*AaSdhB*) were obtained by PCR amplification using two sets of primers designed from both the obtained fragment and conserved sequences of corresponding regions in *A. brassicicola* homologous gene. PCR products were cloned and sequenced as described above.

Allele-specific PCR and PCR-RFLP assays

The polymorphism analysis of the *AaSdhB* gene sequence has revealed that C to T mutation occurred in some *A. alternata* boscalid resistant isolates. A relevant primer pair was designed to specifically prime the *AaSdhB* gene of resistant isolates having this mutation. The reverse primer was designed to match the putative point mutation T996 at the 3'-end of the primer. The primer pair was expected to generate a 250-bp PCR product from resistant isolates only. PCR amplifications were performed in a 50-µl volume containing 2-µl with genomic DNA isolated from each strain as described above, 0.2 µM of each primer, 0.2 mM of each dNTP, 2.5 mM $MgCl_2$, 1X Promega (Madison, WI, USA) Taq polymerase buffer and 2U of Promega Taq polymerase. The PCR amplifications were performed using the following parameters: an initial preheat for 3 min at 95ºC, followed by 40 cycles of denaturation at 94ºC for 40 s, annealing at 65.5ºC for 40 s, extension at 72ºC for 1 min, and a final extension at 72ºC for 10 min. The amplicons of each isolate from AS-PCR assay were analyzed in 1.5% agarose gels in 1x Tris-acetate (TAE) buffer.

In other boscalid-resistant mutants, an A-to-G transition occurred at nucleotide 977 in the *AaSdhB* sequence, thus creating an *Aci*I restriction enzyme site. A relevant primer pair was designed to amplify mutation-containing fragments that were subsequently digested with the enzyme *Aci*I (New England BioLabs Inc, Beverly, MA) according to the manufacturer's instructions. Digestion products were then separated on a 2% agarose gel in 1x TAE buffer.

RESULTS

Phenotypic characterization of *A. alternata* isolates

Sensitivity to boscalid and carboxin was determined by measuring the radial growth on agar media amended with several concentrations of each fungicide. The EC_{50} values for boscalid and carboxin were calculated for individual isolates and values for representative isolates are reported in Table 1. The sampled *A. alternata* isolates were divided into two populations based on their response in the presence of boscalid. The majority of the tested isolates appeared resistant to boscalid (mean EC_{50} > 100 µg/ml). Only few isolates were found sensitive (mean EC_{50} < 1 µg/ml). Cross resistance study showed that the same boscalid-resistant isolates were also cross resistant to carboxin, (mean EC_{50}>50 µg/ml) (Table. 1). It could be noted that the proportion of resistant isolates in 2007 was higher than those observed in the previous years.

Molecular characterization of the *A. alternata* boscalid-resistant isolates

To determine the molecular basis of boscalid resistance in *A. alternata* isolates, we isolated the *AaSDHB* gene encoding the iron sulfur subunit of succinate dehydrogenase. Polymorphism analysis of the deduced amino acid sequence from *A. alternata* boscalid-sensitive and -resistant strains showed that, in some of the boscalid-resistant mutants, a conserved histidine residue (CAC) at position 277 was replaced by either tyrosine (TAC, Y) or arginine (CGC, R). However some other resistant isolates had no mutation in AaSDHB (Table 1).

Table 1: Sensitivity of *Alternaria alternata* to boscalid and carboxin and mutations in AaSDHB sequence

Isolate Code	Origin (Orchard location)	Year of isolation	EC_{50} boscalid (µg/mL)	EC_{50} carboxin (µg/mL)	Mutation in AaSDH B
AaY16	Kern Co, CA	2005	0.28	13.21	none
Aa4	Kern Co, CA	2005	0.26	11.27	none
Aa127	Kern Co, CA	2005	0.56	18.49	none
Aa20	Kern Co, CA	2005	>100	77.37	H277Y
Aa29	Kern Co, CA	2005	>100	54.41	H277Y
Aa65	Kern Co, CA	2005	>100	51.23	H277Y
Aa111	Kern Co, CA	2005	>100	43.58	H277Y
Aa122	Kern Co, CA	2005	>100	65.79	none
AaY1	Fresno Co, CA	2005	>100	38.06	none
Aa2	Kern Co, CA	2006	>100	55.67	none
Aa26	Kern Co, CA	2006	>100	46.64	none
Aa13	Kern Co, CA	2006	>100	46.41	none
Aa40	Kern Co, CA	2006	>100	64.80	none
Aa10	Kern Co, CA	2006	>100	98.53	none
Aa54	Kern Co, CA	2006	>100	68.71	none
Aa19	Kern Co, CA	2006	>100	80.92	H277Y
Aa94	Kern Co, CA	2007	>100	62.17	H277Y
Aa105	Kern Co, CA	2007	>100	63.67	H277Y
Aa37	Kern Co, CA	2006	>100	45.33	none
Aa45	Kern Co, CA	2006	>100	53.51	H277R
Aa33	Kern Co, CA	2006	>100	71.31	H277R
Aa58	Kern Co, CA	2006	>100	80.29	H277R
Aa48o	Tulare Co, CA	2007	>100	40.44	H277Y
Aa26o	Tulare Co, CA	2007	>100	41.47	H277Y
Aa19o	Tulare Co, CA	2007	>100	52.02	H277Y

The genotype (C to T mutation) of the boscalid-resistant isolates was confirmed using As-PCR analysis. DNAs extracted from mutants carrying this mutation were amplified while no products were obtained after amplification of the DNA from boscalid-sensitive isolates and mutants without this mutation. The PCR-RFLP method was successfully used to diagnose the mutants with the A to G mutation. The primer pair amplified an expected 300-bp DNA fragment for all boscalid-resistant and –sensitive isolates tested. The PCR products were then treated using a mutation specific enzyme *Aci*I. The PCR products from resistant mutants carrying the mutated sequence CGC at position 997 were digested into two fragments 210 and 90-bp in length on agarose gels, whereas products from sensitive isolates and other mutants remained undigested.

DISCUSSION

The sampled *A. alternata* isolates were divided into two populations based on their response in boscalid amended medium. These results showed that the use of Pristine® can promote the selection of *A. alternata* boscalid resistant subpopulations that could compromise its use.

Resistant strains are frequently cross resistant to structurally related chemicals or to chemicals with similar mode of action (Delp, 1980). In this study, we have established the existence of a cross resistance relationship between boscalid and carboxin in *A. alternata*. Cross resistance relationship has been demonstrated in the carboxamide group for flutolanil and carboxin (Ito et al., 2004).

The rapid appearance of boscalid resistant populations of *A. alternata* in pistachio orchards, as was the case in the development of resistance to Q_oIs, indicates that it is imperative to monitor boscalid resistance in *A. alternata* in pistachio orchards when boscalid-containing fungicides are used extensively to combat Alternaria late blight. The partitioning in two distinct groups of sensitivity to boscalid suggests that the resistance to boscalid occurred as a result of disruptive selection and is likely to be monogenic in nature, as expected for single-site inhibitor fungicides. This type of resistance is known to be one of the most important factors contributing to the rapid appearance and spread of fungicide resistant alleles (Milgroom, 1990) and a continual increase in the frequency of isolation of the boscalid-resistant isolates, in other pistachio orchards where resistance has not been detected, is thus predictable.

An important step in establishing resistance management strategies consists at elucidating the molecular basis of resistance which will enable to develop a quick method for these monitoring studies. Studies on the molecular mechanisms responsible for the acquisition of resistance to boscalid analog-fungicide in resistant populations of some Basidiomycete and Ascomycete fungi and bacteria have shown that mutations in conserved regions of the genes *SdhB, SdhC* and *SdhD* encoding their molecular targets result in reduced sensitivity (Broomfield & Hargreaves, 1992; Ito et al., 2004; Matsson et al., 1998; Skinner et al., 1998). Given the cross resistance relationship between boscalid and carboxin, we implied that boscalid resistance in *A. alternata* could be governed by similar mutations in the target genes.

In this study we targeted the *SDHB* gene of this fungus and showed that a substitution of a conserved histidine residue within the third Cys-rich cluster that takes part in binding of iron-sulfur centers correlated with the phenotype of resistance as it was described in other fungal species. Other resistant isolates did not have the mutation in the histidine codon. This finding suggests that other loci could confer the resistant phenotype to such mutants. The molecular characterization of boscalid resistance in *A. alternata* isolates will allow the development of full methods that will facilitate sensitivity monitoring

studies, and consequently help in the design of management programs to preserve the use of boscalid.

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Herbivores and the world of tomorrow: Will current pest management strategies be effective in 50 years?

A great deal has been learned about pest management in the past century. Transitioning from calendar-based spray regimens with highly toxic compounds, to field scouting and the utilization of environmentally friendly alternatives saves time, money, and helps preserve soil productivity. Integrated Pest Management (IPM), whereby multiple pest control tactics are employed as part of a comprehensive IPM strategy, is an environmentally-sustainable approach for increasing plant productivity. As a result, IPM tactics have blossomed (e.g., sterile male techniques, natural enemy releases, pheromone mating disruption, genetically modified organisms). Yet a prime concern has always been; how long will these tactics remain effective?

One of the greatest challenges IPM practitioners face is achieving adequate levels of pest control as climate change progresses. As atmospheric composition is changing at an unprecedented rate, we have little predictive ability regarding how the efficacy of currently employed IPM tactics will be altered in future years. Simply put, organisms have not experienced current greenhouse gas levels for millions of years, and never have they experienced such a rapid increase in these atmospheric contaminants. While empirical research on how organisms will respond to increasing levels of greenhouse gases is still in its infancy, some general patterns are beginning to emerge, giving us a glimpse as to what the future may hold for IPM practitioners.

In the vast majority of cases, changes in greenhouse gas levels directly alter plant growth patterns. As many crop plants are currently carbon limited, they have the ability to incorporate additional CO_2 into photosynthate. Plants grown under increased CO_2 levels are frequently larger and more robust, while plants grown under increased O_3 levels are smaller and show signs of visible injury, compared to plants grown under ambient conditions. Even though plants grown under augmented CO_2 levels appear healthier, nitrogen levels are frequently lower resulting in increased rates of herbivores.

Current research at the Aspen Free-Air CO_2 and O_3 Enrichment site (Aspen FACE: http://aspenface.mtu.edu/) in northern Wisconsin, where greenhouse gases are elevated to levels projected for the year 2060, shows that atmospheric effects on trophic interactions may not always be straightforward. Atmospheric composition, associated with global

climate change, has the potential to alter herbivore-natural enemy dynamics in sometimes subtle, but important, ways. For example, one key finding is that greenhouse gases may alter pheromone communication, both within and among species. As pheromones are heavily used in IPM programs, altered efficacy is of key importance to pest management professionals.

Within-species effects: Aphid alarm pheromone responses (Mondor et al. 2004a)

Aphids are ideal study organisms for assessing the effects of enriched CO_2 and O_3 environments on pheromone transmission. Aphids are small, parthenogenetic, and their alarm pheromones have been widely investigated. At Aspen FACE, it is possible to conduct manipulated experiments on aphid populations reared for multiple generations under ambient and elevated levels of CO_2 and O_3, singly and in combination. Thus far, aphid dispersal responses to alarm pheromone have been observed to differ widely depending on atmospheric composition. Aphids are more likely to disperse in response to alarm pheromone under elevated O_3 and less likely to disperse when reared under elevated CO_2, compared to ambient atmospheres. It has yet to be determined whether differences in alarm activity results from differences in pheromone production, pheromone transmission, or pheromone receptivity. If these results can be generalized, much remains to be learned about the utility of pheromones in future IPM programs.

Among-species effects: Aphid-natural enemy interactions (Mondor et al. 2004b)

Pheromones are also important for interspecific interactions. Many organisms adaptively alter their behavior and physiology in response to pheromones from their natural enemies. Aphids, for example, detect pheromone trails left by natural enemies searching for prey. In return, aphids alter offspring phenotypes; producing more winged offspring, capable of dispersing to relative enemy-free space. Research conducted at Aspen FACE, however, shows that atmospheric composition influences aphid responses to natural enemy pheromone trails. Aphids produce more winged offspring in response to predator search tracks under high CO_2, but produce more winged offspring in response to parasitoid search tracks under high O_3 levels, compared to ambient atmospheric conditions. We have yet to fully understand the proximate mechanisms underlying these effects, but subtle changes in interspecific pheromone communication may significantly alter herbivore-natural enemy population dynamics.

In conclusion, we do not yet have the answer to the question, "will current pest management strategies be effective in 50 years?" More research is required to document how plants, herbivores, and their natural enemies will respond to future environmental conditions. There should also be an increased emphasis on how these patterns, and the mechanisms underlying these patterns, will be altered, from an agronomic and agro-economic perspective. Pest managers have previously faced challenges of considerable magnitude (e.g., invasive species, pesticide deregistration, insecticide resistance). A concerted, interdisciplinary approach will be required to attain the solutions to this impending environmental issue.

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Quinalphos Resistance in *Helicoverpa armigera* Hub. : Laboratory Measured vs. Field Control in Cotton

ABSTRACT

The resistance levels to quinalphos in populations around experimental sites varied from 25.0% to 56.5%. The application of recommended dose (RD) of quinalphos at 500g a.i.ha^{-1} resulted in a 34.0 %– 44.0% reduction of *H. armigera* larval populations in the fields. The percent mortality both in field and laboratory were dose effective. The mean percent reduction over control on boll incidence at recommended dose of quinalphos was 36.6, 31.5, 39.4, 40.8 and 27.5 on square, boll, locule, interlocular and bad kapas basis respectively. The measured resistance level in laboratory is almost reflected in the field control for quinalphos validating the discriminate dose technique for monitoring the quinalphos resistance. Application of quinalphos effected a moderate level of sucking pests of *Aphis gossypii* (Glov.), *Amrasca devastans* (Distant), and *Bemisia tabaci* (Genn.).

INTRODUCTION

Cotton is affected by a wide range of pests starting from sowing to harvest. In the early stage, sucking pests like cotton aphids, *Aphis gossypii* (Glov.), leafhoppers, *Amrasca devastans* (Distant), whitefly, *Bemisia tabaci* (Genn.) and thrips *Thrips tabaci* (Hood) and in later stage bollworm complex cause significant damage to the crop. Among the bollworm complex, *Helicoverpa armigera* Hub. was assumed as a major component, with the continued use of synthetic pyrethroids which effectively checked cotton spotted bollworm *Earias vitella* (Fab.) and pink bollworm *Pectinophora gossypiella* (Saundres). Quinalphos was found to be effective against bollworm complex and to some extent against early season sucking pests *viz. A. gossypii*, *A. devastans*, *B. tabaci* and *T. tabaci*. The evaluation of quinalphos against these pest made by various workers had been compiled by Kumar (1995) and subsequently updated by Valarmathi (1997) and Niranjan kumar (2002).

In view of continued predominance of *Helicoverpa armigera* Hub. as a major pest in cotton pest complex, earlier farmers resorted to as high as 25-35 rounds of insecticide applications including tank mixtures. During 1997, many farmers in Andhra Pradesh could not achieve effective control of this pest, the reason being the development of insecticide resistance (Jadhav and Armes, 1996). Subsequently the resistance to pyrethroids, organophosphates, carbamate and cyclodienes was confirmed later in laboratory tests (Dhingra *et al.*, 1988; Mc Caffery *et al*., 1989; Phokela *et al*., 1989; Armes *et al*., 1992, 1994 and 1995; Pasupathy and Regupathy, 1994). Resistance monitoring programs since 1993 under NRI - ICRISAT – ICAR – CFC funded network programs in different locations of India indicated various levels of resistance to quinalphos (Armes *et al.*, 1994 and 1995, Regupathy *et al*.,1994, 1998a,b and 1999, 2003) by topical (bio) assay.

Effective monitoring techniques need to be fixed not only to detect the presence of resistance but also to monitor the changes in resistance frequency for evaluating effectiveness of management programs. Suitability of monitoring technique could be validated by relating the results of bioassays to mortality in the field. Two methods have been used to establish the relationship between monitoring results and field efficacy: a) comparing results directly from field and b) mimicking field applications under more standardized conditions. The second approach offers significant advantages because factors other than pesticides that might reduce or increase population growth such as predators and physical conditions can be controlled experimentally (Dennehy *et al.*, 1987 and Harrington *et al.*, 1989).Studies were carried out to detect the level of resistance to quinalphos and its field efficacy against quinalphos resistant *H. armigera* and sucking pests of cotton as well as to compare and obtain relationship between lab measured resistance and field control.

MATERIALS AND METHODS

Field evaluation

Field trial was laid with MCU –5 cotton in randomized block design with six treatments and four replications, during winter cotton season (September - February) at Agricultural Research Station (ARS), Vaigai Dam ((Latitude $10^o4'$ N; Longitude $77^o9'$ E; Altitude 260 MSL)), Theni District , Tamil Nadu. Quinalphos (Ekalux 25EC ®) was tested at recommended dose (RD) of 500g a.i./ha, 1/10RD, 1/3 RD, 3RD 1/10RD and 10RD against *H. armigera*. One plot measuring 8 x 8 m^2 accommodated around 266 plants with a spacing of 75 x 30cm. Cowpea was sown as bund crop and red gram was sown as border crop. Eggs were collected from heavily infested and unsprayed farmer's fields in local area of the experimental site. The population of that particular local area was maintained in the lab and assessed for the level of resistance through discriminating dose (0.75 /ug) bioassay before field application.

Two hundred eggs were placed in each replication at the rate of two eggs per plant on fresh leaves near young bolls in the top one third portion of the plant and the site of placing eggs was tagged. These 100 plants were examined after a week to know the percent emergence and settling. The egg placement was done again in case of poor emergence. Spraying was taken with different doses of the quinalphos with a backpack mist blower. The quantity of spray fluid was 1.2 l/plot or 200 l/ha during first spray (65 days after sowing- DAS) and 1.8 l/plot or 300 l /ha during second spray (120 DAS). Enough care was taken to avoid drift to adjacent plots. Standard agronomic practices were followed for crop growth.

Observations on Pests *H. armigera*

Early third instar *Helicoverpa armigera* larvae were counted on 100 selected plants prior to the application of insecticide, 2 and 7 days after imposing of treatments. The live larvae were collected 7 days after treatment (DAT), reared through F_1 generation and assessed for resistance by applying DD of 0.75ug/ larva in the laboratory.

Boll worm damage

The extent of damage caused by boll worm was made before and 2, 7, 14 and 21 DAT based on shed squares, bolls, locules, inter locular boring and bad kapas.

The total number of bolls and squares and those damaged by boll worms were counted at ten randomly selected plants per plot. The total number of bolls collected from ten randomly selected plant per plot at each picking was assessed for number of

damaged bolls, number of locules damaged, inter locular boring and the percentage was worked out. The total kapas collected from ten plants was weighed. Bad and good kapas were separated. Bad kapas by good kapas was expressed in terms of percentage (w/w basis).

Sucking pests

Assessment of sucking pest populations of A. gossypii, A. devastans, B. tabaci and T. tabaci was done before and 2, 7, 14 and 21 DAT. Populations of both nymphs and adults of aphids, hopper and thrips and adults of whitefly on three leaves, one leaf each from top, middle and bottom portion in each of the 10 randomly plants per plot were assessed.

Laboratory measurement by bouquet bioassay

Cotton leaves, squares and bolls, were surface sterilized in 0.5% sodium hypochlorite, rinsed in sterile water and shade dried. These shoots were dipped in respective concentration of quinalphos for about 30 sec. And the excess fluid was drained off and allowed for shade drying. The petiole/ stalk of leaves, squares and bolls were surrounded with cotton swab and were kept immersed in water to maintain the turgidity. The entire set up was kept enclosed in Mylar film cage and larvae (30-40 mg weighing) were allowed to feed. Mortality was recorded at 24 h intervals up to 6 days including period of exposure. Separate sets of experiments were conducted using cotton leaves, squares and cotton bolls at different application rates *viz*., one-tenth of recommended dose, one-third of recommended dose, recommended dose (RD) , three times the recommended dose and ten times the recommended dose.

RESULTS AND DISCUSSION

Resistance level

The resistance levels to quinalphos in populations around experimental sites varied from 25.0% to 56.5%; the minimum level of resistance observed during fourth week of October, the maximum during second week of January.

Laboratory measured resistance

The percent mortality at recommended dose of quinalphos (500 g a i/ha) was 56.9, 52.5 and 43.2 on cotton leaves, squares and bolls respectively. Increasing the dose by ten times produced a higher mortality; the range being 82.8 – 93.3 percent. At reduced doses the mortality was 33.2 – 49.2 percent (1/3 RD) and 19.9 – 35.1 percent (1/10 RD) (Table 1).

Table 1: Effects of different doses of quinalphos on *H. armigera* by bouquet bioassay

Treatment	Dose (g a.i./ha)	Cotton leaves					Cotton squares					Cotton bolls				
		No. Dosed	No. dead	Corr. % Mortality	% Resistance	SE	No. Dosed	No. dead	Corr. % Mortality	% Resistance	SE	No. Dosed	No. dead	Corr. % Mortality	% Resistance	SE
1/10 RD	50	56	21	35.1	62.5	6.5	54	18	33.3	66.7	6.5	46	11	19.9	76.1	6.4
1/3 RD	166.7	47	24	49.2	48.9	7.4	50	23	46.0	54.0	7.1	52	19	33.2	63.5	6.7
RD	500	53	31	56.9	41.5	6.8	59	31	52.5	47.5	6.6	50	23	43.2	54.0	7.1
3X RD	1500	59	45	75.4	23.7	5.6	61	42	68.9	31.1	6.0	59	40	66.1	32.2	6.1
10X RD	5000	62	58	93.3	6.5	3.1	64	53	82.8	17.2	4.8	53	47	88.1	11.3	4.4
0	0	54	2	--	96.3	2.6	57	0	--	100.0	0.0	60	3	--	95.0	2.8
Topical bioassay (µg/µl)	0.75	50	29	58.0	42.0	7.1	59	31	52.5	47.5	6.6	54	33	61.1	38.9	6.7

RD – Recommended Dose

Effect on *H. armigera* larval population

The larval population was in the range of 70.0 – 91.0 larvae per hundred plants prior to first application (65 DAS). Application of recommended dose of quinalphos (500 g ai/ha) resulted in a 43.9 percent reduction. Increase in dose by 10 times resulted in a 84.1 percent reduction. The reduction in dose by three and ten times resulted in a 30.6 and 21.7 percent reduction respectively. The resistance levels of spray survived F_1 population ranged from 41.2 – 55.6 percent when compared to 46.6 percent prior to the application (Table 2.).

Table 2. Effect of first application of quinalphos on larval population of *Helicoverpa armigera*

Treatments	Dose (g ai/ha)	Natural infestation Pre count	2DAT		7DAT		% resistance of F_1 field survived population
			Post Count	Corrected % reduction	Post Count	Corrected % reduction	
1/10 RD	50	76	56.5 $(7.48)^{cd}$	15.8 $(17.95)^{e}$	49.2 $(7.04)^{cd}$	21.7 $(26.73)^{e}$	42.4 ± 6.6
1/3 RD	166.7	87	53.5 $(7.34)^{cd}$	29.2 $(32.26)^{d}$	47.7 $(7.10)^{d}$	30.6 $(33.35)^{d}$	41.2 ± 6.3
RD	500	73	41 $(6.42)^{c}$	35.4 $(36.61)^{c}$	34.3 $(5.89)^{c}$	43.9 $(41.53)^{c}$	46.8 ± 6.0
3X RD	1500	78	27 $(5.22)^{b}$	60.2 $(50.97)^{b}$	23.2 $(4.86)^{b}$	63.4 $(52.87)^{b}$	51.7 ± 6.5
10X RD	5000	70	12 $(3.53)^{a}$	80.1 $(63.59)^{a}$	9.2 $(3.11)^{a}$	84.1 $(66.50)^{a}$	55.6 ± 6.2
Control	0	91	80.5 $(8.98)^{e}$	--	76.3 $(8.75)^{e}$	--	42.0 ± 5.4

The laboratory resistance through topical application of discriminating dose before application of treatments is 46.6 ± 7.0.
DAT: Days after treatment, **DAP**: Days after egg placement, **RD**: Recommended dose
Figures in parentheses for post count are square root transformed values.
Figures in parentheses for corrected per cent reduction are arc sin√percentage transformed values.
Means followed by same letters in a column are not significantly different by DMRT.

The larval recovery from 200 implanted eggs before second application of quinalphos ranged from 102 to 136 larvae per hundred plants. Recommended dose of quinalphos resulted in a 42.1 percent reduction. Increase in dose by 10 times resulted in a percent reduction of 84.6 and a decrease in dose resulted in decreased percent reduction; 29.8 (1/3 RD) and 21.4 ($1/10^{th}$ RD). The resistance level of spray survived F_1 population ranged from 46.0 – 58.7 percent when compared to 47.1 prior to the application (Table 3).

Table 3: Effect of second application of quinalphos on larval population of *Helicoverpa armigera*

Treatments	Dose (g ai/ha)	Larval recovery from 200 eggs (5DAP)	Pre count (11DAP)	2DAT		7DAT		% resistance of F_1 field survived population
				Post Count	Corrected % reduction	Post Count	Corrected % reduction	
1/10 RD	50	124	92	69.8 $(8.36)^{e}$	11.1 $(18.65)^{e}$	63 $(7.95)^{de}$	21.4 $(27.36)^{e}$	46.0 ± 6.7
1/3 RD	166.7	106	85.8	57.8 $(7.62)^{d}$	20.5 $(26.35)^{d}$	52.3 $(7.25)^{cd}$	29.8 $(32.88)^{d}$	48.1 ± 6.9
RD	500	136	97	59.5 $(7.40)^{c}$	33.9 $(35.64)^{c}$	50.8 $(6.98)^{c}$	42.1 $(40.39)^{c}$	50.7 ± 6.1
3X RD	1500	102	79.5	36.5 $(6.06)^{b}$	45.3 $(43.16)^{b}$	31.0 $(5.65)^{b}$	54.4 $(47.55)^{b}$	55.7 ± 6.3
10X RD	5000	128	103.8	18.8 $(4.33)^{a}$	79.3 $(63.07)^{a}$	14.0 $(3.76)^{a}$	84.6 $(67.07)^{a}$	58.7 ± 6.4
Control	0	113	82	70 $(8.39)^{d}$	--	71.5 $(8.48)^{e}$	--	49.2 ± 5.4

The laboratory resistance through topical application of discriminating dose before application of treatments is 47.1 ± 7.1.
DAT: Days after treatment, DAP: Days after egg placement, RD: Recommended dose
Figures in parentheses for post count are square root transformed values.
Figures in parentheses for corrected per cent reduction are arc sin√percentage transformed values.
Means followed by same letters in a column are not significantly different by DMRT.

Effect on Bollworm Incidence

The mean percent reduction over control on boll incidence at recommended dose of quinalphos was 36.6, 31.5, 39.4, 40.8 and 27.5 on square, boll, locule, interlocular and bad kapas basis respectively. At the highest dose of 10X RD, the mean percent reduction over untreated check was 51.8, 54.1, 76.7, 65.4 and 58.6 and at lower doses percent reduction over control was 19.3, 9.7, 13.4, 11.3 and 9.2 at 1/3 RD and 30.7, 21.2, 22.3, 21.6 and 14.3 at 1/10 RD on square, boll, locule, inter locular and bad kapas basis respectively (Table 4.).

Table 4: Effect of two applications of quinalphos on bollworm incidence

		Square basis		Boll basis		Locule basis		Inter locular boring basis		Bad kapas basis	
Treatments	Dose (g ai / ha)	% Damage	% Reduction	% Damage	% Reduction	% Damage	Percent reduction over control	Mean	Percent reduction over control	Mean	Percent reduction over control
1/10 RD	50	35.0 $(36.25)^{e}$	19.3	37.6 $(37.77)^{e}$	9.7	16.1 $(23.52)^{e}$	13.4	9.2 (17.68) e	11.3	13.9 (21.31) e	9.2
1/3 RD	166.7	27.5 $(31.58)^{d}$	30.7	32.8 $(34.92)^{d}$	21.2	14.4 $(22.22)^{d}$	22.3	8.2 (16.53) d	21.6	12.6 (20.71) d	14.3
RD	500	25.5 $(30.27)^{c}$	36.6	28.5 $(32.24)^{c}$	31.5	11.3 $(19.42)^{c}$	39.4	6.2 (14.25) c	40.8	10.7 (18.84) c	27.5
3X RD	1500	20.7 $(27.00)^{b}$	45.7	21.7 $(27.75)^{b}$	47.9	7.8 $(16.12)^{b}$	58.1	4.8 (12.51) b	54.2	8.1 (16.35) b	45.1
10X RD	5000	19.9 $(26.64)^{a}$	51.8	19.1 $(25.75)^{a}$	54.1	4.3 $(11.91)^{a}$	76.7	3.6 (10.87)a	65.4	6.1 (13.88) a	58.6
Control	0	42.0 $(40.24)^{f}$	--	41.6 $(40.15)^{f}$	--	18.5 $(25.43)^{f}$	--	10.4 (18.74)f	--	14.7 (22.47)f	--

RD: Recommended dose
Figures in parentheses are arc sine √ percentage transformed values.
Means followed by the same letter are not significantly different by DMRT (P = 0.05).

Effect on *A. gossypii*

The population level prior to application of quinalphos varied from 22.0 to 27.5 per three leaves. At recommended dose the reduction was 41.3 – 41.6 percent and increase in dose up to 10 times affected increased reduction (53.6 – 55.2). At lower doses the mean percent reduction was 20.6 – 24.5 ($1/3^{rd}$ RD) and 12.0 - 13.7 ($1/10^{th}$ RD) (Tables 5).

Table 5: Effect sof first application of quinalphos at different doses on aphids, leafhoppers and whiteflies on cotton – No. per 30 leaves

		Aphids		Leafhoppers		Whitefly	
Treatments	**Dose (g ai/ha)**	**MEAN**		**MEAN**		**MEAN**	
		Population	**% Reduction**	**Population**	**% Reduction**	**Population**	**% Reduction**
1/10 RD	50	20.5	13.7 (21.39)^a	23.4	7.6 (14.91)^a	10.8	15.3 (20.74)e
1/3 RD	166.7	16.8	24.5 (26.27)^b	22.9	12.3 (19.23)^b	11.3	23.3 (22.43)^b
RD	500	13.6	41.6 (34.42)^c	20.3	23.9 (28.44)^c	9.4	38.0 (27.60)^c
3X RD	1500	13.8	48.4 (40.22)^a	16.4	28.4 (31.36)^b	7.1	45.7 (38.44)^b
10X RD	5000	9.9	55.2 (39.99)^b	20.3	31.0 (34.85)^a	7.1	55.3 (40.42)^a
Control	0	24.6	--	24.8	--	15.9	--

RD: Recommended dose; Figures in parentheses are arc sin $\sqrt{}$ percentage transformed values;
Means followed by the same letter are not significantly different by DMRT (P = 0.05).

Effect on *A. devastans*

The population level of leafhoppers per 30 leaves before application of quinalphos varied from 20.3 to 26.5. The mean percent reduction affected by quinalphos was 21.6 - 23.9 (RD), 31.0 – 37.6 (10X RD), 10.6 – 12.3 (1/3 RD) and 7.4 – 7.6 (1/10 RD).

Effect on *B. tabaci*

The population level of whiteflies per 30 leaves before first application of quinalphos ranged between 11.3 and 14.8. At recommended dose the mean percent reduction varied from 38.0 – 39.9. Increase in dose effected increased per cent reduction of 45.7 – 47.6 (3X RD) and 55.0 - 55.3 (10X RD). At lower doses the mean percent reduction was 23.3 – 27.8 (1/3 RD) and 15.3 – 18.2 (1/10 RD) (Table 6).

Table 6: Effect of second application different doses of quinalphos on aphids, leafhoppers and whiteflies on cotton – No. per 30 leaves

		Aphids		Leafhoppers		Whitefly	
Treatments	**Dose (g ai/ha)**	**MEAN**		**MEAN**		**MEAN**	
		Population	**% Reduction**	**Population**	**% Reduction**	**Population**	**% Reduction**
1/10 RD	50	22.8	12.0 (19.73)^a	34.2	7.4 (15.24)^a	12.3	18.2 (24.72)^a
1/3 RD	166.7	17.3	20.6 (26.59)^b	32.5	10.6 (18.40)^b	11.6	27.8 (31.33)^b
RD	500	14.7	41.3 (39.66)^c	26.4	21.6 (26.89)^c	12.0	39.9 (38.68)^c
3X RD	1500	9.4	49.8 (44.87)^b	27.3	32.4 (33.94)^b	8.4	47.6 (43.27)^b
10X RD	5000	10.0	53.6 (47.30)^a	21.8	37.6 (37.32)^a	8.4	55.0 (48.03^a
Control	0	25.1	--	37.3	--	13.9	--

RD: Recommended dose; Figures in parentheses are arc sin $\sqrt{}$ percentage transformed values;
Means followed by the same letter are not significantly different by DMRT (P = 0.05).

T.tabaci population was insignificant; hence inference could not be made.

Yield

Application of quinalphos during winter, 2001 resulted in kapas yield of 1642.3 kg/ha. Increasing dose by 3X and 10X resulted 1781.3 and 1985.5 kg/ha. At lower doses the yield was 1421 (1/3rd RD) and 1218.3 kg/ha (1/10th RD).

The reduction over control reported by earlier workers was 60.6 percent on boll basis and 56.0 percent on locule basis at Ludhiana in 1991 (Dhawan and Simwat, 1997), 41.9 on boll basis, 39.8 on locule basis, 54.6 on inter locular basis and 14.4 on bad kapas basis during winter 1995-96 at 500 g ai/ha dose, 27.1 on boll basis, 38.7 on locule basis, 48.2 on inter locular basis and 33.1 on bad kapas basis during summer 1996 (Valarmathi,1997), 51.1 percent on boll basis, 56.0 on locule basis in 1995 (Dhawan and Simwat, 1997), 26.3 percent on boll basis and 34.2 on locule basis in 1996 in Surat (Vadodaria *et al.*, 2000). 47.4 percent in 1998 in Guntur (Gopalaswamy, 2000), 53.1 on boll basis, 46.3 on locule basis, 26.5 on inter locular basis and 35.4 on bad kapas basis in 1994 (Kumar, 1995). and Valarmathi and Regupathy, 2004, 2007).

The results obtained in the present study due to application of quinalphos when compared to that of earlier reports (26.9 – 60.6) indicates that development of resistance to quinalphos is significant, but low when compared to synthetic pyrethroids as also indicated from laboratory measured resistance. Application of quinalphos effected moderate levels of check on sucking pests *A. gossypii*, *A. devastans* and *B. tabaci* unlike cypermetrin which caused resurgence of *A. gossypii and B. tabaci* (Niranjankumar et. al.,2002)The measured resistance level in the laboratory is almost reflected in the field control for quinalphos as also observed endosulfan (Niranjan Kumar and Regupathy, 2008), profenofos (Niranjan Kumar and Regupathy ,2007), thiodicarb (**Ramasubramanian, and Regupathy. 2003**) and spinosad (**Ramasubramanian and Regupathy, 2003)**. However in the case of cypermethrin the relationship between laboratory measured resistance and field control is not corroborating when discriminating dose of 0.1 µg/ larva was used (Niranjankumar et. al , 2003) indicating the need to relate the level of resistance detected in the laboratory and level of control achieved in field. In the present study the quinalphos DD of 0.75 µg/ larva for resistance monitoring could be used.

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University Based Research on Organic Pest Management for Red Imported Fire Ants, *Solenopsis invicta* Buren (Hymenoptera: Formicidae)

Abstract

Red imported fire ants, *Solenopsis invicta* Buren, are an invasive species that has infested over 300 million acres in the southern United States. Not only is their damage to electrical equipment costly, but their sting can injure or kill humans, wildlife and domestic animals. For these reasons, the use of chemicals is needed to manage their populations. Organic chemical control options are preferred for most clientele, since they are perceived as safe options for the control of pests. However, few scientific studies have been conducted to test home remedies and organic products that are currently on the market, to determine their effects on controlling fire ants. In 2007 and 2008, we were able to test the effects of aspartame, garlic concentrate and two species of nematodes on fire ant populations in Texas. Our results indicate that both sprinkled and watered in aspartame treated plots did not significantly decrease fire ant populations compared to the untreated control plots; after 21 days, 4oz, 6oz, and 8oz rates of garlic concentrate had significantly fewer active fire ant mounds compared to the control plots; and the overall fire ant activity decreased among all nematode treated groups.

Introduction

Red imported fire ants are nuisance insects, making them a desirable pest to control (Drees et al. 2002). On average, Americans spend over $6 billion a year on medical bills, repairing damage to electrical wiring and purchasing insecticides for treatment of fire ants (Drees et al 2008). In the United States, there is a growing interest in using naturally derived, "organic" insecticides for controlling all insects (Drees and Lennon 1998). Most organic treatments are perceived as being safe, but some can be dangerous to apply and can contaminate the environment. Although many home remedies and organic treatments are discussed in main stream media, very few have been scientifically proven to significantly reduce fire ant populations.

Oftentimes products will kill many ants and the colony will abandon the mound. Since the entire colony or queens were not killed, the colony usually establishes another mound in a neighboring area. Some organic treatments that have circulated the internet via email and gardening websites include using aspartame, garlic and nematodes to control fire ant populations. In 2007 and 2008, we were able to conduct several tests to determine if these natural control products used as individual mound treatments actually decreased fire ant populations.

Materials and Methods

Aspartame trial: Two trials were conducted in Williamson County, Texas to determine the effects of aspartame on individual fire ant mounds. On October 3, 2007 a trial was conducted to determine if one tablespoon of aspartame (Equal®) was effective as a mound treatment for fire ants, compared to applying one tablespoon of Ortho® Orthene® Fire Ant Killer (50% acephate) and untreated controls. Another trial, conducted on April 11, 2008, was designed to test if applying one tablespoon of aspartame onto a fire ant mound and then watering it in with one gallon of water was effective at controlling fire ant populations, compared to the application of one tablespoon of Ortho® Orthene® Fire Ant Killer (50% acephate) and an untreated control. A total of 15 plots, each containing five red imported fire ant mounds, with the same width but varying lengths were established for each trial. Treatments were assigned randomly within each replicate, so the total length within treatment groups was roughly equal. Prior to treatment, each active mound was marked with field paint and examined for ant activity using the minimal disturbance method, whereby a mound was considered active if a dozen or more worker ants emerged en masse following mild disturbance. This assessment method was also used to evaluate plots at 3, 7, 14 and 28 days post treatment.

Garlic trial: Garlic concentrate (99.3% garlic, 0.5% citric acid, and 0.2% potassium sorbate) was used to evaluate the reduction of fire ant populations with individual mound treatment rates of 4oz, 6oz and 8oz per gallon of water compared to water and untreated controls. A total of 20 plots, each containing ten red imported fire ant mounds, with the same width but varying lengths were established on September 25, 2008. Treatments were assigned randomly within each replicate, so the total length within each treatment group was roughly equal. Prior to treatment, each active mound was marked with a field flag and examined for ant activity using the minimal disturbance method whereby a mound was considered active if a dozen or more worker ants emerged en masse following mild disturbance. This assessment method was also used to evaluate plots at 3, 7, 14, 21, 28, 42 and 56 days post treatment.

Entomopathogenic nematodes trial: Entomopathogenic nematodes in the genera *Steinernema* and *Heterorhabditis* have been previously used in the biological control of soil-inhabiting insect pests (Neumann & Shields 2008; Shields et al 1999). On October 3, 2008, the species strains *Heterorhabditis bacteriophora* 'Oswego', *Steinernema carpocapsae* 'NY001' and a combination of 'NY001' and 'Oswego' were evaluated to determine their efficacy on fire ant mounds when used as individual mound treatments compared to water and untreated controls. The rate of application was 3000 infective juvenile nematodes per individual fire ant mound. Two sites were utilized for this trial: (1) Clarksville, TX and (2) Elemendorf, TX. At each site, all five treatment groups were evaluated

with the addition of evaluating d-limonene (Safer® Brand Fire Ant Killer- 78.2% d-limonene) at Site 1. At Site 1, a 100 acre hay meadow in Clarksville, TX was divided into two strips, each measuring fifty feet. A total of 20 plots, each containing 10 active fire ant mounds were established. For Site 2, a 20 acre pasture in Elemendorf, TX was divided into 20 plots, each containing seven active mounds, with the untreated control plots containing five active mounds. Treatments were assigned randomly within each replicate so the total length within each treatment group was roughly equal. At Site 1, each active mound was marked with a field flag and examined for ant activity using the minimal disturbance method whereby a mound was considered active if a dozen or more worker ants emerged en masse following mild disturbance. At Site 2, mound activity was determined using a Lickert Scale. After disturbing each mound, a rating of 0-4 was designated after ten seconds: 0 = no ants emerged, 1 = 1-25 ants emerged, 2 = 26-50 ants emerged, 3 = 51-100 ants emerged, and 4 = 100 ants emerged. Evaluations for Site 1 were made at 7, 14, 21, 30 and 60 days post treatment. Evaluations for Site 2 were made at 7, 14, 30 and 71 days post-treatment. In addition, the presence of dead piles of fire ants were noted and collected to evaluate the presence of nematodes.

Data for all of the trials were analyzed using Analysis of Variance (ANOVA) test with means separated using Duncan's Multiple Range Test at $P \leq 0.05$ (SPSS for Windows, Lead Technologies, Version 13.0).

Results and Discussion

Aspartame trial: At all post treatment observations within the two trials, the acephate (Ortho® Orthene®) treated plots had significantly less active fire ant mounds compared to the aspartame (Equal®) treated plots and untreated control (Tables 1 and 2). Mound activity in both sprinkled and watered in aspartame treated plots were not significantly different than the untreated control plots throughout both trials.

Table 1: Mean number of active fire ant mounds at each observation in the sprinkled aspartame trial, Williamson Co, TX, initiated on October 3, 2007.

Treatment	3 Days	7 Days	14 Days	28 Days
Untreated Control	4.80a	4.80a	4.80a	4.60a
Acephate (Ortho® Orthene®)	0.00b	0.00b	0.00b	0.00b
Aspartame (Equal®)	4.80a	5.00a	4.80a	4.40a

[a]Means followed by the same letter within the same column were not significantly different using Analysis of Variance (ANOVA) and means separated using Duncan's Multiple Range Test at $p \leq 0.05$ (SPSS, Windows 11.5).

Table 2: Mean number active fire ant mounds found at each observation in the watered in aspartame trial, Williamson Co, TX, initiated on April 11, 2008.

Treatment	3 Days	7 Days	14 Days	28 Days
Untreated Control	4.60a	4.80a	3.80a	4.00a
Acephate (Ortho® Orthene®)	0.80b	1.20b	0.40b	1.20b
Aspartame (Equal®)	4.00a	4.20a	4.00a	4.00a

[a]Means followed by the same letter within the same column were not significantly different using Analysis of Variance (ANOVA) and means separated using Duncan's Multiple Range Test at $p \leq 0.05$ (SPSS, Windows 11.5).

Garlic trial: At the 3 days and 1 week evaluations, the 8oz treatment of garlic concentrate had significantly fewer active fire ant mounds compared to the other treatments and controls (Table 3). However at the 21, 28 and 56 day evaluations, the 4oz, 6oz and 8oz treatments of garlic concentrate had significantly fewer active fire ant mounds compared to the controls. Future studies should be conducted to compare efficacy of other organic fire ant mound treatments to varying rates of garlic concentrate.

Table 3: Mean number of active red imported fire ant mounds at each observation in the garlic concentrate trial in Clarksville, TX initiated on September 25, 2008 .

Treatment	3 Days	1 Week	2 Weeks	3 Weeks	4 Weeks	6 Weeks	8 Weeks
4 oz	9.00bc	8.25c	7.50b	7.25b	7.00a	7.00ab	6.25a
6oz	8.25b	6.25b	5.25a	6.25ab	6.25a	6.25a	5.75a
8oz	6.25a	4.50a	4.25a	5.25a	6.00a	5.75a	5.25a
Water Control	9.75c	9.75d	9.25c	9.25c	9.25b	8.25b	8.25b
Untreated Control	10.00c	10.00d	9.25c	9.25c	9.25b	8.25b	8.25b

[a]Means followed by the same letter within the same column were not significantly different using Analysis of Variance (ANOVA) and means separated using Duncan's Multiple Range Test at $p \leq 0.05$ (SPSS, Windows 11.5).

Entomopathogenic nematode trial: Site 1: D-limonene treated plots had significantly fewer active mounds compared to all other treatments throughout the study (Table 4). At 7, 14 and 21 day evaluations, there were no significant differences between the nematode treated plots and control plots. At 28 days post treatment, the 'NY100'/'Oswego' combination treated plots had significantly less active fire ant mounds compared to the control and 'Oswego' treated plots. At 60 days post treatment, 'NY100' and 'NY100'/'Oswego' combination treated plots had significantly less active fire ant mounds compared to the 'Oswego' and control plots; however, those treatments were not significantly different than water treated controls, indicating that disturbing mounds with any drench may cause enough agitation to move the mound from the initial location. Site 2: There was no significant difference in mound activity throughout the duration of the study, although overall activity did decrease among all treatment groups (Table 5).

Table 4: Mean number of active fire ant mounds at each observation for the nematode trial in Clarksville, TX initiated on October 3, 2008.

Treatment	7 Days	14 Days	21 Days	28 Days	60 Days
D-limonene	5.75a	3.50a	3.75a	4.00a	3.00a
NY001	9.75b	9.25b	8.25b	7.50bc	7.00b
Oswego	9.75b	9.50b	9.00b	8.75c	8.25c
NY001/ Oswego	9.25b	9.00b	9.00b	7.00b	6.25b
Water Control	9.75b	9.50b	8.75b	8.50bc	8.00bc
Dry Control	10.00b	10.00b	9.25b	9.25c	9.00c

[a]Means followed by the same letter within the same column were not significantly different using Analysis of Variance (ANOVA) and means separated using Duncan's Multiple Range Test at $p \leq 0.05$ (SPSS, Windows 11.5).

Table 5: Mean fire ant activity as determined by a Lickert Scale at each observation for the nematode trial in Elmendorf, TX initiated on October 3, 2008.

Treatment	Pre-treat	7 Days	14 Days	30 Days	71 Days
NY001	3.15a	2.68a	3.07a	2.50b	1.86a
Oswego	3.29a	2.61a	2.71a	2.00ab	1.79a
Combination	3.39a	2.36a	2.43a	2.25ab	1.75a
Water	3.32a	2.14a	2.61a	2.14ab	2.11a
Dry	3.40a	2.25a	2.45a	1.75a	1.95a

[a]Means followed by the same letter within the same column were not significantly different using Analysis of Variance (ANOVA) and means separated using Duncan's Multiple Range Test at $p \leq 0.05$ (SPSS, Windows 11.5).

However, this cannot be correlated with nematode infestation and may be due to the lack of rain and drop in temperatures, which caused the fire ants to move deeper in the soil. Infective juvenile nematodes were discovered from fire ant cadavers collected from a dead pile in the 'NY100' treated group, indicating parasitism occurred (Figure 1). Overall, in both studies, the *Heterorhabditis bacteriophora* 'Oswego' strain did not decrease mound activity, since fire ants thrive in unstable ecosystems; 'Oswego' is more suited for long term insect suppression within stable ecosystems (Sheilds et al 1999).

Figure 1: Fire ant cadaver parasitized by *Steinernema carpocapsae* 'NY100.'

Scientifically tested effective organic control options for controlling fire ants, such as spinosad bait, spinosad concentrate, orange oil and d-limonene, can be found on the Texas Imported Fire Ant Research and Management Project website (http://fireant.tamu.edu). We will continue to evaluate organic options for controlling red imported fire ants on a yearly basis, since it is critical to provide scientific evidence to our clientele.

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Monitoring organophosphorous resistance in pink bollworm using the Attracticide Resistance Monitoring Technique

ABSTRACT

Field and laboratory trials were carried out in two successive cotton seasons (2006 and 2007) and the results were collected in both early and late season. Three different strains, susceptible, El-Behera and Alexandria were chosen along the course of this investigation. The toxicity parameter (LC_{50}) for the susceptible strain indicated that the toxicity of chlorpyrifos-methyl was the most toxic in the two durations (6 hr and 12 hr assessments) with LC_{50} of 14.9 and 4.26 ppm, respectively; followed by profenofos (LC_{50} values = 15.47 and 4.98 ppm) and then the least toxic was chlorpyrifos with LC_{50} values of 16.48 and 6.61 ppm. The statistical analysis data show no significant difference in toxicity of the tested three organophosphorous insecticides. The intensive and continuous use of the tested insecticides in controlling cotton bollworms and especially pink bollworm in Egypt lead to high resistance levels in El-Behera cotton fields reaching 193.97 fold against chlorpyrifos, 117.39 fold against chlorpyrifos-methyl and 86.53 fold against profenofos by late 2007 in a cotton season in the 6hr assessment. The calculated resistance values for Alexandria strain reached 29.98, 60.94 and 151.56 fold by the late 2007 cotton season against chlorpyrifos, chlorpyrifos-methyl and profenofos, respectively. On the other hand, the resistance levels which derived from 12 hr assessment show different values than that of 6 hr assessment for both strains. The resistance values of El-Behera strain reached 61.04, 169.35 and 83.94 fold against chlorpyrifos, chlorpyrifos-methyl and profenofos, respectively; while the resistance values of Alexandria strain reached 50.54, 149.7 and 336.77 fold against chlorpyrifos, chlorpyrifos-methyl and profenofos, respectively.

Key words: Pink bollworm, *Pectinophora gossypiella*, resistance, Organophosphorous.

INTRODUCTION

Cotton, the world's most important fiber is grown on more than 33.9 million hectares in about 100 countries. Four countries alone (China, the USA, India, and Pakistan) account for approximately two thirds of world output. If we added Uzbekistan and Egypt, six countries would account for three fourths of world cotton production, (Anonymous, 2004). The pink bollworm (PBW) *Pectinophora gossypiella* (Saunders) is a worldwide pest of cotton and in some regions of the world is the key cotton pest. Like the boll weevil, the PBW is a well adapted herbivore of cotton, feeding throughout the growing season on the cotton fruit (square and bolls) and burrowing habits. It has caused a loss in yield and costs of insect control, substantial indirect losses occur as result of the destruction of beneficial insects and the development of insecticides resistance in cotton. It has been extremely difficult to control using pesticides but considerable success has been achieved using alternative control tactics.

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 problem 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, organophosphorous have been widely used against cotton pests. However, although organophosphorous insecticides were the most efficient and widely used against bollworms, the onset of resistance developing to these compounds in bollworms have been recently documented (Georghiou, 1983; Haynes *et al*., 1987; Miller, 1990 and Shekeban, 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. Being accurate, easy, rapid and inexpensive, which would aid production, would aid consultants and extension personal in making informed decisions on adequate control measures (Mink & Boethel 1992). The attracticide method was developed in summer of 1985 and was full implementation in 1986 and 1987 as an effective and rapid method to monitor insecticide resistance in pink bollworm adults in cotton fields to a wide range of insecticides (Miller, 1986 and Haynes *et al* ,1986 and 1987).

MATERIALS AND METHODS

1- INSECTICIDES USED: Three organophosphorous insecticides were used :

1.1. Chlorpyrifos (Pestban®)

EC 48% provided by Agrochema, Egypt. Application rate: 1 liter/ feddan

1.2. Chlorpyrifos-methyl (Reldan®)

EC 50% provided by Agrochema, Egypt. Application rate: 1 liter/ feddan

1.3. Profenofos (Seleton®)

EC 72% provided by: Syngenta , Agro, Egypt. Application rate: 0.75 liter / feddan.

2- PHEROMONE USED

Provided by the Ministry of Agriculture, Egypt, as Pink rubber septum containing 1 mg Gossyplure**.**

3- OTHER CHEMICALS USED

Stickum® (sticky adhesive) and acetone.

4- INSECTS USED: Pink bollworm (PBW) *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae).

4.1. The laboratory strain: Male moth population of PBW was supplied by the Bollworm Research Department, Plant Protection Research Institute, Agricultural Research Center, Dokki, and Giza, Egypt, where it has been reared for more than 10 years in conditioned laboratory without exposure to insecticides. The rearing procedure was adopted as that recorded by Abdel Hafizes *et al* (1982).

4.2. Field strains: Male moth populations of PBW from both Kafr El-Dawar District cotton fields, El-Behera Governorate, and Faculty of Agriculture farm, Alexandria Governorate, Egypt were used locally in the present study.

5- ARMT PROCEDURE: The attracticide resistance monitoring technique (ARMT) is that method where delta traps were used with sticky adhesive coated cards containing the insecticide concentrations placed in the trap bottom. Rubber septa with 1mg gossyplure acted as the pheromone source. This technique provided stable LC_{50}'s with low control mortality. This technique was used as described by Miller (1986) and modified by Shekeban (2000).

6- Statistical analysis

6.1. Regression equation and confidence limits:

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

6.2. Resistance Ratio (R.R.):

Resistance ratio (RR) values were measured according to the following equation:

$$\text{Resistance Ratio (R.R.)} = \frac{LC_{50} \text{ of the Field strain}}{LC_{50} \text{ of the susceptible strain}} \text{ (Fold)}$$

RESULTS AND DISCUSSION

1- Toxicity Parameters and Resistance Ratios after 6 hr. of treatment.

Data in table (1) show that the LC_{50} of the tested organophosphorus insecticides at the early 2006 cotton season from the sex hours assessment were differed between the three tested strains where the susceptible strain had the lowest LC_{50} values, while these values were higher in the field strains from the two locations.

Table (1): Toxicity Parameters and Resistance Ratios of the tested organophosphorous after 6 hrs. of treatment at early 2006 cotton season

Insecticides	Strain	Regression Equation	LC_{50} (conf. limits)[1]	LC_{95}	RR[2]
Chlorpyrifos	Susceptible St.	Y= -1.56+1.28 X	16.48 (19.9 - 13.65)	314.8	-
	El-Behera St.	Y = -2.8 + 0.8X	2832.80 (4717.4 - 1709.6)	284724.2	171.89
	Alexandria St.	Y = -4.6 + 1.9X	260.09 (312.8 - 216.1)	1929.85	15.78
Chlorpyrifos-methyl	Susceptible St.	Y = -1.51+1.29 X	14.90 (17.9 - 12.3)	281.9	-
	El-Behera St.	Y = -2.7 + 0.9X	955.10 (1261.2- 723.7)	66800.78	64.1
	Alexandria St.	Y =-3.5 + 1.4X	407.84 (503.5- 330.0)	6661.02	27.37
Profenofos	Susceptible St.	Y = -1.67 +1.4 X	15.47 (18.5 - 12.9)	230.7	-
	El-Behera St.	Y = -4.5 + 1.5X	909.10 1073.3 - 769.9)	11019.74	58.67
	Alexandria St.	Y =-2.5 + 0.9X	955.93 (1277.5 - 715.5)	85872.7	61.79

The LC_{50} values of chlorpyrifos for the three tested strains (susceptible, El-Behera and Alexandria) were 16.48, 2832.8 and 260.09 ppm, respectively; while the corresponding LC_{95} values were 314.8, 284724.2 and 1929.85 ppm, respectively. Comparing the LC_{50} values of the two field strains with that of the susceptible strain showed resistance levels of 171.89 and 15.78 folds for El-Behera and Alexandria respectively. For chlorpyrifos-methyl, the LC_{50} values were 14.9, 955.1 and 407.84 ppm, and the corresponding LC_{95} values were 281.9, 66800.78 and 6661.02 ppm for susceptible, El-Behera and Alexandria strains, respectively; while the calculated resistance values were 64.1 fold for El-Behera strain and 27.37 folds for Alexandria strain. The third tested insecticide, profenofos, LC_{50} values were 15.47, 909.1 and 955.93 ppm and the LC_{95} values were 230.7, 11019.74 and 85872.7 ppm for susceptible, El-Behera and Alexandria strains, respectively; whereas the resistance ratios were 58.67 and 61.79 fold for El-Behera and Alexandria strains, respectively.

Table (2) show that, the obtained data after the 6 hr assessment at the late 2006 cotton season taken the same way where the LC_{50} values for chlorpyrifos were 16.48, 3025.44 and 337.24 ppm for the same order of the tested strains and the corresponding LC_{95} values were 314.8, 110064 and 26.28.87 ppm. The resistance ratios were 183.58 and 20.46 folds for El-Behera and Alexandria strains, respectively. The obtained data for chlorpyrifos-methyl show LC_{50} values of 14.9, 1601.18 and 560.06 ppm and LC_{95} values of 281.9, 42651.1 and 9094.12 ppm for susceptible, El-Behera and Alexandria strains, respectively; while the resistance

values were 107.47 for El-Behera strain and 37.58 for Alexandria strain. The LC_{50} values for profenofos recorded 15.47 ppm to the susceptible strain, 970.16 to Al-Behera strain and 1092.79 to Alexandria strain. The corresponding LC_{95} values were 230.7, 16718.8 and 73257.6 ppm, respectively; while the recorded resistance values were 62.71 and 70.63 fold for El-Behera and Alexandria strains, respectively.

Table 2: Toxicity Parameters and Resistance Ratios of the tested organophosphorous after 6 hr. of treatment at late 2006 cotton season

Insecticides	Strain	Regression Equation	LC_{50} (conf. limits)[1]	LC_{95}	RR[2]
Chlorpyrifos	Susceptible St.	Y= -1.56+1.28 X	16.48 (19.9 - 13.65)	314.8	-
	El-Behera St.	Y = -2.8 + 0.8X	2832.80 (4717.4 - 1709.6)	284724.2	171.89
	Alexandria St.	Y = -4.6 + 1.9X	260.09 (312.8 - 216.1)	1929.85	15.78
Chlorpyrifos-methyl	Susceptible St.	Y = -1.51+1.29 X	14.90 (17.9 - 12.3)	281.9	-
	El-Behera St.	Y = -2.7 + 0.9X	955.10 (1261.2- 723.7)	66800.78	64.1
	Alexandria St.	Y =-3.5 + 1.4X	407.84 (503.5- 330.0)	6661.02	27.37
Profenofos	Susceptible St.	Y = -1.67 +1.4 X	15.47 (18.5 - 12.9)	230.7	-
	El-Behera St.	Y = -4.5 + 1.5X	909.10 1073.3 - 769.9)	11019.74	58.67
	Alexandria St.	Y =-2.5 + 0.9X	955.93 (1277.5 - 715.5)	85872.7	61.79

Regression equation, LC_{50} values, LC_{95} values and the resistance ratios of the tested insecticides for the three strains in early 2007 cotton season after 6 hr of treatment were calculated and then tabulated in table (3). The chlorpyrifos LC_{50} values were 16.48 ppm for the susceptible strain, 3100.1 ppm for El-Behera field strain and 393.51 ppm for Alexandria field strain. The LC_{95} values were 314.8, 43519.2 and 3163.3 ppm; respectively On the other hand, the results show LC_{50} values for chlorpyrifos-methyl of 14.9, 1705.2 and 693.54 ppm and LC_{95} values of 281.9, 20145.6 and 6624.13 ppm, respectively. Also the LC_{50} values of profenofos were 15.47, 1167.03 and 1481.23 ppm while the LC_{95} values were 230.7, 12304.7 and 65143.3 ppm for the previous strains order. The resistance ratios against the three tested insecticides chlorpyrifos, chlorpyrifos-methyl and profenofos were (188.4 and 23.87 fold), (114.44 and 46.54 fold) and (75.43 and 95.74) for both El-Behera and Alexandria Strains, respectively.

Table 3: Toxicity Parameters and Resistance Ratios of the tested organophosphorous after 6 hr. of treatment at early 2007 cotton season

Insecticides	Strain	Regression Equation	LC_{50}	LC_{95}	RR
Chlorpyrifos	Susceptible St.	Y= -1.56+1.28 X	16.48 (19.9 - 13.65)	314.8	-
	El-Behera St.	Y = -4.8 +1.4X	3100.1 (3928.6 - 2118.5)	43519.2	188.4
	Alexandria St.	Y = -4.71 + 1.8X	393.51 (462.9 - 334.4)	3163.30	23.87
Chlorpyrifos-methyl	Susceptible St.	Y = -1.51+1.29 X	14.90 (17.9 - 12.3)	281.9	-
	El-Behera St.	Y =-4.3 + 1.4X	1705.2 (1556- 1048)	20145.6	114.44
	Alexandria St.	Y = -4.8 + 1.7 X	693.54 (810.8 - 593.2)	6624.13	46.54
Profenofos	Susceptible St.	Y = -1.67 +1.4 X	15.47 (18.5 - 12.9)	230.7	-
	El-Behera St.	Y =-4.9 + 1.6X	1167.03 (1375 -989)	12304.7	75.43
	Alexandria St.	Y = -3.2 + 1.0 X	1481.23 (1958.6- 1121.6)	65143.3	95.74

In table (4), the same LC_{50}'s and LC_{95}'s of the tested insecticides for the susceptible strain which mentioned before were installed. The LC_{50}, LC_{95} and the resistance value of chlorpyrifos for El-Behera strain were 3196.62 pm, 49433.2 ppm and 193.97 fold; respectively, while they were 494.1 ppm, 4317.59 ppm and 29.98 fold for Alexandria strain. Chlorpyrifos-methyl LC_{50}, LC_{95} and the resistance value for El-Behera strain were 1749.2 ppm, 31436.5 ppm and 117.39 fold, whereas they were 908.15 ppm, 10932.5 ppm and 60.94 fold, respectively for Alexandria strain. Profenofos LC_{50}, LC_{95} and the resistance value for El-Behera strain were 1338.63 ppm, 18245.1 ppm and 86.53 fold, whereas they were 2344.69 ppm, 26723.3 ppm and 151.56 fold, respectively for Alexandria strain.

Table 4: Toxicity Parameters and Resistance Ratios of the tested organophosphorous after 6 hr. of treatment at late 2007 cotton season

Insecticides	Strain	Regression Equation	LC_{50}	LC_{95}	RR
Chlorpyrifos	Susceptible St.	Y=-1.56+1.28 X	16.48 (19.9 - 13.65)	314.8	-
	El-Behera St.	Y = -4.9 + 1.4 X	3196.62 (4467.6- 2291.4)	49433.2	193.97
	Alexandria St.	Y = -4.7 + 1.7X	494.10 (576.8 - 423.2)	4317.59	29.98
Chlorpyrifos-methyl	Susceptible St.	Y = -1.51+1.29 X	14.90 (17.9 - 12.3)	281.9	-
	El-Behera St.	Y = -4.3 + 1.3 X	1749.20 (2214.7 - 1382.9)	31436.5	117.39
	Alexandria St.	Y = -4.5 + 1.5X	908.15 (1070.5 - 770.5)	10932.5	60.94
Profenofos	Susceptible St.	Y = -1.67 +1.4 X	15.47 (18.5 - 12.9)	230.7	-
	El-Behera St.	Y = -4.5 + 1.5 X	1338.63 (1611.9 - 1112.1)	18245.1	86.53
	Alexandria St.	Y = -5.3 + 1.6X	2344.69 (2940.5 - 1871.1)	26723.3	151.56

Table 5: Toxicity Parameters and Resistance Ratios of the tested organophosphorous after 12 hr. of treatment at early 2006 cotton season

Insecticides	Strain	Regression Equation	LC_{50}	LC_{95}	RR
Chlorpyrifos	Susceptible St.	Y= -0.93+1.11X	6.91 (8.8 - 5.4)	209.3	-
	El-Behera St.	Y = -2.6 + 1.1X	246.25 (339.4 - 178.2)	7718.66	35.63
	Alexandria St.	Y = -4.1 + 1.9X	152.31 (199.9 - 115.8)	596.21	22.04
Chlorpyrifos-methyl	Susceptible St.	Y = -0.66+1.05X	4.26 (5.6 - 3.2)	153.3	-
	El-Behera St.	Y = -2.7 + 1.3X	132.4 (195.4 - 89.4)	2571.21	31.08
	Alexandria St.	Y = -3.7 + 1.6X	243.42 (307.8 - 192.2)	2748.39	57.14
Profenofos	Susceptible St.	Y = -0.73+1.05X	4.98 (6.5 - 3.8)	182.8	-
	El-Behera St.	Y = -4.2 + 1.8X	213.57 (268.3- 169.8)	1729.53	42.88
	Alexandria St.	Y = -2.1 + 0.9X	216.00 (337.8 - 137.5)	15339.43	43.37

2- Toxicity Parameters and Resistance Ratios after 12 hr. of treatment.

The toxicity parameters (LC_{50}, LC_{95}) of chlorpyrifos, chlorpyrifos-methyl and profenofos against the susceptible strain after 12 hr of treatment were obtained and tabulated in all tables of this section as follow: the LC_{50}'s were 6.91, 4.26 and 4.98 ppm, while the LC_{95}'s were 209.3, 153.3 and 182.8 ppm, respectively.

Data in table (5) show that the LC_{50}'s of chlorpyrifos, chlorpyrifos-methyl and profenofos against El-Behera strain were 246.25, 132.4 and 213.57 ppm, while the LC_{95}'s were 7718.66, 2571.21 and 1729.53 ppm, respectively. On the other hand El-Behera strain recorded resistance ratios of 35.63, 31.08 and 42.88 fold against the three insecticides, chlorpyrifos, chlorpyrifos-methyl and profenofos, respectively. Also, the data show that the LC_{50}'s of chlorpyrifos, chlorpyrifos-methyl and profenofos against Alexandria strain were 152.31, 243.42 and 216 ppm, while the LC_{95}'s were 596.21, 2748.39 and 15339.43 ppm, respectively; where the recorded resistance ratios were 22.04, 57.14 and 43.37 fold against the three insecticides, chlorpyrifos, chlorpyrifos-methyl and profenofos, respectively.

Table (6) shows that, the obtained data after the 12 hr assessment at the late 2006 cotton season recorded LC_{50} values of chlorpyrifos equal to 355.38 and 199.7 ppm for El-Behera and Alexandria strains, respectively and the corresponding LC_{95} values were 9342.4 and 1635.2 ppm, while the resistance ratios were 51.42 and 28.9 fold. The obtained data for chlorpyrifos-methyl show LC_{50} values of 410.08 and 280.9 ppm and LC_{95} values of 17877.08 and 2743.4 ppm for El-Behera and Alexandria strains, respectively; while the resistance values were 96.26 fold for El-Behera strain and 65.93 fold for Alexandria strain. The LC_{50} values for profenofos recorded 316.18 ppm against El-Behera strain and 269.97 ppm against Alexandria strain and the corresponding LC_{95} values were 7536.21 and 20228.8 ppm, while the recorded resistance values were 63.48 and 54.21 fold for El-Behera and Alexandria strains, respectively.

Table 6: Toxicity Parameters and Resistance Ratios of the tested organophosphorous after 12 hr. of treatment at Late 2006 cotton season

Insecticides	Strain	Regression Equation	LC_{50}	LC_{95}	RR
Chlorpyrifos	Susceptible St.	Y= -0.93+1.11X	6.91 (8.8 - 5.4)	209.3	-
	El-Behera St.	Y = -2.9+ 1.2X	355.38 (459.5 - 274.4)	9342.40	51.42
	Alexandria St.	Y = -4.1 + 1.8X	199.70 (249.0 - 159.9)	1635.2	28.90
Chlorpyrifos-methyl	Susceptible St.	Y = -0.66+1.05X	4.26 (5.6 - 3.2)	153.3	-
	El-Behera St.	Y = -2.6 + 1.0X	410.08 (545.6 - 307.7)	17877.08	96.26
	Alexandria St.	Y = -4.1 + 1.7X	280.90 (344.6 - 228.7)	2743.4	65.93
Profenofos	Susceptible St.	Y = -0.73+1.05X	4.98 (6.5 - 3.8)	182.8	-
	El-Behera St.	Y = -2.99 + 1.2X	316.18 (418.7 - 238.3)	7536.21	63.48
	Alexandria St.	Y = -2.1 + 0.9X	269.97 (404.8 - 179.4)	20228.8	54.21

The presented data in table (7) discuss the toxicity parameters that were observed after 12 hr of treatment at the early 2007 cotton season. The LC_{50}'s of chlorpyrifos, chlorpyrifos-methyl and profenofos against El-Behera strain were 351.93, 385.05 and 268.82 ppm, while the LC_{95}'s were 12418.7, 9168.18 and 5735.3 ppm, respectively. The recorded resistance ratios were 50.93, 90.38 and 53.97 fold against chlorpyrifos, chlorpyrifos-methyl and profenofos, respectively. Also, the data show LC_{50}'s of chlorpyrifos, chlorpyrifos-methyl and profenofos against the Alexandria strain equal to 213.77, 303.08 and 406.39 ppm, while the LC_{95}'s were 2650.1, 4610.29 and 32773.9 ppm, respectively; where the recorded resistance ratios were 30.96, 71.14 and 81.6 fold against the three examined insecticides, respectively.

Table 7: Toxicity Parameters and Resistance Ratios of the tested organophosphorous after 12 hr. of treatment at early 2007 cotton season.

Insecticides	Strain	Regression Equation	LC_{50}	LC_{95}	RR
Chlorpyrifos	Susceptible St.	Y= -0.93+1.11X	6.91 (8.8 - 5.4)	209.3	-
	El-Behera St.	Y = -2.7 + 1.1X	351.93 (465.7 - 265.5)	12418.7	50.93
	Alexandria St.	Y = -3.5 + 1.5X	213.77 (275.1 - 165.9)	2650.10	30.96
Chlorpyrifos-methyl	Susceptible St.	Y = -0.66+1.05X	4.26 (5.6 - 3.2)	153.3	-
	El-Behera St.	Y = -3.1+ 1.2X	385.05 (490.8 - 301.7)	9168.18	90.38
	Alexandria St.	Y = -3.5 + 1.4X	303.08 (383.9 - 239.0)	4610.29	71.14
Profenofos	Susceptible St.	Y = -0.73+1.05X	4.98 (6.5 - 3.8)	182.8	-
	El-Behera St.	Y = -3.1 + 1.2X	268.82 (357.2 - 201.9)	5735.3	53.97
	Alexandria St.	Y = -2.3 + 0.9X	406.39 (567.9 - 290.2)	32773.9	81.60

The installed data in table (8) shows the LC_{50}, LC_{95} and the resistance value of chlorpyrifos for El-Behera strain were 421.82 pm, 11085.7 ppm and 61.04 fold; respectively, while they were 348.63 ppm, 2778.14 ppm and 50.54 fold for Alexandria strain. Chlorpyrifos-methyl LC_{50}, LC_{95} and the resistance value for El-Behera strain were 721.45 ppm, 21173.3 ppm and 169.35 fold, whereas they were 637.76 ppm, 6538.82 ppm and 149.7 fold, respectively for Alexandria strain. Profenofos LC_{50}, LC_{95} and resistance value for El-Behera strain were 418.04 ppm, 8544.23 ppm and 83.94 fold, whereas they were 1677.16 ppm, 21943.8 ppm and 336.77 fold, respectively for Alexandria strain.

Table 8: Toxicity Parameters and Resistance Ratios of the tested organophosphorous after 12 hr. of treatment at Late 2007 cotton season

Insecticides	Strain	Regression Equation	LC_{50}	LC_{95}	RR
Chlorpyrifos	Susceptible St.	Y= -0.93+1.11X	6.91 (8.8 - 5.4)	209.3	-
	El-Behera St.	Y = -3.0 + 1.2X	421.82 (533.1 - 333.5)	11085.7	61.04
	Alexandria St.	Y = -4.6 + 1.8 X	348.63 (411.7 - 295.1)	2778.14	50.54
Chlorpyrifos-methyl	Susceptible St.	Y = -0.66+1.05X	4.26 (5.6 - 3.2)	153.3	-
	El-Behera St.	Y = -3.2 + 1.1X	721.45 (894.6 - 581.8)	21173.3	169.35
	Alexandria St.	Y = -4.6 + 1.6 X	637.76 (746.3 - 544.9)	6538.82	149.70
Profenofos	Susceptible St.	Y = -0.73+1.05X	4.98 (6.5 - 3.8)	182.8	-
	El-Behera St.	Y = -3.3 + 1.3X	418.04 (525.3 - 332.39)	8544.23	83.94
	Alexandria St.	Y = -4.8 + 1.5 X	1677.16 (2045.3 - 1376.1)	21943.8	336.77

The intensive and continuous use of the tested organophosphorous compounds in controlling cotton bollworms in Egypt either by the application program of the Ministry of Agriculture or by farmers themselves lead to the development of resistance in pink bollworm strains. The obtained data showed that the resistance levels were increased from early to late season and from one season to another. These findings are in agreement with Tabashnik, (1986) who recorded resistance ratios in diamondback moth *Plutella xylostella* equal to 130 folds against DDT. Magaro and Edelson (1990) reported that the RR based on LC_{50} in diamondback moth was 145 folds for methamidophos. The finding of Plapp *et al* (1990) was in harmony with the present data when high levels of resistance were detected in tobacco budworm collected in the spring. Osman *et al* (1991) found that the field strain of PBW reverted to resistance levels close to susceptible laboratory strain at five generations after removing insecticidal pressure. Furthermore, Campanhola *et al* (1996) reported that, removing selection pressure for

one or more generation, alternating insecticides, and changing control strategies decreased the frequency of gene resistance to a level where control can become possible again. Finally, Shekeban (2002) recorded high levels of resistance in pink bollworm field strains by the late 2000 cotton season against profenofos, chlorpyrifos-methyl and chlorpyrifos, these levels were 33.82, 44.79 and 35.16 fold, respectively. Also, reported that by the late 2001 cotton season the resistance levels were increased to be 38.45, 50.26 and 42.3 fold.

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ESTERASE MECHANISMS OF THE RESISTANCE FORMING IN HOUSEFLY (*MUSCA DOMESTICA* L.) TO INSECTICIDES

ABSTRACT

The esterase mechanisms of the housefly resistance in the process of resistance forming to insecticides from three chemical classes: organophosphates, pyrethroids and derivate of benzylphenylurea were researched in this investigation. It is revealed that activity of non-specific esterases increased twice as much in the resistant strains as compared with sensitive strain already in 6-12th generations.

INTRODUCTION

Esterases are ferments, catalised reactions of ethers hydrolysis of alcohols with organic and inorganic acids. The term “esterases” usually mark only hydrolases of ethers of carbon acids. Carboxylesterases and arilesterases are often united in a group of nonspecific esterases. Functional value of insect esterase is defined in their participation in the regulation of hormone titre, metabolism and mobilization of fats, nervous tissue, syntheses and somewhat transport cuticule waxes, reproduction, as well as degradation of different xenobiotics **(**Hrunin, 2001**)**. Insect esterases are able to participate in

metabolizing the three most used, in present class, insecticides – organophosphates (OP), carbamates and pyrethroids. The molecules of all of these contain the ether**s,** which can be hydrolised by esterases.

OP- resistant insects are characterized usually by raised hydrolyze activity, accompanied by increased contents of nonspecific esterases. Currently, there is data about the role of the esterase hydrolysis in pyrethroid metabolism. Different structures of pyrethroids, as well as trance- and cis- izomers are hydrolyzed by means of different esterases. In the majority of insect species, hydrolysis of cis- izomers and cian maintained insecticides occurs slowly. There is information that in majority of dipteras and coleopteras metabolism of pyrethroids is realized by means of monooxygenases, but in lepidopteras and homopteras, by means of esterases (Jshaaya, Casida, 1981).

T. Sparks and B. Hammock (1983) conducted the study of biochemical resistant mechanisms to chitin synthesis inhibitors using as an example diflubenzuron by means of synergist in houseflies. The use of esterase inhibitor DEF (S, S, S-threebutylphosphorothreetioat) and transferase inhibitor dietylmaleat (DEM) has shown the absence of the strong synergism. This means that esterases and transferases do not take part in majority of the process in detoxication of diflubenzuron.

In this investigation, esterase mechanisms of housefly resistance in process of the resistance forming to insecticides of different chemical classes were researched.

MATERIAL AND METHODS

The objects of the studies were imago of one sensitive strain and six selected strains of housefly. The selection used the following insecticides: from class OP - phosmet (phtalophos, 20% e.k.), phoxim (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 determination of resistance index (RI) to selectants conducted was described earlier (Sokolyanskaya, 2007).

The activity of nonspecific esterases was defined in homogenate from whole flies on rate of hydrolysis α-naphtyl acetate (Van Asperen, 1962). Housefly imago were homogenized in 800 мg of China mortar and 4 ml of 0.05M tris-HCl buffer, pH=8.3, consisting of 1mM ditiotreytol, 1mM phenyltiourea, 0.05 mg/l triton X-100. After filtration across 2 layers of gauze, homogenate was centrifuged for 15 minutes by 10000 g. All operations were conducted by a temperature between 0-4°C. Supernatant was used for definition of nonspecific esterase activity. The reaction mixture consisted of 2 ml of 0.1M sodium-phosphate buffer, pH=7.0; 1.0 ml of $6x10^{-4}$ M water solution α-NA (2% acetine) and 50 µl supernatant. After incubation for 15 minutes, the 1 ml mixture consisted of 1% fast blue B and 5% SDS (2:5) was added to stop the reaction. Optic density was measured at 600 nm.

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

RESULTS AND DISCUSSION

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

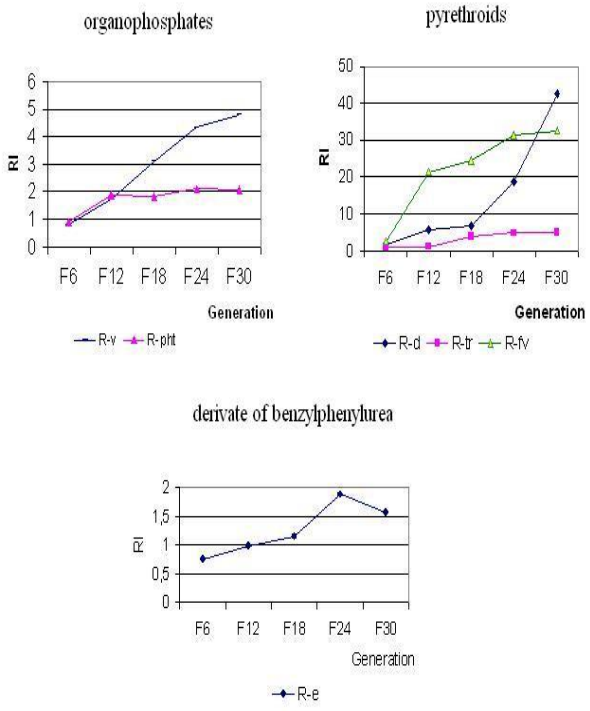

Fig. 1. The dinamic of resistanse formation to insecticides from three chemical classes in the selected strains of housefly.

The esterase activity in process of the selection changed as follows (fig. 2).

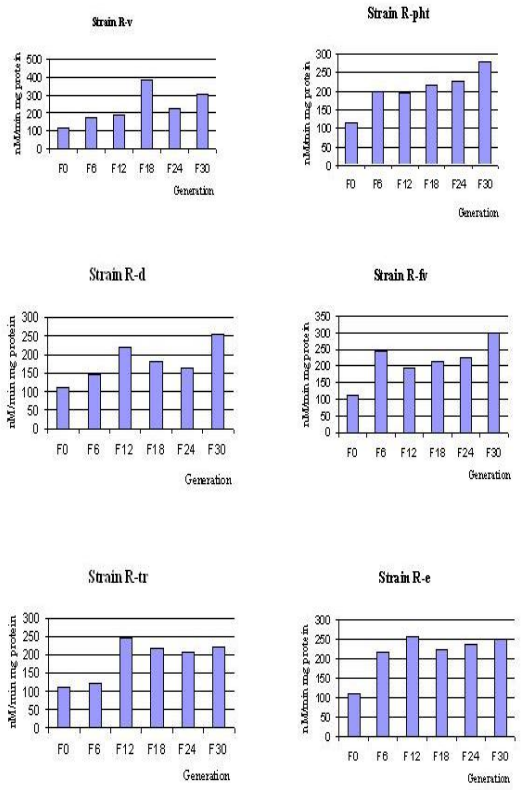

Fig. 2. The change of esterase activity in process of the selection of the housefly (all differences of selected strain with sensitive are reliably, P>0,95, besides of in F6 strain R-tr)

In phoxim-selected strain (R-v) activity of esterases in 6th and 12th generations increased 1.5 times as compared with sensitive strain, in 18th generation - 3.5 times. In the 24th generation activity of the ferment decreased a little, but however it exceeded that twice as much in S strain. In phosmet-selected strain (R-pht) in 6th and 12th generations, activity of the ferment increased nearly 2 times above as compared with sensitive strain, in 18th generation it increased insignificantly and remained on reached level in 24th generation. Increase activity of esterases play a significant role in insects forming resistance to OP. This was noted earlier as for houseflies (Kao et al., 1984), and for the other insects: mosquitoes *Culex pipiens* (Maruyama et al., 1984) and *C. tritaeniorhynchus* (Sun et al., 1988), aphids (Li et al., 2003) and many other species.

The activity of nonspecific esterases in the strain, selected with ethophenprox (R-et), in 6th generation remained at a rate of sensitive strain; up to 12th generation it increased 2 times and remained such until 30th generation. In strain R-d in 6th generation activity of this ferment increased 1.3 times, up to the 12th generation it increased 2 times as compared with initial value. There was the small reduction of activity (in 18th and 24th generations), but in 30th generation it increased again (2.3 times as compared with S strain). In fenvalerate-selected strain (R-fv) activity of esterases increased 2 times already in 6th generation and remained on this level until 24th generation, but in 30th generation the level of activity increased 3 times as compared with sensitive strain. Thereby, nonspecific esterases play the essential role in the resistance forming to pyrethroids in houseflies. Raised esterase activity defines resistance to pyrethroids in many insects: dustpan *Spodortera littoralis* (Riskallah, 1983), aphids *Aphis gossypii* (Tan et al., 1988), as well as tolerance of larvae of the common green (Ishaaya, Casida, 1981).

In flies, selected with chlorfluazuron (strain R-e), activity of nonspecific esterases in 6th generation increased nearly in 2 times as compared with sensitive strain; in 12th generation these esterases increased until the 18th generation when they fell to the level of 6th generation. However, in 24th and 30th they increased slightly.

In the 6-12th generations nearly all variants of the selection activity of nonspecific esterases increased 2 times as compared with sensitive strain. In the processes of the 24-30th generation, activity of esterases changed insignificantlly and by increasing the resistance in some strains reliable increase of activity did not existed. The variety of substrates, in metabolism of which nonspecific esterases take part, probably, is defined by polymorfism of this ferment groups, and, consequently, broadens substrate specificity.

From the data collected it appears that by means of biochemical methods (the determination of activity of nonspecific esterases) resistance forming in a population can be detected earlier, than by means of toxicological methods (tab. 1). In this instance in 6th and 12th generations the resistance forming is registered in only two strains (selected by delthamethrin and fenvalerat), but esterase activity and the amount of citochrome P-450 is higher when compared with the sensitive strain in 6th generation in four strains. The same is true in 12th generation in all six selected strains. This fact of allowing confirmation earlier allowed a researcher (Brogdon et al., 1992) to conclude that biochemical methods provide the reliable factors for determining resistant individuals as well as enables the ability to catch as even earlier periods the resistance forming to systematically applicable pesticides, than toxicological.

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

CONCLUSION

The studies and analysis of literary data shows that esterase play a significant role in resistance forming to insecticides from different classes in insects. These ferments, often forming an insect cross-resistance, were the first enlarge its activity in response to the action used on the selectants. The study of biochemical, in particular esterase, mechanisms forming resistance between insects and insecticides with different mechanisms allows a more valid approach to the choice of high effective preparations, as well as to tactics of its rotation in respect to the insect populations, subjected to constant checking in part by man.

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Study on the resistance insecticides on the cucumber leafminer *Liriomyza sativae* (Blanchard) (Dip: Agromyzidae) under laboratory condition

ABSTRACT

Leafminers of vegetables are the major pests of greenhouse cucumber in Iran. One of the reasons for repeated outbreaks of leafminers during recent years is attributed to application of some ineffective insecticides. A recent study was carried out to determine the toxicity (LC_{50}) of Abamectin 1.8% EC, Cyromazin 75% WP and Spinosad 24% SC insecticides. Bioassay tests were done on first and last-instars larvae and adults under the laboratory conditions 25±1, 65±5% R.H., and 16:8 photoperiod of L: D. Data was analyzed using analysis POlO PC software. Results (based on ppm active ingredient) revealed that for abamectin the LC_{50} for first and last instars larvae and adults were 1.5, 1.8, 14.3 ppm respectively, the values of LC_{50} for cyromazin were 34.8, 38.4, 1295 ppm and the values of LC_{50} for spinosad were 4.4, 12.1 and 137 ppm. The results showed that the toxicity of the three insecticides were higher in larvae stages than adult insects. It also revealed that the cyromazin insecticide was not effective on the control adults, and was only effective on the larvae ages, therefore, it could be concluded that abamectin and spinosad are the only insecticides that have high efficiencies on the different control larvae stages and adult leafminers. After determining the LC_{50} of the three insecticides in comparison with the race source in the method of Denholm & *et al.*, (1984) the ratio of resistance for abamectin on the first instars was 8.8, for cyromazin on the first instars was 5.3 and resistance ratio for spinosad was 3.3-fold. Based on the results, resistance to abamectin on the first instars was available. Additionally, there was no cross-resistance among these three insecticides.

KEY WORDS: *Liriomyza sativae*, Abamectin, Cyromazin, Resistance ratio

INTRODUCTION

The Leaf mining flies (LMF), *Liriomyza trifolii* (Burgess), and *L. sativae* (Blanchard) are important quarantined pests for a wide variety of vegetables and flower crops in different countries such as Iran (Bani Ameri, 2003). The adult flies puncture

the leaves of the plant for feeding and ovipositor, resulting in stippling. The larvae reduce the photosynthetic activity of the leaves by mining, ultimately, the leaves dry out and the plant is defoliated (Parrella *et al.,* 1985). This species (*L. trifolii*) of leafminer fly is tolerant to many insecticides and capable of developing a resistance to products that were originally effective. It is therefore becoming more difficult to control LMF worldwide. In particular, identifying *L. sativae* and *L. trifolii* is important because of their resistance to different insecticides (Spencer, 1990). Two main insecticides are used to control the leafminer on potatoes in Israel, abamectin and cyromazine, both of which are translaminar and affect the larvae. Neem-based insecticides, although effective against *L. huidobrensis* (Weintraub and Horowitz, 1997), are prohibitively expensive for non-organic agriculture in Israel.

The impact of chemicals on beneficial can be more severe than on target pests, resulting in pest outbreaks. Many pesticides, including pyrethroids and organophosphates, are considered ineffective against *L. huidobrensis* because of resistance (Weintraub and Horowitz 1995). Abamectin and cyromazine have provided effective control of *L. huidobrensis* in several countries (Hammad *et al.,* 2000), although these chemicals and in particular abamectin may be harmful to beneficial, including parasitoids (Shipp *et al.* 2000). Abamectin, cartap, and cyromazine have been commonly employed to control serpentine leafminers. However, grower's reliance on a few numbers of insecticides has brought up some insecticide resistance management issues (Minkenberg and Van Lenteren, 1986). The repeated use of these chemicals has the capacity of selecting insecticide resistant leafminer populations (Ferguson, 2004) and thus impairing an important tool to insect control. Also, in several cases, leafminers are not the only pests attacking the crop.

An insecticide that might have those features is the spinosad. Spinosad belongs to a new group of insecticides and it is originated from the fermentation process of soil bacteria *Saccharopolyspora spinosa*. In Brazil, spinosad is already registered to be used on potato crops to control *Liriomyza huidobrensis* (Blanchard) (Diptera: Agromyzidae) at a rate range from 163.2 to 201.6 g ai/ha (Agrofit, 2007). Also, the usage of spinosad can provide a new alternative for controlling *L. huidobrensis* on dry beans. Due to its unique mode of action, spinosad might be used as an active component of insecticide rotation programs.

Due to the low damage threshold on some crops, notably leafy vegetables and ornamentals, the most widely used method of leaf miner control is through the application of insecticides (Cox and *et al.,* 1995). Some insecticides retain effectiveness for only 2 years after introduction (Leibee 1981, Parrella and *et al.,* 1985). In some countries such as Indonesia, the range of insecticides is routinely applied to control leaf miners (Rauf and *et al.,* 2000). Insecticide applications can reduce parasitism by indigenous parasitoid wasps and also decrease the number of the predatory *Coenosia humilis*, overall reducing the control of leafminers. However two insecticides have been used successfully for leafminer control since the mid-1980s: cyromazin, (N-cyclopropyl-1,3,5-triazine-2,4,6-triamine), an insect growth regulator (IGR), is registered on many vegetables (Trigard); abamectin, a GABA agonist, is also registered on many vegetables (AgriMek). An alternative control strategy involving the applications of Abamectin led to a reduction in leafminers without harmful effects on parasitoids and predators. Abamectin (1.8%) applications provide one potential component of an effective *Liriomyza* control strategy for Indonesian potato farmers. It has been suggested that the addition of certain penetrating surfactants may increases translaminar movement and insecticidal activity on pests that mine within leaves and feed on lower leaf surfaces (Larson, 1997).

The wide spectrum of compounds to which *L. sativae* is resistant, suggests that enhanced metabolism of insecticides, altered target site sensitivity, or both, might account for the observed resistance.

In this study, we considered the toxicity of insecticides commonly used against *L. sativae* to both adult and immature stages of the leafminer. In Iran, the translaminar pesticides, abamectin and cyromazine, are commonly used to control *L. sativae*. The studies determined the response of *L. sativae* population to the insecticides from applications of cyromazine, abamectin, and a new leafminer control product, spinosad, and determined the resistance ratio (RR) for the above-mentioned insecticides.

MATERIALS AND METHODS

Host plant culturing

Cowpea was seeded in 10-cm-diampots (4–6 per pot) with holes in the bottom, with soil consisting of equal parts peat moss, vermiculite and sand. Plants were grown at 25±2^{oC}, with a photoperiod of 14:10 h (L: D), until two true leaves were fully expanded, the negin variety for bioassay tests.

Leaf miner rearing

The larvae were collected from infested cucumber from the greenhouses in Pakdasht (adjacent of Tehran) on September 2007. They were transferred to the growth chamber for rearing at 25± 2^{oC}, 60-65% RH, maintained until the emergence of pupa, then transferred in cages that included bean pots.

Insecticide

The insecticides that were used in our experiments were Cyromazine (Trigard 75% WP, Syngenta Crop Protection, Greensboro, NC), that is an insect growth

regulator type of insecticide to control leafminers, Abamectin (Agrimec 0.15 EC, AgriEvo Crop Protection) is a fermentation metabolite of the actinomycete *Streptomyces avermitilis*, a soil inhabiting microorganism, and Spinosad (Spinosin 24% SC) is a fermentation metabolite of the actinomycete *Saccharopolyspora spinosa*, a soil-inhabiting microorganism, and has a novel mode of action that provides excellent crop protection with a relatively low toxicity to non-target organisms, including many beneficial insects.

Bioassays

The bioassay experiments were carried out according to the Cox *et al.* (1995) method and at larval stage of the pest. Additionally, in commercial practice, insecticide applications are directed against the larval stage. Sixty-four young (10-14-d-old) cucumber plants were caged (in lumite-screened cages) and exposed to several hundred (3-4-d-old) flies for an oviposition access period (OAP) of 4-6 h. The short time period for OAP allowed for a synchronous egg hatch and age of the larvae that were present at treatment. After OAP, plants were removed and held in the laboratory condition (25± 2^{oC} and ambient light) for 96 h to allow eggs to hatch and small mines to develop. Cucumber plants were divided into groups containing equal numbers of mines, 75-200 per dose. The leaves and part of the stem were treated by submersion for 15 s into the appropriate serial dilution of insecticide in distilled water. Five to seven doses were used in each bioassay. In the bioassays the leaf-dipping technique was employed against first and last instars larva. Mortality of larva observed continuously and recorded at 24h intervals for abamectin & spinosad, and recorded at 72h intervals for cyromazin. Based on prior experience, bioassays of the larva were conducted with cyromazine at 25 ppm, which was reported to result in ~95% larva- mortality and for abamectin and spinosad at 9 ppm, were reported to result in ~95% larva-mortality.

Data was analyzed using probit analysis procedure and POLO PC software.

RESULTS AND DISCUSSION

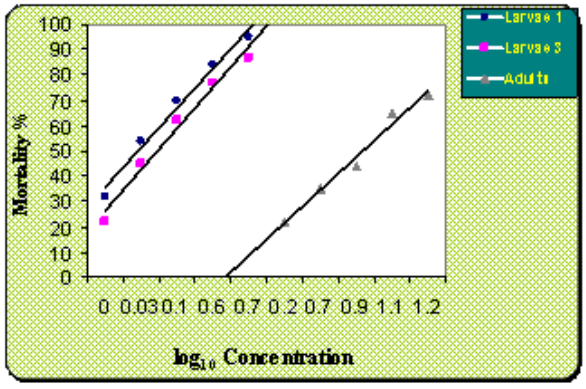
Bioassay results with cyromazine, abamectin, and spinosad are presented in Table 1, 2 and 3. Based on larval mortality, the LC_{50} value with cyromazine, abamectin and spinosad results (based on ppm active ingredient) revealed that for abamectin the LC_{50} for first and last instars larvae and adults were 1.5, 1.8, 14.3 ppm respectively (Table 1). Based on the experiences obtained with abamectin, the toxicity in larvae stages is higher than in adult insects (Figure 1). This is supported by the findings of Mujica *et al.*, (2000) who reported the LC_{50} value of abamectin as 1.1 ppm while it has no adoption with Vanderveire *et al.*, (2002) studies. In his studies the LC_{50} value of abamectin was 6.7 for the first instar larvae. Also, based on Prijono *et al.*, (2004) research the defined LC_{50} value of abamectin was 1.8 for the first instar of leafminer that is adopted with this search. Based on the findings of Ferguson *et al.*, (2004) the LC_{50} values of abamectin on various populations of the leafminer were 0.3, 3.7, 5.2 ppm respectively.

Table 1- Effect of Abamectin on leafminer fly larvae and adult

Stage	n	LC (mg(a.i)/l)			Slop (±SE)	R	Chi-Square	RR*
		LC_{90}	LC_{50}	LC_{10}				
Larvae I	198	8.86	1.51	0.26	1.6 ±0.25	3	0.34	8.8
Larvae III	210	14.4	1.81	0.28	1.19 ±24	3	0.34	-
Adults	180	251	14.3	0.81	1.02 ±0.31	3	0.84	-

* Resistance ratio of the LC_{50} value relative to the UCR reference strain.

Figure 1: Compression the LC_{50} value of abamectin on the leafminer larvae and adults



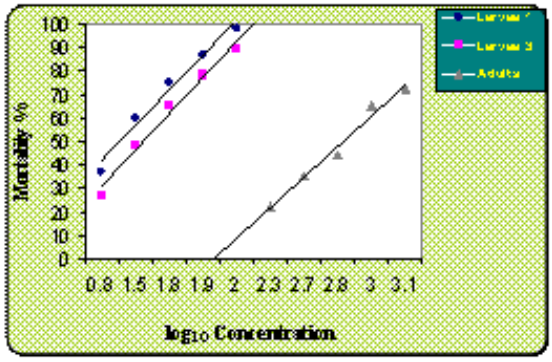
Additionally the results indicated that the LC_{50} value for pure cyromazin for first and last instars larvae and adults were 34.8, 38.4, 1295 ppm respectively (Table 2). Result showed that cyromazin insecticide is not effective to the control adults, and only was effective on the larvae ages, therefore, it could be concluded that cyromazin is the only insecticide that has the highest efficiencies on the control of different larvae stages leafminer (Figurer 2). In the current study, LC_{50} values of a collected strain *L. trifolii* were 34.8 ppm. This is supported by the findings Ferguson *et al.*, (2004) who reported that a LC_{50} value of abamectin 35.1 ppm while it has no adoption with Prijono *et al.*, (2004) studies, in his studies the value abamectin LC_{50} was 12 for the first instars.

Table 2 - Effect of Cyromazin on leafminer fly larvae and adult

Stage	n	LC (mg(a.i)/l)			Slop (± SE)	R	Chi-Square	RR*
		LC_{90}	LC_{50}	LC_{10}				
Larvae I	167	253	34.8	4.8	1.4 ±0.26	3	0.18	5.3
Larvae III	192	561	38.4	6.2	1.09 ±0.36	3	0.15	--
Adults	180	107.3	11.7	1.27	1.3 ±0.31	3	0.72	--

* Resistance ratio of the LC_{50} value relative to the UCR reference strain.

Figure 2: Compression the LC_{50} value of cyromazin on the leafminer larvae and adults



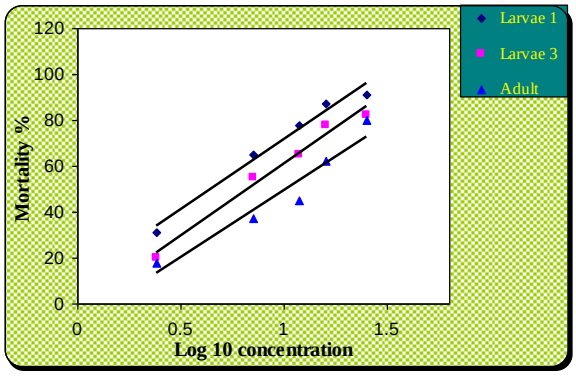
Based on larval mortality, the LC_{50} value with spinosad of the first and last instars larvae and adults were 4.4, 12.1 and 137 ppm respectively (Table 2). This is supported by the findings of Ferguson *et al.*, (2004) the defined LC_{50} value of spinosad was 2.53 and 1.5 for the first instar of leafminer *L. trifolii*. As results show, the toxicity of the three insecticides in larvae stages is higher than in adult insects. Results showed that abamectin and spinosad are the only insecticides that have high efficiencies on the control of different larvae stages and adults leafminers.

Table 3 - Effect of spinosad on leafminer fly larvae and adult

Stage	n	LC (mg(a.i)/l)			Slop (±SE)	R	Chi-Square	RR*
		LC_{90}	LC_{50}	LC_{10}				
Larvae I	218	23.8	4.4	0.8	1.7 ±0.23	3	0.11	3.3
Larvae III	205	81.1	12.1	1.8	1.4 ±0.2	3	0.26	--
Adults	180	107	13.7	2.2	1.3 ±0.31	3	0.57	--

* Resistance ratio of the LC_{50} value relative to the UCR reference strain.

Figure 3: Compression the LC_{50} value of spinosad on the leafminer larvae and adults



After determining the LC_{50} value of the three insecticides in comparison with the race source in the method of Denholm & *et al.*, (1984) it states that the use of laboratory strains to detect and monitor resistance disregards the fact that the response of a natural population changes continuously as the pests come into contact with insecticides, in relation to the reference strain, obtained with the RR (resistance ratio) for abamectin, cyromazine and spinosad.

In the current study, LC_{50} values of the collected strain were in line with those of the UCR reference strain. Bioassay results with cyromazine, abamectin, and spinosad with the UCR reference strain are presented in Table 4. A comparison of the response of the reference strain was made to the St. Lucie-tomato strain, collected from a commercial tomato field. Bioassays were conducted on the F1 of the field strain (Ferguson *et al.*, 2004). Based on the experiences obtained, LC_{50} values for abamectin for first instars larvae were 1.5 ppm which that of the LC_{50} values UCR strain was 0.17 ppm (Table 4). The resistance ratio of the LC_{50} value is relative to the UCR reference strain. Thus for abamectin, there was an approximate 8.8-fold increase in LC_{50} value in the UCR strain. This was evidently highly resistant in comparison to the UCR strain. The cyromazine for first instars larvae was 34.8 ppm, which is a 5.3-fold increase in LC_{50} value of the UCR strain, (Table 2, 4). With spinosad, there was an approximate 3.3-fold increase in LC_{50} value in the reference strain over that of the UCR strain.

Table 4– Response of *L. trifolii* UCR reference strain to cyromazin, abamectin and spinosad

Stage	Cyromazine				Abamectin				Spinosad			
	n	χ^2	Slope (±SE)	LC_{50} ppm (95% CI)	n	χ^2	Slope (±SE)	LC_{50} ppm (95% CI)	n	χ^2	Slope (±SE)	LC_{50} ppm (95% CI)
Larvae	120	3.4	8.33 (0.75)	6.46 (5.92–7.0)	120	19.3	4.31 (0.52)	0.17 (0.13–0.21)	120	18.4	10.72 (3.56)	1.35 (0.57–1.62)
Pupuria	120	9.3	3.89 (0.75)	3.13 (2.42–4.04)	120	32.5	1.52 (0.30)	0.21 (0.13–0.35)	120	6.1	4.24 (0.90)	1.16 (1.0–1.53)

Based on significant and dramatic increased LC_{50} values compared with a laboratory and field-collected population, our results indicated that *L. sativae* from cucumber greenhouse production operations had developed resistance to the very different modes of action between these three compounds (an IGR [cyromazine] and a nerve toxicant [abamectin] and biotic insecticide [spinosad]). This would also indicate that cross-resistance would not be expected. Given the different modes of action and no cross-resistance among these three insecticides, they should be considered in rotation for leaf miner control in an insecticide resistance management strategy.

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Abstracts in Resistance Management

Baseline Susceptibility of Oriental Fruit Moth (Lepidoptera: Tortricidae) to Selected Insecticides

The oriental fruit moth (OFM), *Grapholita molesta* (Busck), has been a serious pest of peaches, apples, and other fruit crops since its introduction into North America in 1916. Severe infestations were reported in IL for apples in 2006, and OFM populations in southwestern IL are thought to be

resistant to pyrethroids. Populations in other parts of North America have been shown to exhibit resistance to pyrethroids as well as organophosphates (Kanga et al. 2003; Kanga et al. 1997). Recent increased losses to OFM threaten the viability of apple and peach production in southern and southwestern IL and the lower Midwest.

The focus of an ongoing project at the University of Illinois is to describe baseline susceptibility for larvae of two OFM colonies to key insecticides, including newly available reduced-risk insecticides and insecticides approved by OMRI (Organic Materials Review Institute).

Initial efforts have focused on chlorantraniliprole, spinetoram, and acetamiprid. To assess concentration-response relationships for these insecticides neonates were placed on diet blocks containing a range of concentrations (approximately 200 larvae per dose). Mortality was assessed after 96 hours. Two colonies are being tested separately, a long-term lab colony from Rutgers University and a colony established from a southwestern Illinois field population (Calhoun County) in the summer of 2007. The LC50s expressed as ppm in diet, for Rutgers and Calhoun colonies respectively were 0.077, 0.078 for chlorantraniliprole; 0.040, 0.057 for spinetoram; and 0.300, 0.402 for acetamiprid. The LC90s expressed as ppm in diet for Rutgers and Calhoun colonies respectively were 0.281, 0.382 for chlorantraniliprole; 0.136, 0.207 for spinetoram; and 0.470, 0.667 for acetamiprid.

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Spatial Analyzed Region of Risk to Dengue Hemorrhagic Fever (DHF) and A*edes aegypti* Linn Resistance to *Temefos* in Padang

The objective of this research is to investigate the *Aedes aegypti* resistance to *temefos* in sub-district and also know the region of risk to occurrence of DHF. The research is observing the larvae density based on the *Breteau Index* (BI), and *quasi experimental* 3th and 4th larvae of *Aedes aegypti* with ovitrap, which were colonized for biochemical tests with colour eye score and *Elisa reader l* 450 nm.

The resistance was detected from esterase enzyme activity (*a-acetat napthyl* and β-*acetat napthyl*) showing a change of colour as a reaction to the homogenate micro-plate and filter paper spot technique. The results went from uncoloured to deep blue, as according to resistance level from sensitive (SS), tolerant (RS) to resistance (RR). Region of risk based distribution data contained water.

The results of the research for esterase enzyme *activity a-acetat napthyl* is tolerant (RS) by a colour eye score mean of 2, with ELISA reader (SS), AV <0,700 900 =" 18.66"> 0.900 = 3.67 %. While that β-*acetat napthyl* is sensitive (SS) by a colour eye score mean of 2.05 and with ELISA reader (SS) = 82.34 %, (RS) = 14.67 % and (RR) = 2.99 %.

The results analyzed by *Oneway* ANOVA significantly indicated that there was statistically significant *Aedes aegypti* resistance to *temefos*, and a multi comparison test of Post Hoc by Turkey's HSD got results of a resistance difference between sub-districts Andalas, Jati, and Teluk Bayur with other sub-districts.

Spatial analyzed the results of the region of risk occurrence of DHF based on *Breteau Index* (BI). The distribution data obtained by the results were 42.86 % of sub-districts at medium risk and 21.43 % in danger. For the Padang city (BI), the mean was 35 which is a high risk.

Keywords: *Aedes aegypti – Resistance – Temefos – Spatial - Breteau Index*

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