Resistant Pest Management Newsletter

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

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Letter from the Editors

Dear Newsletter Subscribers,

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

Sincerely,
Abbra Puvalowski
RPMNews Coordinator

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

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

Conclusion

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

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

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

ABSTRACT

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

these products are exerting selection pressure in Bt-cotton. Consequently, a systematic resistance management program to detect changes in bollworm population susceptibility is needed in order to preserve the effectiveness of chemical products if Cry1Ac resistance is developed.

Keywords: Helicoverpa zea, Bacillus thuringiensis, Bt-cotton, response base lines, remedial action plan.

INTRODUCTION

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

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

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

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

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

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

MATERIALS AND METHODS

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

Susceptibility of three H. zea populations was evaluated. The Comarca Lagunera population (LAG) was established by collecting 300 larvae from transgenic cotton fields in the Ejido Patrocinio, Municipio de San Pedro, Coahuila. The CESTAM population was initiated with 200 larvae collected from maize in the Estación Cuauhtémoc, Municipio de Altamira, Tamaulipas (CESTAM). The susceptible Stoneville population (STON) came from the USDA-ARS Southern Insect Management Research Unit (SIMRU), which has been sustained without exposure to insecticides for over 25 years. It has also been maintained as a breeding colony since 2003 in the Entomology laboratory of CESTAM and was used as a reference population.

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

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

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

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

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

RESULTS AND DISCUSSION

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

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

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

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

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

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

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

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

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

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

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

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

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

If traditional insecticide control practices against H. zea continues, and Bt-cotton and other transgenic crops like Bt-corn are widely use, is possible that the selected agent (insecticide or Bt resistance) may increase to unmanageable levels for which there would be no effective tools for controlling resistant H. zea populations. This situation is a matter of concern given that the development of new products with different modes of action to what is currently available has not kept up with the demand, and those that are developed will be progressively less effective.

CONCLUSIONS

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

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

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

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

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

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

MATERIALS AND METHODS

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