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TOXICITY OF *BACILLUS THURINGIENSIS* CRY1-TYPE INSECTICIDAL TOXIN TO GEOGRAPHICALLY DISTANT POPULATIONS OF TOMATO PINWORM

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The tomato pinworm (TPW), *Keiferia lycopersicella* (Walsingham), is an important pest of tomato in southern California (Oatman 1970), Texas (Wellik et al. 1979) and Florida (Poe 1974). In Florida this insect pest increases to tremendous numbers late in the tomato season (namely in spring) when the plants mature. In a number of instances, dense populations (2 to 10 larvae/leaf) have resulted in large scale death of the plants, despite frequent insecticide applications (D.R.S, field observation).

Currently growers use chemical insecticides to manage TPW in commercial fields; but the degree of control is not satisfactory in large measure because of the deficiencies in the current spray programs and detection method. The use of broad-spectrum insecticides for TPW control may induce development of resistance, or induce outbreaks of secondary pests (Smith 1970; Brown & Pal 1971). Cultural practices such as burning of crop residues, crop rotations, manipulation of planting dates, etc. helped suppress the TPW population (Elmore & Howard 1943; Poe 1974). However, insecticides are the only tool for pest management that is reliable for emergency action when insect pest populations approach or exceed the economic threshold (Metcalf 1975). Therefore, efforts are needed to evaluate less detrimental insecticides for control of TPW.

Bacillus thuringiensis has become an effective insecticide in the management of various insect pests (Burgerjon & Martouret 1971); however, resistance to *B. thuringiensis* toxin has been documented through laboratory studies in 12 species of Lepidoptera, 2 species of Coleoptera and 5 species of Diptera (Tabashnik 1994). When compared to the response of a fully susceptible strain the level of resistance observed in most instances did not exceed 50- to 100-fold.

For coping with resistance to *B. thuringiensis* protein toxins, it will be very important to determine the level of resistance that a given population can develop, and the cross-resistance spectrum of this resistance. Considerably more information of benefit to management would be obtained if the mode of inheritance of the resistance could also be determined. The overall objective of the present study was to characterize the toxicity of a selected *B. thuringiensis* toxin to a number of geographically diverse populations of the TPW.

Insects. The study was conducted in a private quarantine facility at Labelle, Florida. Mixed instars of TPW were collected from tomato over a period of time from 4 locations in Florida (Tropical Research and Education Center, Homestead; commercial field, Homestead; BHN Laboratories, Naples; commercial field, Naples); three commercial locations in California (Cameron, Hurron, California-South) and three commercial locations in Sinaloa, Mexico (2 locations at Guasava on two dates, and LaPalma). TPW larvae from each location were shipped to Labelle in strict accordance with quarantine procedures. All shipments were secured in the quarantine facility to separate the live TPW larvae. A colony from each geographic population was established on 'Flora-Dade' tomato transplants within the quarantine facility in a separate room to prevent any intermixing. The escape of live insects was prevented by not handling any insects outside the quarantine facility. Each colony was maintained for 2 generations prior to use in the bioassay study. Sufficient numbers of the required developmental stage of TPW were collected from the cultures to run bioassays using the selected *B. thuringiensis* toxin. At the end of each bioassay all live specimens were destroyed by heating in an autoclave.

Bacillus thuringiensis toxins. Sufficient amount of selected toxin of *B. thuringiensis* var. *kurstaki* (Cry 1, Monsanto) was supplied by BHN Research, Bonita Springs, Florida. The toxin was stored in a refrigerator at 6°C at Tropical Research and Education Center, Homestead, Florida for future use.

Bioassay procedure. A leaf-dip bioassay was conducted in the laboratory. Five concentrations of the selected *B. thuringiensis* toxin (0, 6.25, 12.50, 25.00, 50.00 and 100.00 µg/ml of water) were prepared in 0.02% Tween 20 (Sigma St. Louis, MO). The concentrations of *B. thuringiensis* protein were prepared following a serial dilution method, where a factor of 0.5 was used in each dilution step. Each concentration was made up to a total volume of 20 ml in a test tube. Freshly cut tomato leaflets (eight leaflets/concentration) were immersed in each suspension for one minute. The leaves were removed and air dried. To avoid leaf desiccation, the petiole of each leaf was wrapped with moist cotton, which was kept moist by adding a drop of distilled water daily.

Two treated leaflets from each concentration were placed in a petri dish (9 cm. diam.) and infested with 8 freshly molted 2nd/3rd instars (4 larvae/leaflet). Bioassays were repeated four times. Bioassays were maintained at 28 ±1.2°C, 75-81% r.h., & 14 h photoperiod in the quarantine facility. All concentrations of *B. thuringiensis* toxin were tested at one time against a specific population and placed on a bench in a randomized complete block design.

Evaluation of experimental treatments was made by recording mortality of the TPW larvae at 24-h intervals for 4 days after initiation of the experiment. Any larva that failed to move when touched repeatedly was considered dead.

Statistical analysis. The LC_{50} values and confidence limits were obtained by probit analysis (POLO-PC, LeOra Software 1987). In the present study, nontreated control mortality was below 20%, hence Abbott's formula (Abbott 1925) was not used to correct TPW mortality data.

LC_{50}s among the different populations were similar with a greatest difference of 3.17-fold. The slope values generated from dose-mortality response varied significantly among the TPW populations. The slope value of TREC population is almost 3-5 times greater than other experimental populations (Table 1). This high slope value is not unusual for a laboratory culture reared for several generations, resulting in a more homogeneous population. The lower slope values for the field population are typical, indicating greater heterogeneity. Hemingway et al. (1993) concluded heterogeneity in resistant strains of German cockroaches based on the shallow slopes of the probit lines.

The LC_{50} value for the TREC population was similar to that of the LaPalma population, but the confidence interval for the LC_{50} of the LaPalma population was wider than that of the TREC population (Table 1). The LC_{50} values of the TREC and LaPalma populations did not differ from the commercial field population at Homestead and the BHN laboratory population at Naples. The LC_{50} values of the rest of the experimental populations were higher than populations of Homestead (laboratory and field), BHN and LaPalma. With the increase of LC_{50} values in other populations, confidence intervals increased. The confidence interval reflects the extent of variability in the response of individuals of a population to a certain concentration.

In contrast to the LC_{50} value, the LC_{90} value of LaPalma population was 3 times greater than the TREC population (Table 1). With the TREC colony, the highest concentration tested was at least 3 times higher, and significantly different based on the upper limit of 95% confidence limit (CL), than the concentration needed to cause 90% mortality in the same population (Brewer et al. 1990; Brewer & Trumble 1991; Sanderson & Roush 1992). With the rest of the experimental populations, the concentrations of toxins needed to cause 90% mortality were 3 to 30 times more than the highest concentration tested.

Field to susceptible ratios (FS) were >1.0 for most of the populations when 50% of a population was considered to cause mortality (Table 1). FS (LC_{90}) values were 2 to 4 times greater than the corresponding FS (LC_{50}) values.

Based on the present study, TPW is susceptible to the Cry1-A endotoxin of *B. thuringiensis*. The concentrations needed to cause adequate mortality of TPW must be determined based on the variability of susceptibility in widely separated geographical populations.

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TABLE 1. SUSCEPTIBILITY OF TOMATO PINWORMS, *KEIFERIA LYCOPERSICELLA*, FROM VARIOUS GEOGRAPHICAL AREAS TO INSECTICIDAL CRYSTAL PROTEIN FROM *BACILLUS THURINGIENSIS* VAR. *KURSTAKI*.

Population[*]	Slope + SE	LC_{50} ($FL_{95\%}$[2])	LC_{90} ($FL_{95\%}$)	FS[1] (LC_{50})	FS (LC_{90})
1. Lab., Homestead	9.97 ± 2.26[3]	17.68 (14.88-21.00)	23.77 (20.15-32.44)	—	—
2. Field, Homestead	2.61 ± 0.54	22.74 (16.88-31.33)	70.59 (46.38-170.89)	1.28	2.96
3. BHN Lab.	1.75 + 0.37	27.15 (18.34-45.05)	146.94 (107.73-1449.83)	1.53	6.18
4. Field, Naples	1.69 + 0.40	44.45 (29.38-83.68)	255.39 (119.00-1782.65)	2.51	10.74
5. Cameron	2.20 + 0.46	32.59 (22.92-47.75)	124.51 (75.38-366.56)	1.84	5.23
6. Huron	1.72 + 0.41	42.54 (28.00-72.87)	237.12 (117.18-1428.03)	2.40	9.97
7. LaPalma	1.94 + 0.49	17.79 (12.58-30.79)	81.58 (41.66-534.43)	1.01	3.43
8. Guasava 1	1.72 + 0.44	56.00 (36.83-116.93)	311.16 (138.68-3047.21)	3.17	13.01
9. Guasava 2	3.32 + 0.39	42.18 (18.97-833.68)	102.62 (39.54-32504.63)	2.39	4.31

*1: TREC, Homestead laboratory colony; 2: commercial field, Homestead, FL; 3: BHN Laboratory, Naples, FL; 4: commercial field, Naples, FL; 5: Cameron, CA; 6: Huron, CA; 7: LaPalma, Sinaloa, Mexico; 8: Guasava 1, Sinaloa, Mexico; 9: Guasava 2, Sinaloa, Mexico.

[1]FS (Field to susceptible ratio: lethal concentration (LC) value of the field population divided by the lethal concentration value of the reference susceptible strain.

[2]95% CI (confidence interval) were calculated from probit analysis.

[3]Data are expressed in micrograms of toxin per ml of water. Number of replications for each population: 4.

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SUMMARY

The susceptibilities of nine geographically distant populations of tomato pinworm, *Keiferia lycopersicella* (Walsingham), to Cry1-A protein produced by *Bacillus thuringiensis* var. *kurstaki* are presented. LC_{50} values were similar with a difference of 3-fold. The slope values for different populations varied significantly, indicating a variability in the susceptibility among the populations to the Cry1-A. This information will establish a basis for selecting a proper concentration of *B. thuringiensis* toxin to be used for the control of this economically important pest on tomato genetically engineered for resistance to this pest. The results also provide baseline data for monitoring resistance.

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