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TESTING THE SUSCEPTIBILITY OF FIELD-COLLECTED BOLLWORM AND TOBACCO BUDWORM POPULATIONS TO A HOMOGENEOUS CRY1AC-INCORPORATED DIET

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Abstract

Bollworm and tobacco budworm populations were evaluated for resistance to CryIAc endotoxins using an incorporated diet bioassay. Mortalities were assessed following a seven day exposure period. CryIAc endotoxins were less effective against bollworm larvae as compared to tobacco budworm larvae. Bollworm LC_{50} values ranged from 42 ppm to 145 ppm. An LC_{50} value of 1.0 ppm was recorded for the tobacco budworm population. These values were approximately 2-3 times greater than LC_{50} values obtained from comparable populations collected between 1996 through 1999. It is imperative that Bt resistance monitoring remain a priority for future studies.

Introduction

Laboratory and field results have indicated that resistance to *Bacillus thuringiensis* (Bt) endotoxins has developed in a number of lepidopteran pests (Stone et al. 1989, Gould and Anderson 1991, Tabashnik et al. 1991, Gould et al. 1992). In addition, variations in susceptibility to Bt endotoxins have been documented in tobacco budworm populations (Stone et al. 1991), and resistance to one Bt toxin may confer cross-resistance to other Bt toxins (Gould et al. 1992). During the mid-1990s, a multi-state Bt resistance monitoring project was initiated by Dr. Dick Hardee and colleagues at the USDA-ARS Southern Insect Management Research Unit in Stoneville, MS. The USDA-ARS Project called for the collection of male bollworm and male tobacco budworm moths from the field using pheromone-baited traps. The male moths were then shipped to the USDA-ARS facility in Stoneville, MS where they were mated with virgin female moths from a laboratory-maintained cultures to produce progeny for testing. Because of this, the project was often criticized for not providing an accurate reflection of tobacco budworm and bollworm responses to Bt insecticides in field-collected populations. Therefore, this study was designed to help detect and quantify fluctuations in the responses of 'wild-type', field-collected tobacco budworm and bollworm populations to Cry1Ac Bt proteins using a standard insecticide/diet incorporation protocol.

Materials and Methods

Bollworm and tobacco budworm larvae/moths were collected from a variety of sites throughout south Georgia (Figure 1) and Virginia. Bollworm populations were collected from Burke County (Bur), Dooly County (Doo), Early County (Ear), Miller County (MilA; collected from corn and MilB; collected from cotton), Mitchell County (Mit), Turner County (Tur) and Virginia (Vir). Tobacco budworm populations were collected from Tift County (Tif). Larvae were transferred to artificial diet and maintained in a controlled environment (27˚ C, 14:10 light:dark cycle and 60% relative humidity). Moths, whether reared from field-collected larvae or collected directly from the field, were sexed and mated in 1 gallon paper cylinder breeding chambers equipped with a muslin cloth top for oviposition. Eggs were collected from the breeding chambers and held until hatched. Neonate, unfed larvae were evaluated for susceptibility to CryIAc proteins of MVPII® by placing one larva on CryIAc-incorporated diet that had been dispensed into a 30 ml plastic solo cup. Twenty larvae were evaluated per rate for five rates plus a control; therefore, 120 larvae were evaluated per replicate. Three to five replicates were conducted per test; therefore, 360-600 larvae were evaluated per test.

To decrease variability in the tests, Cry1Ac-incorporated diet was prepared and provided by the USDA-ARS facility in Stoneville, MS. Bollworm larvae were evaluated at 0, 0.1, 1.0, 10, 100 and 250 ppm CryIAc. Tobacco budworm larvae were evaluated at 0, 0.05, 0.1, 1.0, 10, and 100 ppm CryIAc. Treated larvae were maintained in a controlled environment at 27˚ C, a 14:10 light:dark cycle and 60% relative humidity. Mortality was assessed each day for a period of 7 days. Larvae were weighed prior to evaluation and at the conclusion of the 7 day period.

Results and Discussion

MVPII® was less effective against bollworm larvae as compared to tobacco budworm larvae (Table 1, Figure 2; Polizzi and Payne 2004). On average, bollworm larvae were ca. 100X more tolerant of MVPII® than tobacco budworm larvae. Average

LC_{50} values for bollworm populations collected in 2003 were 2-3 times greater as compared to LC_{50} values for bollworm populations collected in 1996-1997, and the slopes of the dosage-mortality regressions were more shallow in 2003 (Table 1; Polizzi and Payne 2004). These results indicated that bollworm populations in 2003 were more heterogeneous in their response to treatment with MVPII® than previously evaluated populations collected during 1996-1997 and suggest an increase in the number of Bt-resistant bollworms within these populations. When larvae were continuously exposed to the MVPII-treated diet for a period of seven days, larval feeding behavior and weight gain were significantly affected (data not shown). At rates representing the LC_{50} values, bollworm and budworm larval weights were reduced ca. 98-99% over the seven day period. For example, the average weight of Tift County tobacco budworm larvae exposed to "control" (untreated) diet for a period of seven days was 32.8 mg; the average weight of Tift County tobacco budworm larvae exposed to diet containing CryIAc at a concentration of 1 ppm (the LC_{50} for the Tif population) for a period of seven days was 0.26 mg. The average weight of Mitchell County bollworm larvae exposed to untreated diet for a period of 7 days was 44.3 mg; the average weight of Mit bollworm larvae exposed to diet containing CryIAc at a concentration of 100 ppm (the LC_{50} for the Mit population) for a period of seven days was 0.8 mg. The data generated during the 2003 season will serve as baseline data for future comparisons. In addition, these populations should be evaluated against standard USDA laboratory-maintained CryIAc-susceptible bollworm and tobacco budworm populations. These evaluations will be made in the future and the protocol will be modified to incorporate these tests into the 2004 test season.

Because of the time investment in conducting these susceptibility tests and collecting and recording weight data, comparisons were made to evaluate the number of replicate experiments required. First, comparisons were made between various combinations of three replicate experiments and five replicate experiments (Table 2, Table 3). Each population was evaluated five times for susceptibility to CryIAc-incorporated diet. Each evaluation consisted of five rates plus an untreated control, and 20 larvae were evaluated per rate for a total of 120 larvae per evaluation. Larvae were placed in cups (1 larva per cup) and mortality was assessed daily for a period of seven days. LC_{50} values and confidence intervals were calculated and compared for all combinations of three replicate evaluations and for the five total evaluations. LC_{50} values generated for each of the combinations of three replicate evaluations were comparable to the LC_{50} value obtained from data for the five total evaluations (Table 2). Although Table 2 shows data collected for the Burke County bollworm population, comparable results were obtained for each population tested. In addition, mean LC_{50} values for all combinations of three replicate evaluations were compared to the LC_{50} value obtained for the five total evaluations for each population tested (Table 3). Again, there was overlap of the 95% confidence intervals indicating that the LC_{50} values were not significantly different. Although Table 3 shows data collected for the Burke County, Early County and Virginia bollworm populations, comparable results were obtained for each population tested.

Summary

The LC_{50} values obtained were only 2-3 times greater than LC_{50} values obtained from earlier studies on populations collected from the same areas; however, the slopes of the dosage-mortality regressions were shallower in 2003 indicating an increased heterogeneity in response to CryIAc endotoxins. In the event that this study is extended into the future, these data will represent valuable baseline data to help address a key issue concerning the long-term monitoring of bollworm and tobacco budworm populations for susceptibility to CryIAc endotoxins in Georgia. Finally, these data should be compared with data obtained through independent studies conducted by participating colleagues at Auburn University, Tamaulipas, Mexico, and the USDA-ARS SIMRU Research Facility.

References

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Table 1. Susceptibility of bollworm and tobacco budworm larvae to CryIAc-incorporated diet.

Strain	LC_{50}, ppm (C.I)	LC_{95}, ppm	Slope
Bollworm			
Burke (cotton)	96.6 (71-137)	2759	0.7
Mitchell (cotton)	97.6 (76-126)	1115	0.8
Early (corn)	145 (107-210	2997	0.7
MilA (corn)	125 (89-186)	3006	0.6
MilB (cotton)	129 (86-212)	11260	0.5
Turner (corn)	41.8 (27-67)	9007	0.4
Dooly (corn)	138 (83-163)	2663	0.6
Virginia (cotton)	53.8 (39-75)	1894	0.6
Tobacco Budworm			
Tift (tobacco)	1.0 (0.2-4.5)	44.0	0.5

Table 2. 5 reps vs. 3 reps? or How many times must this experiment be repeated? Susceptibility of the Burke County bollworm larvae to CryIAc-incorporated diet.

Replicate Combinations	LC_{50}, ppm (95%C.I.)
1,2,3	79.8 (52.2-130)
1,2,4	82.6 (56.1-127)
1,2,5	88.8 (58.4-144)
1,3,4	95.8 (62.0-161)
1,3,5	105 (65.7-186)
1,4,5	106 (69.1-177)
2,3,4	95.4 (38.1-291)
2,3,5	101 (69.5-153)
2,4,5	106 (77.4-146)
3,4,5	118 (81.0-181)
1,2,3,4,5	96.6 (70.8-137)

Table 3. 5 reps vs. 3 reps? or How many times must this experiment be repeated? Susceptibility of the Burke County, Early County and Virginia bollworm larvae to CryIAc-incorporated diet.

	5 Replicates	3 Replicates*
Strain	LC_{50}, ppm (95%C.I.)	LC_{50}, ppm ± S.E.
Burke	96.6 (70.8-137)	97.8 ± 3.80
Early	145 (107-210)	148 ± 7.83
Virginia	53.8 (39.0-75.4)	62.3 ± 9.56

* Mean LC_{50} value for all combinations of 3 replicates

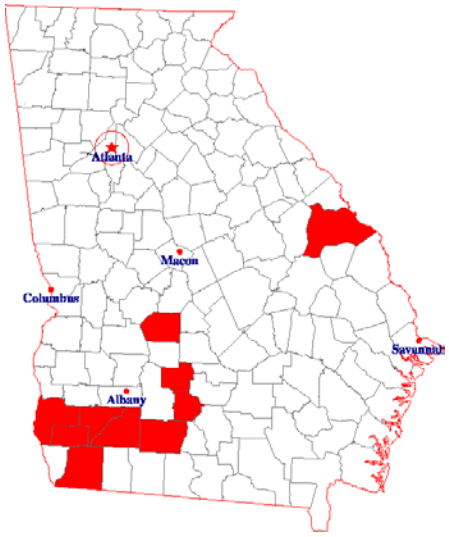

Figure 1. Bollworm and tobacco budworm collection sites in Georgia.

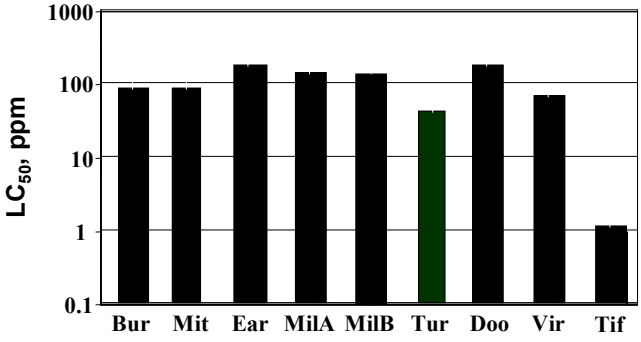

Figure 2. Susceptibility of bollworm and tobacco budroom larvae to CrylAc-incorporated diet.