AN EVALUATION OF RESISTANCE TO Bt TOXINSIN NATIVE POPULATIONS OF THE PINK BOLLWORM

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Abstract

Bolls infested with pink bollworm larvae were collected from cotton fields in five locations in Arizona (Stanfield, Yuma, Buckeye, Parker, and Marana). Eggs were then gathered from the adult pink bollworms resulting from those collections and newly hatched larvae from those eggs were subjected to artificial diet containing doses of a purified solution of the *Bacillus thuringiensis* (bt) entomotoxin cryIA(c). The dosages of toxin were 0.00125, 0.0025, 0.005, 0.01, 0.02 0.04, and 0.08 μg/ml. Newly hatched larvae from two established laboratory strains, APHIS (a bt toxin susceptible strain) and SOOTY-BTX (a bt toxin resistant strain) were also exposed to the same doses of bt toxin. No mature individuals (individuals which were able to pupate) were produced from any of the native strains or from the APHIS strain at toxin doses exceeding 0.005 μg/ml. Mature individuals were found at all doses administered to the resistant strain. Some third instar larvae were found at toxin doses higher than 0.005 μg/ml, but none which completed development during the duration of the tests.

Introduction

In 1996, 20-25% of the cotton acreage in Arizona was planted with transgenic varieties of cotton (DP&L NuCOTN varieties) which contain the Bollgard™ gene (Hardee and Herzog 1997). Transgenic cottons that express insect control protein genes from *Bacillus thuringiensis* (bt) are highly resistant to pink bollworm, *Pectinophora gossypiella* (Saunders), (pbw) attack (Wilson et al. 1995). The dosage of the expressed δ-endotoxin remains high and relatively constant during the growing season. The toxic effects of these plants on pbw populations hold the promise of reducing or even eliminating the high number of insecticide treatments required to control this destructive pest.

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 2:885-888 (1997)
National Cotton Council, Memphis TN

Unfortunately, the very effectiveness of these transgenic plants will also assure that high selective pressures are placed on the insect to adapt to that toxic environment. Other insects, such as the indianmeal moth and the diamondback moth have developed resistance to bt formulations applied to their host plants for insect control (Bartlett 1995).

During 1994 Watson and Kelly-Johnson (1995) monitored pbw from Safford, and Yuma, Arizona in order to establish the level of susceptibility of the pink bollworm to bt toxins prior to the widespread introduction of transgenic cotton into the Arizona ecosystem. They found that a dose of 0.375 μg/ml of purified cryIA(c) toxin was sufficient to stop all development of pbw larvae and doses above 0.047 μg/ml stopped development beyond the third instar. Additionally, they found that increasing the dose of toxin affected both the ability of the insects to pupate, and the rate of development of the larvae.

The tests reported here were designed to examine the baseline level of susceptibility of the pbw to bt toxins at the end of the first year of commercial deployment of transgenic strains of cotton.

Materials and Methods

Fourteen to twenty-eight day-old bolls infested with pbw larvae were collected from cotton fields in five locations in Arizona. The collections (Stanfield, Yuma, Buckeye, Parker, and Marana) were designated according to the town or city most closely located to the fields where the bolls were picked. All of the fields where bolls were collected were planted in non-transgenic varieties. Mature larvae from the bolls were allowed to pupate and emerge as adults. Eggs were then collected from the adults from each of those collections. Newly hatched larvae were then placed on artificial diet containing doses of a purified solution of the *Bacillus thuringiensis* (bt) entomotoxin cryIA(c) in order to test the effects of the toxin on development of the larvae. Newly hatched larvae from two established laboratory strains, APHIS (a bt susceptible strain) and SOOTY-BTX (a bt toxin resistant strain) were exposed to the same doses of bt toxin.

The cryIA(c) toxin was supplied to us by Dr. Steven Sims (Monsanto Corporation) in vials containing 1 ml of an aqueous solution at a concentration of toxin of 2 μg/ml. This material was diluted at a ratio of 1 ml of the concentrate to 99 ml of sterilized nanopure water producing a toxin concentration of 0.02 μg/ml. This stock solution was then added to liquid wheat-germ diet to produce the toxin-laced diet that subsequently was fed to the larvae in bioassays. The final dosages were 0.00125, 0.0025, 0.005, 0.01, 0.02, 0.04, and 0.08 μg/ml. These dosages were obtained by adding 0.625, 0.00125, 0.0025, 5, 10, 20, and 40 ml of bt toxin stock solution to one liter of diet. The diet was allowed to harden and age for one day and then

shredded. One hundred grams of the shredded diet was placed into a 16 oz (473 ml) paper cup so that each liter of diet produced 10 (or more) cups of diet.

Diet cups were each infested with either 25 (for the native strains) or 100 (for the laboratory strains) newly hatched larvae. This difference in number of infested larvae was merely a function of the number of neonate larvae available for a given strain. The larvae were incubated at a constant temperature of 28 ± 2◦ C for 21 days. This is the length of time necessary for over 95% of normal pbw to reach pupation in our laboratory at this temperature. At the end of 21 days the diet cups were examined and the numbers of larvae, size of larvae, and numbers of pupae or adults (number mature) in each cup were recorded. In the following tables the number of individuals reaching 3rd or higher instars divided by the total number of neonate larvae placed on a treated diet is referred to as % of total number implanted that developed.

No statistical tests were done on the data because of a lack of replication for most of the strains. Differences noted are merely trends.

Results

The numbers of eggs produced by the native strains were quite low in spite of the large numbers of adults. Therefore, only the Parker and Marana strains were tested over the whole range of doses and only one cup for each dose was infested for the Parker strain. The Marana strain had one to three cups per dose infested with larvae. Some of the Marana larvae are still being placed on test diets.

Table 1 shows the numbers of pupae and adults from the few doses tested for the Yuma, Buckeye, and Stanfield strains. The numbers of F_1 pbw produced even in the control diet were quite low, but of those produced, 83 - 86% reached maturity. None of the larvae placed on doses of toxic diet reached maturity. A few larvae placed upon toxic doses of diet did develop to the third instar. We do not know if these larvae would have ever reached pupation because the artificial diet was quite dry and hard after 21 days at 28◦C so these larvae were discarded.

The response of the Parker and Marana strains of native insects to doses of bt toxin is shown in Table 2. The numbers shown in this table are totals of 1 - 3 replications of 25 larvae per diet dosage, so the percentage of larvae (not absolute numbers of individuals) can be compared to Table 1. The Parker strain reared on the control diet (87% mature) responded much as the other native strains, while the Marana strain lagged in development even in the control diet (69% mature). In contrast to the other native strains, some Parker individuals completed development on a dosages of 0.0025 and 0.005 μg/ml. Additionally, some 3rd instar larvae were produced at all doses of bt toxin that were fed to the Parker strain. The Marana strain behaved very similar to the other native strains, with no mature individuals produced at any dose of toxin, but some 3rd instar larvae were produced at all doses except 0.02 and 0.08 μg/ml.

The susceptible APHIS laboratory strain responded to bt toxin in a manner similar to some of the native strains (Table 3). Doses of toxin up to 0.005 μg/ml allowed 74 - 97% of the larvae to reach maturity. Because of the larger numbers of neonate larvae available for testing we were able to estimate the effect of the toxin on mortality of the larvae. In the APHIS strain the control diet allowed 53% of the neonate larvae placed on the diet to reach 3rd instar or greater stage of development (column 3). As the dose of toxin increased, the % of total larvae implanted that developed to 3rd or greater stage of development of the total larvae implanted that developed on the diet decreased, reaching 0% for all doses over 0.02 μg/ml. At doses of toxin exceeding 0.005 μg/ml, none of the developing larvae reached maturity. The percentage of larvae (given that any larvae developed) increased with increasing dosage of toxin.

The SOOTY-BTX strain has been selected for resistance to the bt endotoxins found in transgenic cotton (A. C. Bartlett, unpublished results). Table 4 shows the response of this strain to doses of purified bt CryIA(c) proteins placed in the rearing media. In this strain 81% of the neonate larvae place on control diet developed to 3rd or higher instar. This percentage of neonates placed on treated diet never reached zero for the SOOTY-BTX strain as it did for all of the other strains in this test. Additionally, there were mature individuals produced for every dose of toxin. In contrast to the native and APHIS strains, the percent of SOOTY-BTX individuals which did not develop beyond 3rd instar was extremely variable over doses but did not show the typical increase as the dosage increased until the dosage was over 0.01 μg/ml and even then it leveled out at about 75%.

Conclusions

In limited tests in 1996 neonate pbw larvae produced by adults collected from bolls from five areas in Arizona reacted to the purified bt entomotoxin CryIA(c) very similar to that found by Watson and Kelly-Johnson (1995) although the tests were carried out somewhat differently. Resistance, as measured by the ability of larvae to reach maturity at doses over 0.04 μg/ml, was not apparent in these populations. As the dosage of the toxin increased the number of individuals able to survive and develop in the diet decreased.

As the dosage of toxin was increased, the percentage of developing individuals that remained larvae (i.e. did not pass 3rd instar) increased. None of the neonates from native strains or from the susceptible APHIS laboratory strain were able to complete development when the dose of toxin exceeded 0.005 μg/ml. However, some 3rd instar larvae were produced at doses up to 0.08 μg/ml in the Parker strain, but none pupated.

Several factors should be considered as a result of these tests. Even though small samples of individuals were tested for some of the native collections, there was obvious variability in the response of neonate larvae to the bt toxin. Some of the larvae in the Parker strain survived high doses of bt toxin in their food supply and remained viable. This suggests that if population density was high enough in the field and the bolls remained capable of supporting larval growth (i.e. did not become too dry or hard for the larvae to survive) then the Parker strain may have a high potential to develop resistance to the toxins in transgenic cotton. Since laboratory strains of the pbw (SOOTY-BTX) have acquired resistance to bt toxins under an artificial selection regime (Bartlett 1995), it does not seem judicious to ignore such a possibility. The development of effective resistance management tactics must be quickly accomplished and then actually carried out in the field.

Acknowledgements

Special thanks are due to Amanda Simmons, for her work in collecting infested bolls and extracting larvae from the bolls; Lillian J. Lewis, for extracting larvae from the bolls, collecting eggs, and preparing the diets; and Theresa Rodriquez, for collecting eggs, preparing the diets, implanting first instar larvae, and counting the resulting larvae and pupae from each diet.

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Table 1. Response of three native strains of pbw to bt toxins (25 first instar larvae/dose, 1 - 3 replications per dose).

Strain	Dose of Toxin	% of total larvae implanted that developed	Total at 21 days	% Larvae at 21 days	% Mature at 21 days
Buckeye	control	28	7	14	86
	0.005	8	2	100	0
Yuma	control	8	3	17	83
	0.0025	0	0	0	0
	0.005	8	2	100	0
Stanfield	control	4	6	17	83
	0.005	4	2	50	50
	0.04	0	0	0	0

Table 2. Response of the native Parker and Marana strains of pbw to bt toxin (25 first instar larvae per dose).

Strain	Dose of Toxin	% of total larvae implanted that developed	Total at 21 days	% Larvae at 21 days	% Mature at 21 days
Parker	control	60	15	13	87
		28	7	57	43
	0.0025				
	0.005	12	3	66	33
	0.01	8	2	100	0
	0.02	8	2	100	0
	0.04	8	2	100	0
	0.08	8	2	100	0
Marana	control	4	9	31	69
	0.00125	3	0	100	0
	0.0025	1	0	100	0
	0.005	1	0	100	0
	0.01	2	0	100	0
	0.02	0	0	0	0
	0.04	3	0	100	0
	0.08	0	0	0	0

Table 3. Response of the APHIS laboratory (susceptible) strain of pbw to bt toxin (100 larvae per replication, 3 replications per dose).

Dose of Toxin	% of total larvae implanted that developed	Total number at 21 days	% Larvae at 21 days	% Mature at 21 days
Control	53	23	4	96
0.00125	33	11	9	91
0.0025	29	51	16	84
0.005	14	27	26	74
0.01	4	7	100	0
0.02	1	1	100	0
0.04	0	0	0	0
0.08	0	0	0	0

Table 4. Response of the SOOTY-BTX laboratory (bt resistant) strain of pbw to bt toxin (100 larvae per replication, 3 replications per dose).

Dose of Toxin	% of total larvae implanted that developed	Total number at 21 days	% Larvae at 21 days	% Mature at 21 days
Control	81	161	26	74
	66	131	15	85
0.00125				
	48	95	9	91
0.0025				
0.005	39	39	18	82
0.01	17	41	49	51
0.02	21	52	75	25
0.04	3	7	71	29
0.08	5	9	78	22