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FITNESS EVALUATIONS OF HELICOVERPA ZEA (BODDIE) FROM BOLLGARD™ COTTON IN SUBSEQUENT GENERATIONS

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Abstract

A delay in Bt resistance evolution in the cotton bollworm (*Helicoverpa zea*) could be enhanced if a fitness cost were associated with those individuals that have resistance alleles. Fitness comparisons were made over two generations measuring the responses between a bollworm strain found on Bollgard cotton and one found on non-Bt cotton plant, BG and NBT respectively. Measurements of larval growth and mortality were made for both strains after exposure to Cry1Ac and technical grade cypermethrin. Our expectation would be a decrease in survival after pyrethroid exposure and a decrease in growth on the non-toxin diet in the BG strain if a there were fitness cost due to Bt resistance. We did not find a statistically significant difference between strains in our pyrethroid evaluations, although there was a difference in LD_{50} values for the F1 and F2 generation. Our Cry1Ac evaluations showed BG survival was higher than NBT larvae in the F1 generation, but not for the F2 generation. In contrast to our expectation, the BG strain larvae grew larger than the NBT for the F1 generation in the non-toxin control diet. Similar results have been reported in the past and are discussed within a context of environmental and maternal effects. Future experiments with an *H.zea* strain that has substantial Cry1Ac resistance should improve understanding of whether differences between strains are due to genetic or environmental causes.

Introduction

A large number of factors are expected to affect the rate at which insects evolve resistance to Bt toxins. The major factors considered in most models of resistance evolution are: number of generations an insect is exposed to a transgenic crop per year, percentage of insect population exposed to Bt per generation, mortality of heterozygotes caused by toxin, larval and adult mobility, mating patterns, initial frequency of resistant alleles in population, fitness costs to individuals carrying resistance genes (Mellon and Rissler 1998), dominance of alleles conferring resistance, and presence of refugia (Georghiou and Taylor 1977).

The high dose refuge strategy which is generally recommended to delay the development of resistance is dependant on low initial frequency of resistant alleles, extensive mating between resistant and susceptible adults, and effectively recessive inheritance of resistance in the field (Carrière and Tabashnik 2001). Bollgard cotton is toxic to the bollworm but cannot be characterized as a high dose because a significant number of larvae survive on it under field conditions. (Jackson et al. 2002, Mahaffey et al. 1995)

Although, initial frequencies of Bt resistance alleles in the cotton bollworm (*Helicoverpa zea*) to Cry1Ac are low, the resistance alleles examined have been characterized as dominant, or incompletely dominant (Burd et al. 2003). Given this scenario, the frequency of resistance alleles should increase rapidly unless a large refuge is present (Jackson et al. 2003b). Local alternate crop hosts, such as corn and soybean in the southeastern United States may provide substantial refugia for *H. zea* during the cotton production season (Jackson et al. 2003a), and *H. zea* migrating from other areas may also contribute to the refuge population (Gould et al. 2002) The frequency of resistance alleles has not increased in field populations of bollworm in Eastern North Carolina (Jackson et al. 2002, Burd et al. 2003), despite evidence that *H. zea* has the genetic potential for resistance development (Burd et al. 2000).

In addition to presence of large refuges, the delay of resistance evolution in *H. zea* could, in part, be due to a fitness cost to individuals that have resistance alleles (Carrière and Tabashnik 2001). Unfortunately, available information on fitness costs remains limited. The present study was initiated to provide more information on the fitness cost to individuals carrying Bt resistance alleles. In the current study, intergenerational fitness of two North Carolina bollworm field strains was evaluated. One of the two strains was initially selected on Bollgard cotton in the field and the second strain was derived from larvae that fed on conventional cotton varieties. We evaluated growth of both strains on unadulterated artificial diet and the response of both strains when exposed to the Bt toxin, Cry1Ac, and to technical grade cypermethrin.

A fitness cost due to Bt resistance would be expected to decrease survival after exposure to pyrethroids and to decrease growth on non-toxin diet. Our results indicate a more complex situation.

Materials and Methods

Large fourth and fifth instar bollworm larvae were collected from Bollgard cotton fields in Eastern North Carolina in July-August 2003. These larvae were transported to the laboratory where they were placed individually into 30 ml plastic cups containing approximately 10 ml of a corn-soy blend artificial insect diet until they pupated. A total of 137 individuals were collected from Bollgard cotton plants, this populations was labeled BG. Additionally, a group of 89 individuals were collected from non-Bt conventional cotton varieties and this population was labeled NBT. Pupae were placed into ½ gallon mating buckets in groups of 20-30 individuals, where successfully emerged adults were allowed to mate. Adults were fed a 5% sucrose solution. A cheesecloth cover over the top of each bucket served as an oviposition substrate. Cheesecloths were removed twice a week and placed into 12 oz. plastic containers where eggs were allowed to hatch. The same general rearing procedures were used in the F1 and F2 generations.

Newly hatched first instar bollworm larvae of both strains were evaluated in the F1 and F2 generations for growth and survival on Cry1Ac as produced in the Mycogen product MVPII. The test involved a five fold serial dilution of Cry1Ac at concentrations of 0.0, 0.064, 0.32, 1.6, 8, 40, 200 µg/ml of diet. Larvae were placed singly into 30 ml plastic cups with the Cry1Ac toxin incorporated diet. Each bioassay consisted of 30 individuals per concentration and was incubated at 27ºC with a light:dark photophase 14:10 hours. Mortality and larval weights were recorded after ten days; (larval weight data for 200 µg/ml could not be collected because there were no survivors at that concentration). Growth differences between the strains on the 0.0 µg/ml control treatment were used as an indication of fitness cost.

Technical grade samples of cypermethrin were tested on both bollworm strains to determine the effects of Bollgard resistance on bollworm susceptibility to pyrethroids. The third instar larvae were topically treated on third abdominal terga with cypermethrin dissolved in 1.0 µl of actetone at the following concentrations: 0.0, 0.125, 0.1875, 0.25, 0.375, 0.5, 1.0 mg/ml. Each larva was placed back into its diet cup and was incubated at 27ºC and light:dark photophase of 14:10 hours for 72 hours before mortality was assessed. Bioassay sample size ranged from 15 to 34 individuals per concentration.

Mortality data from cypermethrin and Cry1Ac tests were analyzed with SAS Probit analysis to determine LD_{50}s and confidence intervals. Larval weight data was converted to log weight and analyzed with SAS two-way ANOVA with strain as a fixed variable and concentration as a random variable (SAS 1999).

Results

In the F1 generation Cry1Ac mortality assay (Table 1), the BG strain had a statistically higher LC_{50} than the NBT strain but this difference was not seen in the F2 generation. This change in the between strain comparison seems to be due to the fact that in the F2 generation the BG strain had a lower LC_{50} than it did in the F1 generation.

In the F1 generation, larval weight of the BG strain was significantly higher than that of the NBT strain concentrations of 0.0, 0.064, 0.32, and 1.6 µg/ml Cry1Ac (Graph 1); but there was no significant difference at 8 and 40 µg/ml. In the F2 generation, larvae of the BG strain were significantly larger at concentrations of 0.064, 0.32, 1.6, and 8 µg/ml; but there was no significant difference at 0.0 and 40 µg/ml (Graph 2).

For the cypermethrin evaluations there was a significant difference between LD_{50}s for the F1 and F2 generation, but no difference between strains within a generation was found (Table 2).

Discussion

Our initial assumptions that a fitness cost associated with resistance to Cry1Ac in the BG strain would lead to slower growth on regular diet and higher mortality when exposed to a pyrethroid were not borne out by our data. Our results are more complex and point to a small increase in Cry1Ac resistance in the BG strain (in the F1 generation) due to genetic or maternal effects. This increased tolerance for Cry1Ac was not accompanied by a decrease in pyrethroid tolerance or in slower growth on normal artificial diet.

Although BG survival on Cry1Ac was higher than for NBT larvae in the F1 generation, this difference was not found in the F2 generation. The LC_{50} value of the NBT strain for Cry1Ac was constant in the F1 and F2 generation. The significant decrease in LC_{50} values that was observed between the F1 and F2 generation for the BG strain could be explained by a cost to resistance in the BG strain that was unselected for the Po and F1 generations. If such a cost was present, it was certainly not expressed as lower growth on the normal artificial diet. Instead, the F1 larvae of the BG strain grew larger than the NBT larvae on the control diet. Similar results were found in Lambert et al. 1998 where larval weight of the BT strain was greater than the NBT strain for F1 larvae that were fed a non-toxin diet. Lambert et al. 1998 also compared F1 larvae from the NBT and BT reared on Bt diet and attributed the smaller size of the BT strain on Bt diet to environmental or maternal effects. Carrying this experiment further onto the F2 generation, Lambert found that parents that were stressed from developing on a

Cry1Ac diet, had larger offspring than unstressed parents when both sets of offspring were allowed to develop on a non-toxin diet. Lambert proposed the stressed parents were able to produce more fit offspring when stressed and cites this as "negative maternal effects". Both Lambert's results and the data presented here are perplexing and cannot differentiate whether differences in the growth of the strains on Bt and non-Bt diet are due to genetic or maternal Bt resistance. Unfortunately, further information needed to clarify this enigma are absent from the current literature. It is hoped that fitness evaluations currently being performed on a highly selected strain of bollworm (Marcus et al. unpublished results) will provide the information needed to understand the discrepancies between the two strains.

Our results indicate a lack of difference between the two strains in pyrethroid tolerance in the F1 and the F2 generations. However, both strains have a decrease in tolerance to cypermethrin between the F1 and F2 generations. One possible explanation for loss of pyrethroid resistance by both strains is the absence of selection pressure in the F1 larvae that gave rise to the F2 generation. An example of a similar loss of resistance after bringing a field population into the laboratory, was documented with tobacco budworm (*Heliothis virescens*) pyrethroid resistance (Campanhola et al. 1991). The fact that the two strains did not differ from each other in pyrethroid tolerance suggests that there was no fitness cost from the small amount of Bt tolerance in the BG strain. The fact that the BG strain was not more tolerant of the pyrethroid than the NBT strain indicates lack of cross-resistance between Cry1Ac and the pyrethroid. These results must be viewed cautiously because given the low level of tolerance to the Bt toxin in the BG strain, lack of significant differences may simply be due to the low statistical power of our experiment. Again, future experiments with a *H. zea* strain that has substantial Cry1Ac resistance (Marcus et al. unpublished results) should provide more rigorous results.

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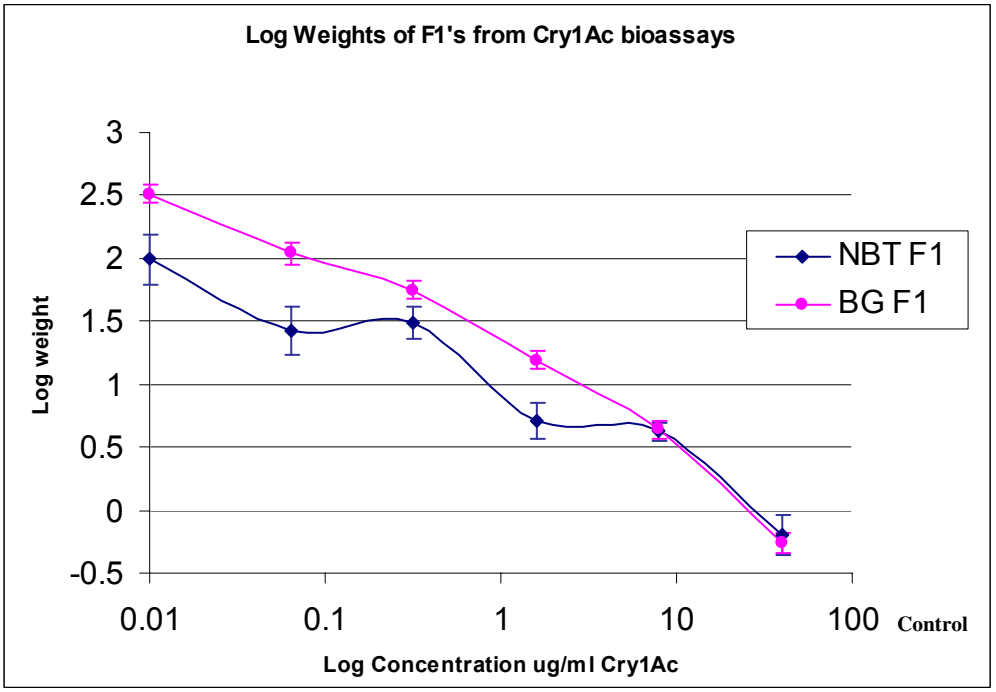
Table 1. Susceptibility of *Helicoverpa zea* larvae to Cry1Ac endotoxin.

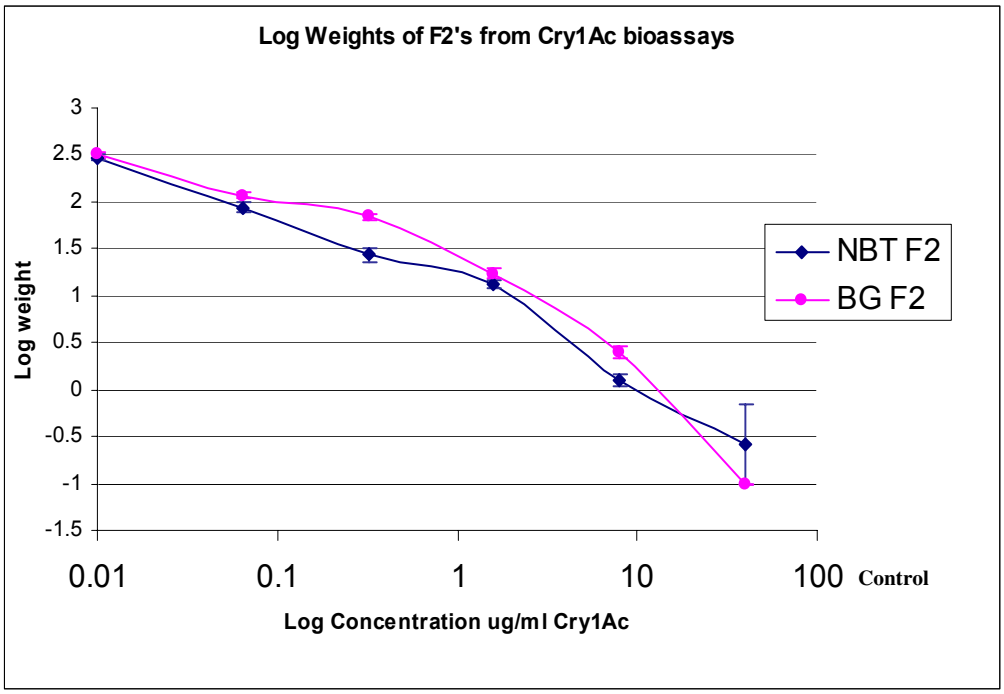
Strain	Generation	LC_{50}s (µg/ml Cry1Ac)	Fiducial Limits	
			Upper	Lower
NBT	F1	16.3	21.8	12.3
BG	F1	30.6	37.9	25.5
NBT	F2	13.2	27.9	7.41
BG	F2	15.6	19.6	12.8

Table 2. Susceptibility of third instar *Helicoverpa zea* larvae to technical grade cypermethrin.

Strain	Generation	LD_{50}s (mg/ml)	Fiducial Limits	
			Upper	Lower
NBT	F1	0.623	0.819	0.504
BG	F1	0.806	2.82	0.528
NBT	F2	0.179	0.217	0.137
BG	F2	0.150	0.191	0.0690



Graph 1. Log weight larval comparisons of Non-Bt and Bollgard strains for F1 generation.



Graph 2. Log weight larval comparisons of Non-Bt and Bollgard strains for F2 generation.