BOLLWORM (HELICOVERPA ZEA): ADAPTATION TO BT TOXIN?

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Abstract

The development of insect resistance to the *B.t.* endotoxin is a concern associated with the widespread adoption of *B.t.* cottons. Laboratory trials were conducted in 1996 to determine if the ability of the bollworm, *Helicoverpa zea* (Boddie), to establish and complete larval development in *B.t.* cotton was due to adaptation to the endotoxin in the field population. In addition, the adaptation to *B.t.* endotoxins that occurs from selection of single generations in the field was measured in the laboratory to determine if and how rapidly the onset of such resistance may occur. Results with field-collected bollworm larvae suggested that adaptation to the *B.t.* endotoxin had not occurred in the area of collection but that there was potential for increased tolerance to *B.t.* (as a result of field exposure) in bollworm in as few as 3 generations. Additional lab trials will be conducted to determine if the basis for increased larval weights and pupal weights and decreased durations of pupation of bollworm larvae originally reared on *B.t.* cotton is indeed genetic or if environmental factors (i.e., maternal effects) influenced these differences.

Introduction

The recent commercialization and widespread adoption of insect-resistant transgenic cottons which express the delta-endotoxin protein gene from a soil bacterium, *Bacillus thuringiensis* Berliner var. *kurstaki* (*B.t.*) present many opportunities as well as challenges. The planting of *B.t.* cottons provides season-long protection, a lack of dependence on application timing for treatment success, and opportunities in areas where conventional insecticide applications are often restricted (Benedict 1996). Fewer insecticide applications is healthier for the environment, frees producers' time and labor, and reduces human exposure to insecticides (Fischhoff 1996). Furthermore, the reduction or elimination of the need for conventional insecticides may be especially helpful in areas where tobacco budworm, *Heliothis virescens* (F.), populations have become increasingly resistant to most conventional synthetic insecticides. However, field trials conducted in North Carolina from 1993 to 1996 where bollworm, *Helicoverpa zea* (Boddie), comprised 95-98% of the larval pest populations indicated that *B.t.* toxin levels in transgenic cotton plants may not be high enough to adequately suppress very high numbers of bollworms and thereby prevent significant yield loss (Lambert et al. 1996, 1997). These transgenic *B.t.* cotton trials demonstrated approximately a 15% yield loss due to damage by bollworm when pyrethroids were not applied (Lambert et al. 1996, 1997, Mahaffey et al. 1994, 1995).

The levels of toxin in Bollgard™ cotton that was commercialized in 1996 were expected to be high enough to control approximately 99% of the tobacco budworm population and 90-95% of the bollworm population (J. R. Bradley, Jr., N. C. State Univ., personal communication). This "high-dose" toxin expression strategy (Gould 1997) was also part of Monsanto's resistance management plan for Bollgard™ cotton. This strategy will work if toxin expression is effective enough to eliminate all or almost all susceptible or partially resistant insects (Gould 1997). According to Gould (1994), a "high dose" is 25 times the amount of toxin needed to kill 99% of susceptible insects. With the current levels of *B.t.* endotoxin in transgenic cotton plants, this high dose strategy will be ineffective for bollworm. In studies of geographic susceptibility of bollworm and tobacco budworm to *B.t.* endotoxin in pinto bean diet, Stone and Sims (1993) reported a greater range of LC50's for bollworm than for tobacco budworm. MacIntosh et al. (1990) and Sims (1995) have also reported a greater tolerance to *B.t.* and/or higher survival after exposure to the *B.t.* toxin for bollworm. Because *B.t.* toxin levels have not been high enough to adequately control some bollworm populations in Bollgard™ cotton, a concern associated with the widespread adoption of this technology is that insect pests such as bollworm may quickly develop resistance to the *B.t.* endotoxin.

Laboratory trials were conducted in 1996 to determine if the level of bollworm tolerance of *B.t.* endotoxins increased due to exposure of a single generation of bollworm to Bollgard™ cotton.

Materials and Methods

B.t. Tolerance of Larvae from *B.t.* and Non-*B.t.* Cotton

Third and fourth instar bollworms were collected in a cotton field in Martin Co., N.C., on August 20, 1996 from *B.t.* (BT) and non-*B.t.* (NBT) cottons. Each larva was weighed with a digital scale upon collection and larval weights (g) were recorded. Each larva was then placed in a 30-ml transparent plastic cup containing approximately 10 ml of a diet containing 8 µg /ml of cryIA(c) toxin from MVP (Mycogen Corp., San Diego, CA), and cups were sealed with an unwaxed paper lid. MVP is a commercially available microbial insecticide that contains a *B.t.* toxin almost identical to the cryIA(c) in Bollgard™ cotton. Diet cups containing larvae were placed in a rearing room (14:10 light:dark cycle, 27°C) for 4 days. Each larva was weighed after 4 days on diet. The number of larvae collected from

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each cotton type (BT or NBT), the initial and final larval weights (g), and the differences in these weights are recorded in Table 1.

Selection for Adaptation

Fifth instar bollworm were collected in *B.t.* (BT) and non-*B.t.* (NBT) cotton at the C. A. Martin Farm in Martin Co., N.C., on August 20, 1996. Larvae were placed in individual rearing chambers with cotton bolls from their respective plots. These chambers consisted of a 100-ml transparent plastic container with a flip lid on top and a hole punched in the bottom through which a standard floral water pick with a rubber cap was inserted. The stem of a cotton boll was supported by the opening in the cap of the water-filled pick, and water picks were placed approx. 3 in. apart on styrofoam boards according to cotton type (BT or NBT) . The boards were placed in a rearing room (14:10 light:dark, 27°C) where larvae completed development. Each container was examined daily for pupae, and pupae were removed and weighed (g). Each pupa was then placed in a 30-ml transparent plastic cup with an unwaxed paper lid. The duration of pupation for each bollworm was measured as the number of days from eclosion until emergence. Upon emergence, the sex of individual moths was determined, and males and females were placed, according to cotton type they originated from (BT or NBT), in 1-gal. cardboard buckets and allowed to mate. The opening of each bucket was covered with a piece of cheesecloth which served as an oviposition substrate. Each bucket contained a dish with a cotton ball that had been soaked in a sucrose solution (160:1 sugar:water). The cheesecloth from each bucket (BT or NBT) was collected daily. The egg-infested cloth was placed in a transparent plastic bag with a piece of moistened filter paper and the bag was labeled according to cotton type and date.

Neonate larvae from these collections were placed singly in 30-ml transparent plastic cups containing one of two diets. Half of the hatchlings were placed on control (NBT) diet and the other half were placed on diet containing 0.10 µg/ml of cryIA(c) toxin from MVP (BT). These first generation (G1) larvae were weighed after 12 days on diet and were returned to their respective diet cups until pupation. Pupal weights and durations of pupation were measured for this generation of bollworms as with the G0 generation. Upon emergence, male and female moths were again placed in cardboard buckets based on G0 larval diet (BT or NBT cotton) and G1 larval diet (BT or NBT diet). The four buckets were labeled as follows: BT-BT, BT-NBT, NBT-BT and NBT-NBT. Eggs from each bucket were collected daily, and these G2 neonate larvae from each group were placed on BT or NBT diet in individual diet cups. Larval weights, pupal weights, and durations of pupation were also measured for the G2 generation as for previous generations.

Larval weights, pupal weights, and/or durations of pupation for bollworm larvae in both experiments were subjected to ANOVA using PROC GLM (SAS Institute 1990), and means were separated ($P \leq 0.05$) using the LSMEANS procedure of SAS.

Results

***B.t.* Tolerance of Larvae from *B.t.* and Non-*B.t.* Cotton**

Field-collected third and fourth instar bollworms from NBT cotton weighed significantly more than bollworm larvae from BT cotton upon collection (Initial Weight) and after 4 days on a diet containing 8 µg /ml of cryIA(c) toxin (Final Weight) (Table 1). However, there were no significant differences in weight gain between the larvae from the two types of cotton (Table 1).

Selection for Adaptation

Bollworm larvae field-collected from NBT cotton had significantly greater pupal weights (g) than larvae field-collected from BT cotton, but there were no significant differences in the durations of pupation (days) for the two groups of bollworms (Table 2).

Larval weights, pupal weights, and durations of pupation for G1 are recorded in Table 3. Bollworm larvae of the G1 generation reared on BT diet whose parents fed on BT cotton in the field (BT-BT) weighed significantly less than all other groups of G1 larvae. However, larvae reared on NBT diet whose parents fed on BT cotton (BT-NBT) weighed more than all other groups of G1 larvae. Similarly, pupal weights were significantly lower in the BT-BT group than all other groups and significantly higher in the BT-NBT group than all other groups. BT-BT insects also had significantly longer durations of pupation, and BT-NBT insects had significantly shorter durations of pupation than all other groups. Within the two groups of larvae whose parents fed on NBT cotton in the field, G1 larvae reared on BT diet (NBT-BT) weighed less as larvae and pupae and had significantly longer durations of pupation than G1 larvae reared on NBT diet (NBT-NBT).

Larval weights, pupal weights, and durations of pupation for the final generation (G2) insects are recorded in Table 4. G2 larvae reared on BT diet were significantly smaller as larvae and pupae than G2 larvae reared on NBT diet irrespective of larval diet in the previous generations (G0 and G1). G2 larvae reared on NBT diet whose parents (G1) were reared on BT diet (BT-BT-NBT and NBT-BT-NBT) were significantly larger as larvae and pupae than all other groups of insects. G2 larvae reared on BT diet with G1 and G0 reared on NBT and BT diets, respectively, (BT-NBT-BT) were significantly larger as larvae and pupae and had significantly shorter durations of pupation than G2 insects reared on BT diet with G1 and G0 reared on NBT diet and NBT cotton, respectively (NBT-NBT-BT).

Discussion

Results with field-collected bollworm larvae suggest that field populations did not have high frequencies of larvae adapted to *B.t.* cotton. Not only were there no significant differences in weight gain (g) among larvae field- collected from BT and NBT cotton, but bollworm larvae collected from BT cotton were significantly smaller upon collection and after 4 days on BT diet than larvae from NBT cotton (Table 1). This absence of adapted larvae reflects the lack of bollworm exposure to the *B.t.* endotoxin in the field to that point. In fact, the sales and usage of foliar-applied *B.t.* products over the last decade has been limited to less than 1% of the total insecticide market (Deaton 1993). Although resistance to *B.t.* in the laboratory has been reported for a number of insects including tobacco budworm, only the diamondback moth, *Plutella xylostella,* has reportedly evolved high levels of resistance to *B.t.* in the field due to repeated use of commercial *B.t.* products (McGaughey and Whalon 1992). The smaller sizes of larvae collected from BT cotton also demonstrate the potential for BT cotton to delay development of surviving insects.

Several interesting observations were made during the laboratory selection experiment. First, although larvae collected from BT cotton weighed significantly less than larvae collected from NBT cotton, there were no significant differences in the durations of pupation between the two groups of insects (Table 2). It appeared that the BT cotton delayed larval development of the insects surviving on this cotton but did not affect their pupal period. It should also be noted that the number of bollworms successfully completing development as larvae and pupae on BT cotton (25 and 17, respectively) was lower than the number surviving through pupation on NBT cotton (49 and 35, respectively).

The fact that bollworm larvae reared on a BT diet for 2 generations (BT-BT) were smaller than G1 larvae reared on BT diet with parents (G0) from NBT cotton in the field (NBT-BT) (Table 3) may be explained by the "maternal effects" hypothesis. Maternal effects (or parental effects) are defined as the non-nuclear contributions of a parent to its offspring (Farnsworth 1978). Numerous examples of these effects have been cited in the literature (e.g., Ginzburg and Taneyhill 1994, Kirkpatrick and Lande 1989, Mousseau and Dingle 1991, Rossiter 1991). It is therefore possible that a poor nutritional source (*B.t.* cotton) in the G0 generation negatively impacted larval development in the G1 generation. However, the opposite occurred when G1 larvae were reared on NBT diet in the lab. G1 larvae reared on NBT diet whose parents (G0) were reared on BT cotton were larger than larvae reared on NBT diet whose parents (G0) were reared on NBT cotton in the field and had significantly shorter durations of pupation. This is a contradiction to the aforementioned maternal effects hypothesis and may in fact be described as a "negative maternal effect" (Rossiter 1991). These effects were observed in studies with gypsy moth, *Lymantria dispar*, where the pupal weights of offspring were higher when male and female parents were fed damaged leaves during larval development (Rossiter 1991). The mechanism for this negative maternal effect was unknown, but Rossiter (1991) suggested that females produce qualitatively better provisioned eggs by using the information supplied by damaged leaves. Perhaps this is the mechanism by which bollworm larvae are better able to grow on regular diet after exposure to *B.t.* toxin in the previous generation. Clearly, two contradictory events were occurring in this portion of this study. It appears that the larval weights, pupal weights and durations of pupation of the G1 generation were not influenced solely by larval diet in the G1 generation but by an interaction of G0 larval diet and G1 larval diet.

Larval and pupal weights of G2 insects reared on BT diet were significantly lower than weights of insects reared on NBT diet in this generation regardless of diets in previous generations (Table 4). This may be expected because larvae were exposed to sublethal doses of *B.t.* toxin in diet; even low levels of toxin may slow the growth of these insects and delay larval development. However, exposure to BT diet in the G1 generation resulted in significantly higher larval and pupal weights for G2 insects reared on NBT diet regardless of which cotton type larvae were reared on in the G0 generation. This is the same reaction to a BT-NBT (G0-G1) diet combination observed in the G1 generation that may be explained by a negative maternal effect. In fact, G2 larvae reared on NBT diet with any prior generation having been exposed to a BT diet (cotton or diet) were significantly larger as larvae and pupae than the colony that had never been exposed to the *B.t.* toxin (NBT-NBT-NBT).

Finally, the most important observation with G2 insects was that of significantly higher larval and pupal weights and significantly shorter durations of pupation for the BT-NBT-BT insects than the same measurements for NBT-NBT-BT insects (Table 4). It appeared that exposure to BT in the first generation afforded the BT-NBT-BT colony a greater tolerance to *B.t.* toxin in diet than the NBT-NBT-BT colony which had no prior exposure to *B.t.* before the third generation. Growth of larvae from field collections indicated that bollworm populations had not yet adapted to the *B.t.* endotoxin in the field. However, subsequent laboratory trials suggested that progeny of larvae surviving on BT cotton in the field might have genes for higher tolerance of the *B.t.* toxin than progeny of larvae surviving on NBT cotton.

It is apparent from the relative ease in collecting from BT cotton the bollworm population which was used in these lab tests that a high dose of toxin is not present. However, sublethal effects were expressed in the lab and always seemed to contribute to dwindling bollworm populations after several generations in the laboratory. Furthermore, although larvae in the adaption study were exposed to "sublethal" doses of *B.t.* toxin in diet, it is believed that

some mortality due to *B.t.* did occur. While collection of these insects in the field prior to laboratory studies is tedious and time-consuming, extremely high numbers of insects should be collected for these types of studies in order to prevent such "bottlenecking" of caterpillar populations in the laboratory and to be able to sustain laboratory colonies for many generations. The mating of groups of insects is also recommended over single-pair matings unless the study requires that characteristics within a single parental line be observed.

The next step in this study would be to determine if the basis for increased larval weights and pupal weights and decreased durations of pupation for a bollworm colony originally reared on *B.t.* cotton is indeed genetic or if environmental factors (i.e., maternal effects) influenced these differences. The answers to these types of questions are critical to the successful design and implementation of resistance management programs that will prevent, or at least delay, the onset of resistance and thus sustain the long-term use of transgenic *B.t.* cottons.

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Table 1. Mean initial and final weights (g) of third and fourth instar G0 bollworm collected from BT and NBT cotton in the field after 4 days on a diet containing 8 µg /ml of cryIA(c) toxin

Cotton Type (N)	Initial Weight (g)[a]	Final Weight (g)[a]	Weight Gain (g)[a]
BT (91)	0.0626 b	0.0918 b	0.0292 a
NBT (73)	0.1008 a	0.1211 a	0.0203 a

[a/] Means followed by the same letter within a column are not significantly different according to LSMEANS Procedure ($P \leq 0.05$).

Table 2. Mean pupal weights (grams) and durations of pupation (days) of fifth instar G0 bollworm collected from BT and NBT cotton.

G0 Larval Diet	Pupal Weight (g)[a] [N]	Duration of Pupation (days)[a][N]
BT	0.2744 b [25]	9.76 a [17]
NBT	0.3327 a [49]	9.83 a [35]

[a/] Means followed by the same letter within a column are not significantly different according to LSMEANS Procedure ($P \leq 0.05$).

Table 3. Mean 12-day larval weights (g), pupal weights (g), and durations of pupation (days) of G1 bollworm larvae reared on BT or NBT diets (G0 reared on BT and NBT cotton bolls).

G0 Larval Diet (cotton)	G1 Larval Diet	G1 Larval Weight (g)[a] [N]	G1 Pupal Weight (g)[a] [N]	G1 Duration of Pupation (days)[a] [N]
BT	BT	0.0790 c [64]	0.2607 d [35]	12.29 a [6]
BT	NBT	0.6158 a [66]	0.4970 a [23]	9.28 c [18]
NBT	BT	0.1285 b [98]	0.3082 c [66]	11.29 a [24]
NBT	NBT	0.5738 a [128]	0.4360 b [90]	10.15 b [67]

[a/] Means followed by the same letter within a column are not significantly different according to LSMEANS Procedure ($P \leq 0.05$).

Table 4. Mean 12-day larval weights (g), pupal weights (g), and durations of pupation (days) of G2 bollworm larvae reared on BT or NBT diets (G0 larvae reared on BT or NBT cotton bolls, G1 larvae reared on BT or NBT diet).

G0 Larval Diet (cotton)	G1 Larval Diet	G2 Larval Diet (N)	G2 Larval Weight (g)[a] [N]	G2 Pupal Weight (g)[a] [N]	G2 Duration of Pupation (d)[a][N]
BT	BT	BT	0.0840 cd [51]	0.3083 c [40]	13.56 ab [18]
BT	BT	NBT	0.6104 a [60]	0.4413a [52]	12.55 b [44]
BT	NBT	BT	0.1056 c [46]	0.2939 c [33]	12.30 b [10]
BT	NBT	NBT	0.4028 b [71]	0.3618b [59]	13.78 a [32]
NBT	BT	BT	0.0625 d [34]	0.2336d [19]	13.00 ab [3]
NBT	BT	NBT	0.6030 a [40]	0.4293a [37]	13.95 a [20]
NBT	NBT	BT	0.0570 d [78]	0.2563d [34]	13.46 ab [13]
NBT	NBT	NBT	0.3653 b [111]	0.3666b [86]	12.60 b [30]

[a/] Means followed by the same letter within a column are not significantly different according to LSMEANS Procedure ($P \leq 0.05$).