SUSCEPTIBILITY OF COTTON BOLLWORM, *HELICOVERPA ZEA*, COLLECTED FROM GENUITY VT3 PRO FIELD CORN ON DUAL-GENE TRANSGENIC COTTON

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Abstract

Bollworm larvae were collected from Non-Bt and VT3P field corn. Larvae were evaluated on fitness costs of pupal duration and pupal weight. Reciprocal crosses were arranged to determine dose mortality curves for neonate larvae on lyophilized Bollgard II cotton. Male bollworm larvae collected from VT3P had a longer pupal duration compared to males collected from Non-Bt. Female pupal duration did not significantly differ. All larvae collected from VT3P had higher pupal weights than larvae collected from Non-Bt. Progeny from females reared on VT3P had higher LC50 values compared to progeny resulting from females reared on Non-Bt regardless of paternal host.

Introduction

Bollworm, *Helicoverpa zea* (Boddie), has an extensive host range, being collected from a total of 238 plant species within 36 plant families (Kogan et al. 1989). Of these, field corn is not only the most preferred but the most suitable host (Isley 1942, Gore et al. 2003). Larval development is more rapid and adults are more fecund when reared on field corn compared to other hosts or artificial diet. The most damaging outbreaks in cotton occur where corn and cotton are in comparable acreage. However, damage to cotton is the most common complaint because it has never been economically advantageous to treat field corn in order to control bollworm (Isley 1926). Silking corn is largely favored during early summer but as corn begins to mature, bollworm populations begin to transition into cotton (Lincoln 1972). The first transgenic corn and cotton varieties utilizing *Bacillus thuringiensis* (Bt) were implemented for control of tobacco budworm, *Heliothis virescens*¸ in cotton and several corn borer species attacking field corn. Bollworm susceptibility has been highly variable (Greenplate et al. 1998, Adamczyk et al. 2001) and researchers have begun to notice an increase in the frequency of bollworm outbreaks in Bt cotton. Trait packages in both corn and cotton containing two or more Bt genes utilize the same or similar proteins in both corn and cotton.

Trait Packages (abbreviations)*	Lepidoptera Active Traits
Cotton	
Bollgard II	Cry1Ac + Cry2Ab
Widestrike	Cry1Ac + Cry1F
Corn	
VT Double PRO (VT2P*), VT Triple PRO (VT3P*)	Cry1A.105 + Cry2Ab
Smartstax (GENSS* or SSX*)	Cry1A.105 + Cry2Ab + Cry1F
Agrisure Viptera 3110, Agrisure Viptera 3111	Cry1Ab + VIP3A

Figure 1. Bt corn and cotton trait packages in commercial production for use against bollworm.

The vast majority of corn acreage in Mississippi is planted to varieties containing at least one Bt protein. Furthermore, a large percentage of the bollworms infesting Bt cotton completed at least one generation in field corn (Jackson et al. 2008, Head et al. 2010). These factors are cause for concern about the potential selection pressure placed on bollworm populations completing development in Bt field corn and then transitioning into Bt cotton. To address these concerns, the objective of this research was to determine the susceptibility bollworms collected from Non-Bt and Genuity VT3 PRO field corn, and their reciprocal crosses on Bollgard II cotton. Fitness parameters such as pupal weight and pupal duration were also evaluated and will be presented.

Materials and Methods

Bollworm larvae were collected from Non-Bt and Genuity VT3 PRO field corn were collected across Mississippi, placed on artificial diet, and stored in a rearing facility on Mississippi State University. Pupal weight (recorded within a day of pupation) and pupal duration were recorded. Pupae were sexed by determining the presence of a ventral v-shaped suture on the females and two circular pads near the tip of the abdomen in the males. After segregating pupae by sex, backcrosses were done with each colony. Additionally, reciprocal crosses were done to determine if bollworm ability to survive on a Bt host is sex-linked. The following crosses were done:

Non-Bt (F*) x Non-Bt (M)

Non-Bt (F) x VT3P (M)

VT3P (F) x Non-Bt (M)

VT3P (F) x VT3P (M)

*Denotes the sex of pupa

Non-Bt and Bollgard II leaf tissue was collected in 1 gal zip-lock bags and placed in a -80°F freezer for approximately 48 hr. Tissue was then lyophilized and ground until dry powder would pass through a 40-mesh sieve. For bioassay, 100 mg of powder was diluted with 5.0 ml of 0.2% agar solution to make a 20 mg/ml stock solution. Further dilutions were performed from the stock solution to develop eight treatment concentrations to apply to artificial diet. Bioassay arenas were arranged by adding 0.5 ml of warm artificial diet to each well in a 128-well bioassay tray and allowed to cool at room temperature. Each well then had 50 µl of one of the treatment concentrations pipetted onto the diet surface for a total of 16 wells per concentration for each tray. Trays were again allowed to dry at room temperature. Once samples were dry, each well received one neonate larvae (16 larvae per concentration) with one tray of larvae per cross for each tissue type. Wells were then covered with perforated, adhesive tray covers and placed in a rearing room at 84°F.

Larval mortality was rated seven days after initiation. Mortality was assessed by determining larvae that had not molted into the second instar (larvae weighing <10 mg). Bioassay data were analyzed using probit analysis (Proc Probit SAS version 9.2). Fiducial limits that did not overlap were considered significantly different. Fitness data were analyzed using analysis of variance (Proc Mixed SAS version 9.2, α=.05).

Results

Overall pupal duration was significantly longer for individuals collected from VT3P compared to bollworms developed from Non-Bt. However when segregated, there was no difference in pupation time between females collected from VT3P and Non-Bt. Male pupal duration was significantly longer for larvae collected from VT3P; taking approximately 2 days longer to eclose compared to Non-Bt male pupae (Fig. 2). Male and female pupal weight of bollworm larvae collected from VT3P was significantly higher than those larvae collected from Non-Bt (Fig. 3). Progeny form the backcross of the VT3P colony and the reciprocal cross with the female from the VT3P colony had higher LC50 values on Bollgard II cotton than the backcross with the non-Bt colony and the reciprocal cross with the female from the non-Bt colony.

Discussion

In these colonies, males took longer to eclose than females as is the case in feral populations. Males appear to be more influenced by larval development on VT3P corn compared to females. Though there is a numerical trend, females did not statistically differ in pupal duration. Differences in female eclosion may be masked if larvae had not been removed from host plants and placed on artificial diet at a range of different instars. This delay in pupal development lends to the observation of prolonged adult bollworm moth flights in recent years. Bollworm moth flights have not only extended for longer duration, they have seemingly intensified with the introduction corn varieties utilizing Bt proteins aimed at bollworm control.

Pupal weights were higher for all individuals collected from VT3P corn, which indicate one of two possibilities for this occurrence. First and most likely, VT3P corn may be selecting for the largest, or most fit, individuals. Second, higher pupal weights may be an indication of enhanced tolerance of bollworms to Bt toxins. Regardless of mechanism, VT3P corn is selecting for the largest individuals to infest cotton.

LC50s were elevated in both crosses in which the female was collected on VT3P corn. There are a few functions that may be at play and determining the reason behind this observation will require further research. Given the current data set, it is unclear whether the mechanism responsible is a sex-linked trait or maternal influence. This research and crude analysis is preliminary and further steps are being evaluated in minutia in order to determine the exact mechanism of enhanced tolerance to dual-gene cotton.

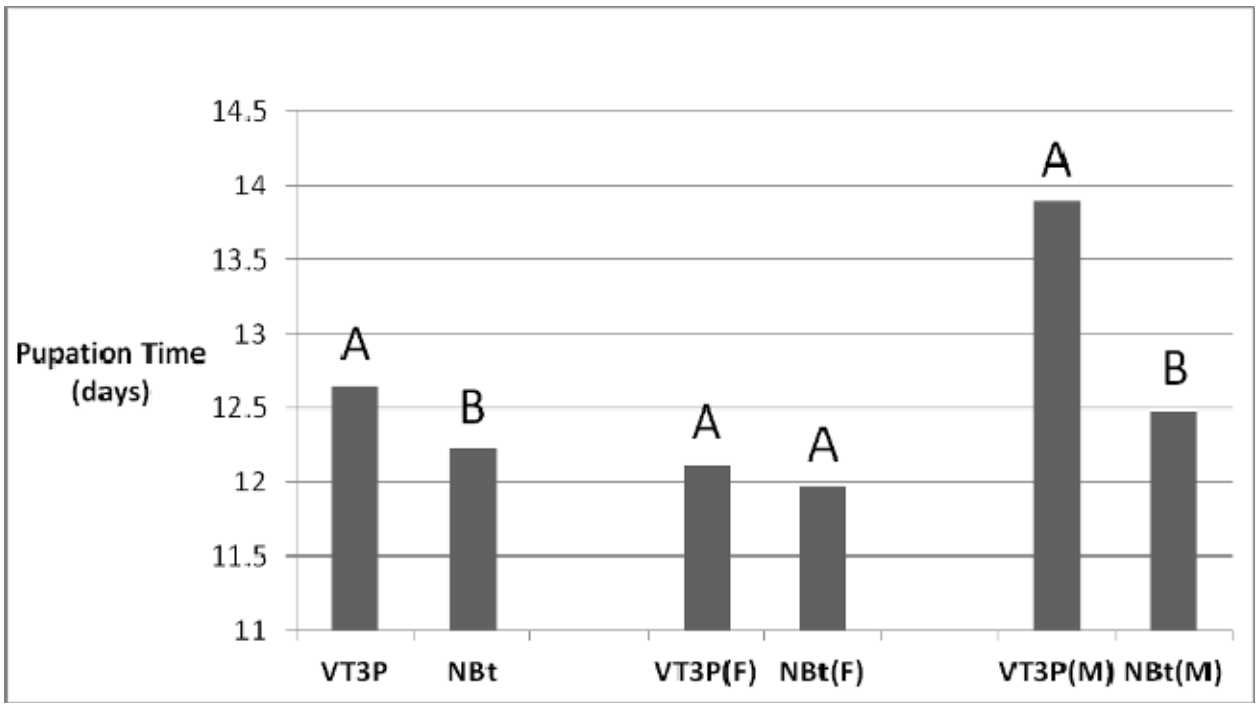

Figure 2. Pupal duration of larvae collected from Non-Bt and VT3P field corn. Column groupings with the same letter grouping do not significantly differ.

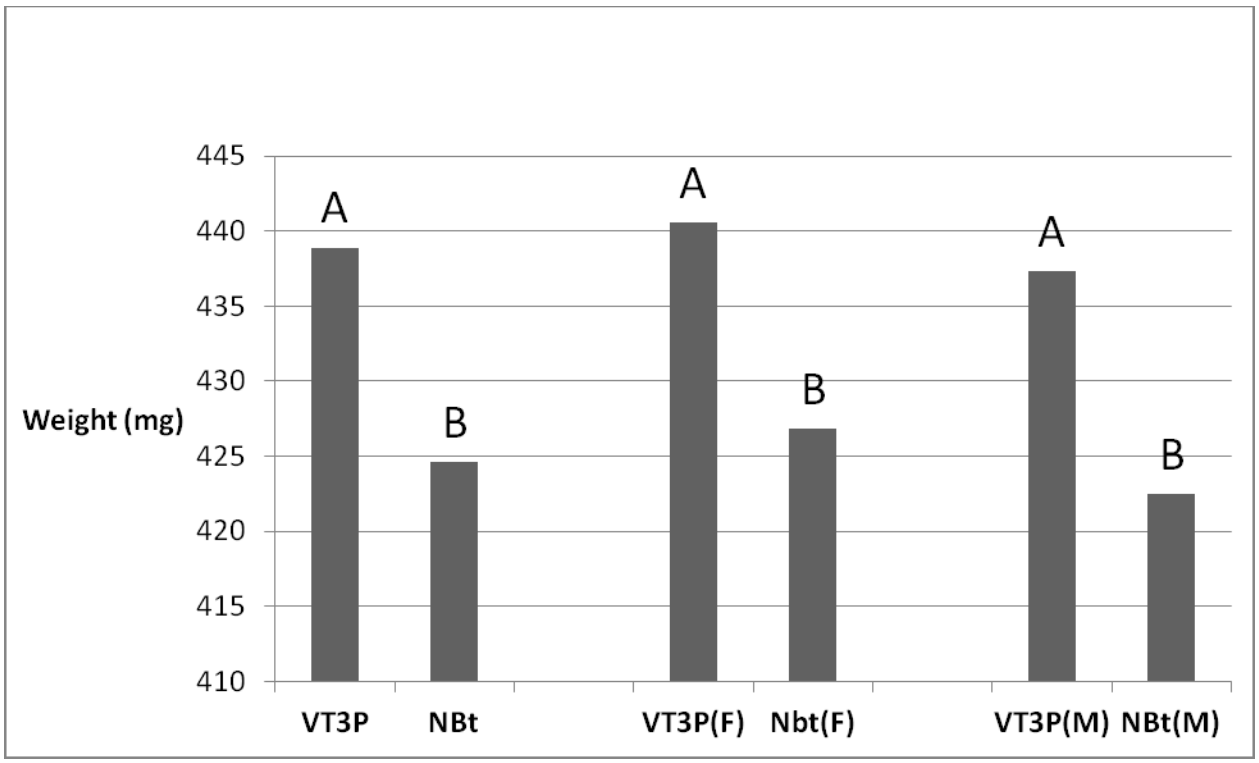

Figure 3. Pupal weight of larvae collected from Non-Bt and VT3P field corn. Column groupings with the same letter grouping do not significantly differ.

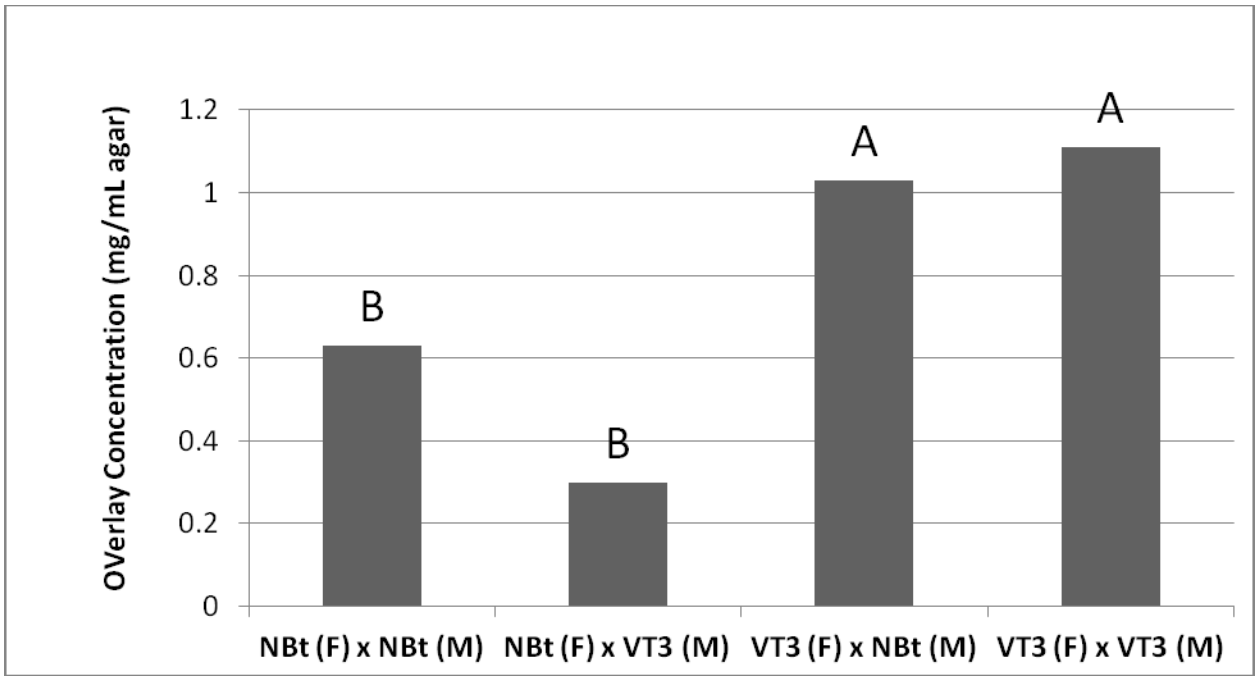

Figure 4. LC50s for Reciprocal Crosses of CEW Collected from VT3P and Non-Bt on BGII Lyophilized Tissue. Columns with the same letter grouping do not significantly differ.

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