FUNCTIONAL NON-TARGET DIFFERENCES BETWEEN BT AND CONVENTIONAL COTTON

Steven E. NARANJO

USDA-ARS, Western Cotton Research Laboratory
4135 East Broadway Road
Phoenix, AZ 85040, U.S.A.

snaranjo@wcrl.ars.usda.gov

ABSTRACT

A five-year field study was conducted in Arizona to assess the long term impact of transgenic cotton expressing the Cry1Ac δ- endotoxin of Bacillus thuringiensis (Bt) on population densities of 22 taxa of foliar-dwelling arthropod natural enemies and on the effect of the natural enemy community on key pests in the system. Multi-year analyses of arthropod abundance revealed small, but statistically significant, reductions in five common arthropod predator taxa in unsprayed Bt compared with unsprayed non-Bt cotton. In contrast, the use of conventional insecticides led to large reductions in 13 predator taxa. Furthermore, functional studies conducted over a three-year period indicated that the small reductions in abundance observed in Bt cotton may have little ecological meaning. Sentinel eggs and pupae of P. gossypiella experienced the same rates of mortality, primarily from predation, in both Bt and non-Bt cotton and cohort-based life tables for B. tabaci demonstrated that rates of sucking predation, parasitism and dislodgement (chewing predation in part) were unchanged between Bt and non-Bt cotton. Results demonstrate that long-term and multi-factor studies are required to examine meaningful non-target effects in the field.

INTRODUCTION

Transgenic crops expressing the insecticidal proteins of Bacillus thuringiensis (Bt) have been commercially available in the U.S. since 1996 and their adoption continues to expand rapidly in the U.S. and other parts of the developed and developing world (James 2004). In 2004 it was estimated that Bt cotton represented about 46% of all upland cotton production in the U.S. (USDA 2004). Use rates are much higher in Arizona where Bt cotton was grown on 81% of the upland cotton acreage in 2003, most of it (74%) in a stacked configuration with transgenes conferring glyphosate resistance (Tronstad et al. 2004). The primary target of Bt cotton in Arizona and southern California is the pink bollworm, Pectinophora gossypiella (Saunders), a caterpillar that feeds within the cotton fruit and is difficult to control with conventional insecticides (Henneberry and Naranjo 1998). Bt cotton is extremely effective in controlling this pest (Flint and Parks 1999).
As with any new technology, both benefits and risks are associated with transgenic crops in agricultural production systems. The use of transgenic crops have led to significant reductions in conventional, broad-spectrum insecticides, improved suppression of target pests, improved yields, reductions in production costs leading to increased profitability, and increased opportunities for biological control (Cannon 2000; Edge et al. 2001; Federici 2003; Shelton et al. 2002). Some of the potential risks include outcrossing through pollen drift, horizontal transfer of transgenes to other organisms, food safety, loss of susceptibility to Bt toxins in target pests, and effects on non-target organisms and biodiversity (Cannon 2000; Conner et al. 2003; Marvier 2001; Shelton et al. 2002; Wolfenbarger and Phifer 2000). Despite the long history of safety associated with the topical use of Bt endotoxins (Federici 2003; Glare and O’Callaghan 2000) the season-long expression of these toxins in crop plants has prompted research to address potential ecological concerns.

A growing number of studies have examined non-target effects in both the laboratory and field (see reviews by Glare et al. 2001, Lovei and Arpaia 2005; Pilson and Prendeville 2004; O’Callaghan et al. 2005; Schuler et al. 1999) with most concluding that Bt crops are highly selective. Laboratory studies have tended to focus on defining the effects of direct exposure or indirect exposure via trophic interactions to Bt toxins on the biology of non-target species while most field studies have focused primarily on changes in abundance and diversity of non-target taxa. Relatively few studies have examined predator/prey or host/parasitoid interactions, especially in the field (Bourguet et al. 2002; Orr and Landis 1997; Sisterson et al. 2004) and all have been relatively short-term in duration and have examined only a few of the potential interactions that may occur between natural enemies and their prey or hosts in transgenic crops.

Cotton hosts a rich diversity of parasitoid and arthropod predator species (van den Bosch and Hagen 1966; Whitcomb and Bell 1964), and these natural enemies are known play an important role in regulating pest herbivore populations (e.g., Eveleens et al. 1973; Naranjo and Ellsworth 2005; Stoltz and Stern 1978). Conventional cotton production relies heavily on the input of insecticides which typically have broad toxicity to both pests and their natural enemies. The pattern of insecticide use in cotton is one of the most severe constraints to realizing the potential of natural biological control in this system. Improving the compatibility between chemical and biological control depends on minimizing the effects of insecticides on natural enemies through reductions in use of broader-spectrum materials and adoption of more selective compounds (Hull and Beers 1985; Newsom et al. 1976). Transgenic Bt crops have the potential to contribute to natural enemy conservation through both their selective activity and associated reductions in the broad-spectrum insecticides they replace. However, longer-term and more inclusive studies are needed to define any potential unintended effects of transgenic crop production.

A five-year field study was conducted in Arizona to assess the long term effects of Bt cotton expressing the Cry1Ac dδ-endotoxin on natural enemy abundance and on the potential impact of the natural enemy community on pest populations. The objectives were to compare: 1) populations of a large group of common natural enemy taxa and several key target and non-target pests between Bt and non-Bt cottons and to contrast any potential
effects relative to conventional production practices using an array of selective and broad-spectrum insecticides, and 2) rates of natural enemy-induced mortality on two key pests between unsprayed Bt and non-Bt cotton using sentinel prey and field life table studies.

MATERIALS AND METHODS

STUDY SITE AND EXPERIMENTAL DESIGN
Cotton plots were established at the University of Arizona, Maricopa Agricultural Center, Maricopa, Arizona between 1999-2003. All plots were planted in early April of each year and grown according to standard agronomic practices for the area. Each year included a contrast between Deltapine NuCOTN 33B, a transgenic cultivar expressing the Cry1Ac insecticidal protein of B. thuringiensis, and its non-transgenic parent cultivar Deltapine 5415. A randomized complete block design with four replications was used in all years; plot size varied from 0.12-0.17 ha. Studies in 2001 and 2002 included positive control treatments which consisted of split plots of Bt and non-Bt main plots that were spayed for P. gossypiella, other lepidopteran pests, Bemisia tabaci (Gennadius) and Lygus hesperus Knight based on established action thresholds (Ellsworth and Barkley 2001; Ellsworth et al. 1996; University of California 1996). Applications were made on 12 and 20 July and 2 August in 2001, and 12 and 25 July and 16 and 28 August in 2002 consisting of organophosphates, pyrethroids, carbamates and insect growth regulators.

ARTHROPOD NATURAL ENEMY AND PEST DENSITY
Studies in all years tracked the density of a consistent, selected complex of 22 taxa of foliage-dwelling arthropod natural enemies, primarily predators, along with densities of various key pests including P. gossypiella, B. tabaci and L. hesperus. Most arthropods were sampled using a standard sweep net (38-cm diameter). Two sets of 25 sweeps were collected weekly in each plot between early June and mid-September each year. Densities of immature aphelinid parasitoids attacking B. tabaci (Eretmocerus spp. and Encarsia spp.) were estimated by weekly leaf samples (20-30 per plot) from the seventh mainstem node below the terminal. Densities of B. tabaci nymphs and adults were estimated weekly from early July through mid September each year using standard methods (Naranjo and Flint 1994; 1995). Densities of P. gossypiella larvae were estimated by counting all larvae inside 100 hostable green bolls per plot every two weeks from early July onward. Adult moth density was monitored weekly with beginning in June with pheromone traps. The abundance of other larval lepidopterans as well as L. hesperus were estimated from sweep net samples.

PEST MORTALITY STUDIES
Mortality of P. gossypiella and B tabaci was examined in unsprayed plots of Bt and non-Bt cotton from 2001-2003. To examine mortality of P. gossypiella eggs, small cards containing 20 eggs (1 d old) obtained from the USDA-ARS rearing facility in Phoenix, AZ were pinned under the bracts of cotton bolls that were approximately 20 day old to simulate oviposition by female moths. Twenty cards (one per plant) were placed in each plot and left exposed for 24 hours after which they were examined under magnification in the laboratory for evidence
of predation. Intact eggs that remained were then held at 27°C for an additional 6 d to evaluate parasitism. The experiment was repeated 3-4 in 2001-2003. The mortality of *P. gossypiella* pupae (USDA-ARS rearing facility in Phoenix, AZ) were studied on four dates each in 2002-2003. Individual pupae were placed at the base of 20 cotton plants in each plot and left exposed for 24 hours. This simulated a type of pupation site used by larvae exiting bolls. Pupae that remained were returned to the laboratory and examined under magnification for evidence of predation. Intact pupae were held for three weeks to evaluate parasitism. Appropriate controls were run for both sentinel egg and pupae. Morality of *B. tabaci* nymphs was examined using an *in situ* life table approach (Naranjo and Ellsworth 2005). Briefly, the method involves marking the location of individual settled 1st instar nymphs (≥ 50 per plot) with a non-toxic felt pen on the underside of leaves and then repeatedly observing these nympha every 2-3 d until death or adult emergence. Mortality due to dislodgment (from wind, rain and chewing predators), sucking predation, parasitism, and unknown causes was recorded for each of the four nymphal instars. Life table studies were repeated twice each year between mid-July and early September in 2001-2003.

**ANALYSES**

Yearly analyses were conducted for all arthropod taxa (Naranjo 2005a) but only multiyear analyses will be highlighted here. These analyses were conducted by calculating seasonal mean densities for all taxa for each replicate plot in each year and entering block and year as random effects. Arthropod counts were transformed by \((x+0.5)^{0.5}\) or \(ln(x+1)\) throughout as necessary to achieve normality and homoscedasticity before analyses; untransformed means are presented. The response variable for egg and pupal mortality of *P. gossypiella* was the proportion missing, eaten or parasitized. Marginal mortality rates were calculated from *B. tabaci* life table data based on apparent mortality using the methods outlined by Elkinton et al. (1992) (see Naranjo and Ellsworth 2005 for details) to correct for mortality due to contemporaneous agents. Mixed model ANOVA was used to test for treatment effects in each year where block and trial were entered as random effects. Mixed model ANOVA was also used for multi-year analyses with block, year and trial within year entered as random effects. Proportional and marginal mortality values were transformed by arcsin prior to analyses as needed.

**RESULTS**

**ARTHROPOD ABUNDANCE**

Yearly analyses revealed few differences between *Bt* and non-*Bt* cotton in any natural enemy taxa and no differences for the natural enemy community as a whole (Fig. 1). However, multiyear analyses revealed significant \((P < 0.05)\) declines in seasonal densities of five predator taxa in *Bt* compared with non-*Bt* cotton including a group of miscellaneous spiders, *Hippodamia convergens* Guérin-Méneville, *Geocoris punctipes* (Say), *Nabis alternatus* Parshley and *Drapetis* nr. *divergens* (Table 1). In general, the changes in density were smaller than those observed in individual years; however, the increased sample size of the analyses improved power considerably allowing smaller changes to be detected. Overall, the mean decline in these five taxa was around 19%. In contrast, the use of insecticides resulted in signifi-
cant \((P < 0.05)\), and larger declines in 13 individual taxa averaging nearly 48% (Table 1). As expected, there were essentially no \(P. gossypiella\) larvae in \(Bt\) cotton and densities of other lepidopterans were reduced in most years (Fig. 1). However, populations of two other key pests, \(B. tabaci\) and \(L. hesperus\) were similar in \(Bt\) and non-\(Bt\) cotton (Fig. 1).

**MORTALITY OF PEST INSECTS**

Eggs of \(P. gossypiella\) were readily preyed upon in both \(Bt\) and non-\(Bt\) cottons with no significant differences \((P > 0.05)\) in rates of predation in each of three individual years or all years combined (Fig. 2). No parasitism was detected in eggs that survived predation in any year. Pupae of \(P. gossypiella\) placed on the soil surface beneath plants also experienced high rates of mortality and there was no significant difference \((P > 0.05)\) in rates of pupal mortality between \(Bt\) and non-\(Bt\) cottons in either year or both years combined (Fig. 2). Most pupae disappeared presumably from the action of chewing predators and no parasitism was observed in intact pupae held in the laboratory after field exposure.

**Figure 1.** Seasonal mean density of arthropod natural enemies and pests over all sample dates. Error bars are 95% confidence intervals. From Naranjo (2005a).
Table 1. Overall change in mean densities of arthropods (per 50 sweeps) in Bt and non-Bt cottons (5 years) and in sprayed and unsprayed cottons (2 years), Maricopa, AZ, 1999-2003. Data from Naranjo (2005a).

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Non-Bt densitya</td>
<td>Prop. (\Delta(P)b)</td>
</tr>
<tr>
<td>Dictyna reticulata</td>
<td>Araneida: Dictynidae</td>
<td>0.62±0.05</td>
<td>0.121 (0.56)</td>
</tr>
<tr>
<td>Gertsch and Ivie</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Misumenops celer</td>
<td>Araneida: Thomisidae</td>
<td>2.59±0.28</td>
<td>-0.038 (0.42)</td>
</tr>
<tr>
<td>(Hentz)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salticidae</td>
<td>Araneida: Salticidae</td>
<td>0.33±0.07</td>
<td>-0.268 (0.07)</td>
</tr>
<tr>
<td>Other Araneida</td>
<td>Araneida</td>
<td>0.63±0.14</td>
<td>-0.233 (0.02)</td>
</tr>
<tr>
<td>Collops vittatus (Say)</td>
<td>Coleoptera: Melyridae</td>
<td>1.65±0.29</td>
<td>-0.062 (0.51)</td>
</tr>
<tr>
<td>Hippodamia convergens</td>
<td>Coleoptera: Coccinellidae</td>
<td>1.20±0.13</td>
<td>-0.189 (0.04)</td>
</tr>
<tr>
<td>Guérin-Meneville</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anthicidae</td>
<td>Coleoptera</td>
<td>1.48±0.23</td>
<td>-0.095 (0.33)</td>
</tr>
<tr>
<td>Other Coccinellidae</td>
<td>Coleoptera</td>
<td>0.59±0.18</td>
<td>-0.132 (0.56)</td>
</tr>
<tr>
<td>Geocoris punctipes (Say)</td>
<td>Heteroptera: Lygaeida</td>
<td>7.30±1.69</td>
<td>-0.176 (0.01)</td>
</tr>
<tr>
<td>Geocoris pallens (Stål)</td>
<td>Heteroptera: Lygaeida</td>
<td>4.30±0.79</td>
<td>0.058 (0.38)</td>
</tr>
<tr>
<td>Orius tristicolor (White)</td>
<td>Heteroptera: Anthocoridae</td>
<td>4.89±0.67</td>
<td>0.054 (0.21)</td>
</tr>
<tr>
<td>Nabis alternatus</td>
<td>Heteroptera: Nabidae</td>
<td>2.53±0.25</td>
<td>-0.238 (&lt;0.01)</td>
</tr>
<tr>
<td>Parshley</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zelus renardii</td>
<td>Heteroptera: Reduviidae</td>
<td>0.71±0.20</td>
<td>-0.011 (0.77)</td>
</tr>
<tr>
<td>Kolenati</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sinea spp.</td>
<td>Heteroptera: Reduviidae</td>
<td>0.01±0.01</td>
<td>0.370 (0.74)</td>
</tr>
<tr>
<td>Lygus hesperus Knight</td>
<td>Heteroptera: Miridae</td>
<td>18.8±2.23</td>
<td>-0.073 (0.35)</td>
</tr>
<tr>
<td>Pseudatomoscelis</td>
<td>Heteroptera: Miridae</td>
<td>10.3±2.33</td>
<td>0.044 (0.98)</td>
</tr>
<tr>
<td>seriatus (Reuter)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spanogonicus</td>
<td>Heteroptera: Miridae</td>
<td>2.99±0.53</td>
<td>0.052 (0.38)</td>
</tr>
<tr>
<td>albofasciatus (Reuter)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhinacloa forticornis</td>
<td>Heteroptera: Miridae</td>
<td>0.26±0.07</td>
<td>-0.160 (0.31)</td>
</tr>
<tr>
<td>Reuter</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chrysoperla carnea s.l.</td>
<td>Neuroptera: Chrysopidae</td>
<td>2.27±0.21</td>
<td>-0.042 (0.56)</td>
</tr>
<tr>
<td>Stephens</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drapetis nr. divergens</td>
<td>Diptera: Empididae</td>
<td>19.3±4.31</td>
<td>-0.118 (0.02)</td>
</tr>
<tr>
<td>Aphelinid parasitoids</td>
<td>Hymenoptera: ApheLINIidae</td>
<td>6.18±1.26</td>
<td>-0.273 (0.21)</td>
</tr>
<tr>
<td>Other Hymenoptera</td>
<td>Hymenoptera</td>
<td>1.72±0.20</td>
<td>0.039 (0.59)</td>
</tr>
</tbody>
</table>

* Overall means (±SE) based on seasonal means in four replicate main plots in each of five years for Bt and non-Bt contrasts (n=20) and two years for unsprayed and sprayed contrasts (n=8).

b Prop. \(\Delta\) is the proportional change in density in Bt cotton relative to non-Bt cotton or sprayed cotton relative to unsprayed cotton. Numbers in parentheses following prop. \(\Delta\) are P-values; values < 0.05 are bolded.
Nymphs of *B. tabaci* were subject to high rates of predation, moderate rates of dislodgement, and low to moderate rates of parasitism over the three years of study (Fig. 3). Marginal rates of predation did not differ significantly (*P* > 0.05) between *Bt* and non-*Bt* cotton in any year or all years combined. Rates of dislodgement, which included the effects of chewing predation and weather (Naranjo and Ellsworth 2005) varied over years but not as a result of the use of *Bt* cotton. Parasitism was generally low but there was no difference (*P* > 0.05) in rates of parasitism between the two cottons.
DISCUSSION

Although separate analyses each year generally indicated no negative effects, combined analyses across the five years revealed a significant average decline of about 19% in five predator taxa representing four orders including *H. convergens*, *G. punctipes*, *N. alternatus*, *D. nr divergens* and a group of miscellaneous spiders. With very few exceptions there was a numerical decline in seasonal population density of all these taxa in *Bt* compared with non-*Bt* cotton in all five years. Combining the data sets simply allowed for a larger sample size and correspondingly greater statistical power to discern smaller changes in density.

The causes for these declines are uncertain but could be associated with sampling error, declines in target or non-target prey abundance, or sublethal effects resulting from exposure to *Bt* toxins. Sampling error seems to be an unlikely cause because populations were consistently lower in *Bt* cotton for the five taxa in the majority of years. There was also no obvious difference in the canopy structure between *Bt* and non-*Bt* cotton that could have affected sampling efficiency. Many stages of *P. gossypiella* are relatively invulnerable to natural enemies (Henneberry and Naranjo 1998) and so it is unlikely that the absence of this prey in *Bt* cotton would have measurable effects on generalist predator populations. *B. tabaci* was the most abundant prey for the affected predators but densities of immature and adult stages were similar in both *Bt* and non-*Bt* cotton. Other potential caterpillar prey occurred at low densities and differed relatively little between *Bt* and Non-*Bt* cotton, however, because all are foliage feeders they are susceptible to predation and reductions in their density may have influenced predator populations. Direct feeding on the plant by *G. punctipes* and *N. alternatus* could expose these predator to *Bt* toxins, however, Armer et al. (2000) found no negative effects for *Geocoris* and *Nabis* spp. feeding directly on *Bt* potato foliage. Pollen feeding may be an avenue of exposure to the predaceous bugs as well as *H. convergens* but this has not been examined in these species. *D. nr. divergens* largely specialize on adult *B. tabaci* which are phloem feeders and unlikely to possess *Bt* toxins in their bodies. However, Ponsard et al. (2002) observed modest declines (H"27%) in longevity of adult *G. punctipes* and *O. tristicolor* (but not *Nabis* spp.) feeding strictly on *Bt* intoxicated *S. exigua* compared with larvae feeding on non-*Bt* cotton in the laboratory. Although, these predators would not feed exclusively on such caterpillars in the field it does suggest a potential explanation that merits further study.

The biological relevance of these declines in *Bt* cotton is also uncertain. Clearly, conventional alternatives to the use of *Bt* cotton, as represented by the positive controls in this study, are many times more damaging to the natural enemy community, causing much larger reductions in density and affecting a broader range of taxa. Such disruptions by broad-spectrum insecticides have been shown repeatedly to compromise the natural biological control of cotton pests (e.g., Eveleens et al. 1973; Stoltz and Stern 1978). On the contrary, the results of functional studies here demonstrated that the overall contribution of the natural enemy community to mortality of *P. gossypiella* eggs and pupae and *B. tabaci* nymphs was equal in *Bt* and non-*Bt* cotton that received no additional insecticide applications. In addition there was no indication of resurgence by key pests in the system (*B. tabaci* or *L. hesperus*) that might indicate a reduction in natural control. Thus, the small declines in several taxa of natural enemies in *Bt* cotton observed here may not be ecologically meaningful in terms of at least some trophic interactions.
The lack of association of reductions in density of some predator taxa and rates of natural enemy mortality on two key pests in the system may be explained by the general feeding behavior of most of these predators. Reductions in the density and associated activity of any one species in the complex is offset or replaced by the activity of other members of the community. Life table studies with \textit{B. tabaci} revealed that most of the mortality from any one source is replaceable (Naranjo and Ellsworth 2005). Thus, a reduction in predation \textit{G. punctipes} or \textit{N. alternatus}, for example, could be easily replaced by \textit{Orius tristicolor} White or \textit{Zelus renardii} Kolenti. However, there are limits to the amount of mortality that can be replaced by the natural enemy community. Life table studies with \textit{B. tabaci} also have shown that the use of broad-spectrum insecticides which cause large reductions in natural enemy density can significantly reduce the contribution of these natural enemies to pest mortality leading to a situation where the continued use of insecticides are required for pest suppression (Naranjo 2001; Naranjo and Ellsworth unpublished data). In contrast, the use of selective insecticides for \textit{B. tabaci} only slightly reduces the abundance of various natural enemies (Naranjo et al. 2004) but allows the complex to continue contributing significant mortality that enables long-term pest suppression in the absence of additional insecticides (Naranjo 2001; Naranjo and Ellsworth unpublished data).

Transgenic \textit{Bt} cotton appears to represent a highly effective and selective technology for lepidopteran pest control. The long-term studies described here suggest that negative effects of \textit{Bt} cotton on non-target arthropods, particularly natural enemies, are minimal and that even small declines in density of some taxa do not appear to be associated with any meaningful changes in the function of the overall natural enemy community. The use of \textit{Bt} cotton and other selective methods of pest control will continue to advance the important role of biological control in cotton IPM in the western U.S. and elsewhere.

**ACKNOWLEDGMENTS**

I thank Kim Beimfohr, Rebecca Burke, Luis Cañas, Melanie Charney, Scott Davis, Greg Owens and Jeffrey Rivas for expert technical assistance and Peter Ellsworth (Univ. Arizona) for assistance with insecticide applications. This study was funded entirely by public funds appropriated to the USDA-ARS through CRIS project 5344-22620-015-00D.

**REFERENCES**


