ARTHROPOD COMMUNITIES AND TRANSGENIC COTTON IN THE WESTERN UNITED STATES: IMPLICATIONS FOR BIOLOGICAL CONTROL

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INTRODUCTION

Cotton, transgenically modified to express the insecticidal proteins of *Bacillus thuringiensis* (*Bt*), has been available commercially in the United States since 1996. *Bt* cotton is widely used throughout the cotton belt (Layton *et al*., 1999), and more than 65% of the acreage in Arizona has been planted to *Bt* cotton since 1997. In the low desert production areas of Arizona and California, *Pectinophora gossypiella* (Saunders), the pink bollworm, is the major target of *Bt* cotton. A number of other lepidopterous species occur in this area, but they are sporadic secondary pests of cotton whose population outbreaks are typically induced by indiscriminate use of broad-spectrum insecticides. As a result of the adoption of *Bt*-cotton and the coincident introduction and adoption of selective insect growth regulators for suppression of *Bemisia tabaci* (Gennadius), insecticide usage in Arizona cotton over the past decade as declined from a high of 12.5 applications per acre in 1995 to 1.9 in 1999 (Ellsworth and Jones, 2001). These reductions in insecticide use have broadened opportunities for all biological control approaches in cotton.

Beyond concern for the maintenance of susceptibility in target pest populations there also are a number of ecological and environmental questions associated with use of transgenic crops, one of the most prominent being effects on non-target organisms. Although *Bt* crops are putatively selective, potential effects on non-target insects have been found, the most sensational involving the monarch butterfly (*Danaus plexippus* [L.]) and *Bt* corn (Losey *et al.,* 1999; Hansen and Obrycki, 2000). Non-target effects in *Bt* cotton have received relatively little attention. A number of parasitoid and arthropod predator species naturally inhabit cotton fields in the western United States (van den Bosch and Hagen, 1966; Gonzales *et al*., 1977), and it is generally recognized that they play an important role in regulating pest populations (Whitcomb, 1980). Here, we present preliminary results from on-going comparative field studies in Arizona to evaluate non-target effects of *Bt* cotton. We will emphasize natural enemy abundance, overall arthropod diversity, and the ecological function of natural enemy complexes.

MATERIALS AND METHODS

The research presented here is a combination of two independent and on-going programs of investigation conducted in central Arizona; however, the general methods used in each were similar and they will be described together. Field research plots were established at the University of Arizona, Maricopa Agricultural Center, Maricopa, Arizona in 1999-2001. Experimental designs were randomized complete blocks with four replications. Individual plots ranged in size from 0.03 to 0.15 ha, depending on the study. In every case, we contrasted single transgene events (Cry1Ac; Bollgard®, Monsanto, St. Louis, Missouri) with the non-transgenic parent cultivar. In some cases, both single and two gene events (Cry1Ac and Cry2Ab; Bollgard II, Monsanto) were examined. In all instances, there were treatments of both *Bt* and non-*Bt* cottons that received no additional insecticide applications for any pests. In some cases, studies also included treatments that received additional insecticide applications for both lepidopterous and other pests as needed. All cotton was maintained according to accepted agronomic practices for the area. A variety of sampling methods were used, including standard sweep nets (38-cm diameter, 50 sweeps per plot), beat-buckets, whole plant inspections, and pitfall traps (12 by 9.5 cm diameter; buried flush with the soil surface within rows and shaded with a white plastic cover). In this report we will discuss only results from sweep nets and pitfall traps in 1999 and 2000. ANOVA was used to test for treatment differences. The program EstimateS 5 (Colwell, 1997) was used to characterize arthropod community structure for pitfall trap data.

Additional studies were conducted in 2001 to examine comparative rates of predation and parasitism on *P. gossypiella* eggs and *B. tabaci* nymphs in *Bt* and non-*Bt* plots that received no additional insecticides. For studies with *P. gossypiella*, we placed small egg cards (15-20 eggs per card, 1 d old) under the bracts of cotton bolls to simulate the oviposition behavior of female moths. Eggs were obtained from the USDA ARS rearing facility in Phoenix, Arizona. Twenty cards were placed per plot and were exposed to predators and parasitoids for 24 hours. Cards were collected and examined in the laboratory for predation. They were then held for an additional 6 days to evaluate parasitism. The experiment was repeated three times during the season. For analyses we calculated proportional attack rates. For *B. tabaci*, we used an *in situ* life table approach (Naranjo *et al.*, 1998) to estimate sources and rates of mortality on nymphs. Briefly, the method involves marking the location of settled first instar nymphs with a nontoxic felt pen on the underside of leaves and then repeatedly observing these nymphs every 2-3 days until death or adult emergence. The experiment was repeated twice during the season. For analyses we calculated marginal rates of mortality from predation and parasitism.

RESULTS

Natural Enemy Abundance

In studies that did not involve the use of additional insecticides, we generally found that the seasonal abundance of natural enemies estimated with sweep nets was not significantly affected by *Bt* cotton in comparison to non-*Bt* cotton. Results for 10 of the most common predators and for parasitic hymenoptera as a group are shown in Table 1. Seasonal densities of *Nabis alternatus* Parshley were significantly lower ($P = 0.03$) in *Bt* plots in 1999 and densities of *Zelus renardii* Kolenati were marginally lower ($P = 0.08$) in *Bt* plots in the same year. These larger predators can attack caterpillars and reductions of such prey in *Bt* cotton may have influenced the movement or reproduction of these predators. However, the effect was not consistent, as no differences for these species were found between *Bt* and non-*Bt* cottons in 2000. We also found no differences in the season-long density of parasitoids.

In studies that involved the use of additional insecticides for control of caterpillars and other pests, we found that insecticide use, but not *Bt* cotton, affected the abundance of natural enemies by causing a dramatic reduction in their populations in sprayed plots. There was no difference in natural enemy abundance between *Bt* cottons with one versus two toxin genes (Fig. 1).

Table 1. Mean season-long abundance[a] of common arthropod predators and parasitic Hymenoptera in non-*Bt* and *Bt* cotton, 1999-2000, Maricopa, Arizona.

	1999			2000		
Species	Non-Bt	Bt	P[b]	Non-Bt	Bt	P[b]
Misumenops celer (Hentz) (Araneida: Thomisidae)	378	377	0.98	300	284	0.70
Collops vittatus (Say) (Coleoptera: Melyridae)	187	166	0.37	80	104	0.22
Hippodamia convergens Guérin-Méneville (Coleoptera: Coccinellidae)	179	156	0.35	110	101	0.79
Geocoris spp (Heteroptera: Lygaeidae)	2036	1576	0.41	603	620	0.88
Orius tristicolor (White) (Heteroptera: Anthocoridae)	664	762	0.34	337	402	0.18
Nabis alternatus Parshley (Heteroptera: Nabidae)	385	220	0.03	171	155	0.66
Zelus renardii Kolenati (Heteroptera: Reduviidae)	94	54	0.08	13	36	0.16
Spanogonicus albofasciatus (Reuter)(Heteroptera: Miridae)	359	304	0.62	217	231	0.78
Chrysoperla carnea Stephens (Neuroptera: Chrysopidae)	246	208	0.24	195	229	0.16
Drapetis sp. (Diptera: Empididae)	5611	4530	0.28	1345	1410	0.39
Total Predators	10960	9005	0.20	3599	3767	0.63
Hymenoptera	257	220	0.57	75	90	0.51

[a] Abundance measured as the area under the seasonal incidence curve (weekly samples, 50 sweeps per plot).

[b] The P-value from ANOVA for a randomized block design; df = 1, 5.

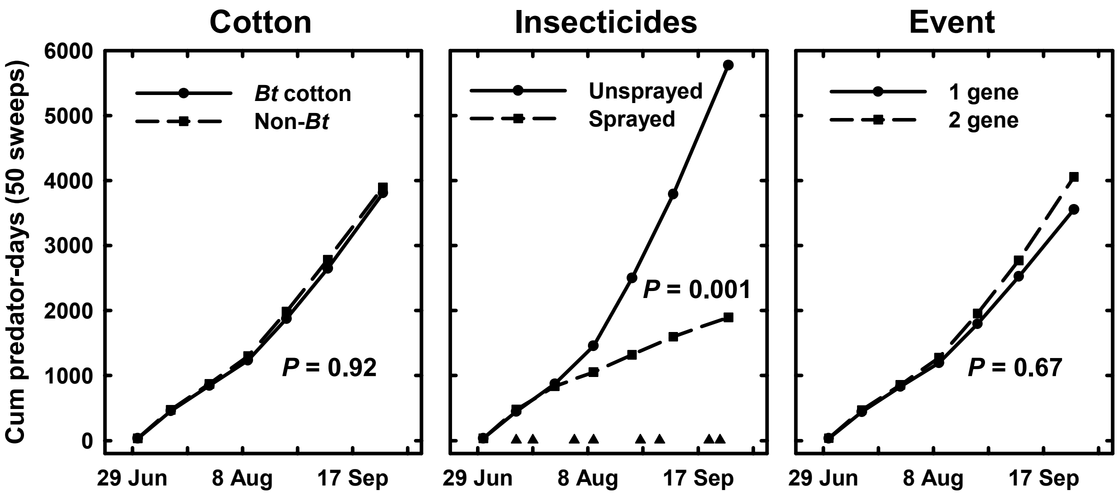

Figure 1. Factorial analysis of the effect of cotton type, insecticide use, and transgenic event on the abundance of predatory arthropods over the season. The *P*-value is indicated for the difference in season-long abundance, 1999, Maricopa, Arizona. Triangles along bottom denote dates of insecticide applications.

Arthropod Community Structure

Preliminary analyses of community structure were completed for all arthropods collected in pitfall traps over the entire season for both 1999 and 2000. Three characteristics were examined: (1) *species richness*, which we calculated simply as the number of species observed, (2) *evenness*, which measures the equitability of abundance across species (here we use the Shannon evenness index), and (3) *diversity*, which attempts to integrate both richness and evenness (here we use the Fisher alpha index). Factorial ANOVA was used to examine the effects of cotton type, insecticide use, and the number of transgenes (Table 2). Species richness varied between years, but was only significantly different between sprayed and unsprayed plots in 2000, with a dramatic reduction in richness with insecticide use. Likewise, diversity varied by year, but was only significantly lower in plots treated with insecticides in 2000. This result follows directly from lower richness and higher evenness. Evenness was more variable, but significant differences were generally restricted to contrasts involving use of insecticides. In both years, evenness increased with the use of insecticides. It would appear that insecticide use acts to increase equitability among the species present, probably by reducing the density of the most abundant species. Further analyses of shared species (not shown) indicated that only insecticide use significantly altered the species composition by eliminating some species. This was especially apparent in 2000.

Table 2. Analysis of community composition of arthropods captured in pitfall traps (season long), in relation to cotton type, insecticide use, and number of transgenes, 1999-2000, Maricopa, Arizona.

	1999			2000		
	Richness[a]	Diversity[a]	Evenness[a]	Richness	Diversity	Evenness
Non-Bt	70	16.2	0.54	57	15.3	0.59
Bt	68	17.8	0.60	57	14.9	0.58
P	0.62	0.11	0.03	0.98	0.75	0.69
df = 1, 21						
No-insecticides	70	16.9	0.55	74	16.9	0.52
Insecticides	68	17.5	0.61	39	13.2	0.64
P	0.58	0.57	0.02	<0.01	<0.01	<0.01
df = 1, 21						
1 Bt gene	67	16.9	0.58	58	14.8	0.54
2 Bt genes	69	18.7	0.62	55	15.1	0.60
P	0.59	0.14	0.22	0.75	0.85	0.19
df = 1, 13						

[a] Richness = number of species; Diversity = Fisher's alpha; Evenness = Shannon evenness (range 0-1). All indices calculated with EstimateS 5 (Colwell, 1997). Interaction terms nonsignificant.

Enemy Function

We have seen that changes in natural enemy abundance and overall changes in community structure may be subtle between *Bt* and non-*Bt* cottons. However, from the perspective of biological control, perhaps the most significant question is whether the use of transgenic crops alters the existing function of the natural enemy complex. Studies conducted in 2001 were designed to address this issue by contrasting mortality of two key pests in the system due to predators and parasitoids. No additional insecticides were used in these studies. We found no change in the level of predation on eggs of *P. gossypiella* between *Bt* and non-*Bt* cotton plots. Consistently, over 50% of the sentinel eggs we placed in the field were consumed by either chewing or sucking predators (Table 3). No parasitism was observed in either type of cotton. Similarly, marginal rates of predation on *B. tabaci* nymphs did not differ and were roughly 50% in both *Bt* and non-*Bt* cotton. Parasitism on whitefly nymphs was low and did not differ between the cottons. These high rates of predation and low rates of parasitism are consistent with previous extensive life table studies in Arizona cotton (Naranjo, 2001).

Table 3. Comparative rates of predation and parasitism on two key insect pests in non-*Bt* and *Bt* cotton receiving no additional insecticides, 2001, Maricopa, Arizona.

	Pectinophora gossypiella eggs[a]		*Bemisia tabaci* nymphs[b]	
	Predation	Parasitism	Predation	Parasitism
Non-Bt	0.57	0.0	0.46	0.028
Bt	0.51	0.0	0.51	0.029
P	0.44	-	0.82	0.84

[a] Proportion of sentinel eggs on cotton bolls attacked over three separate trials; factorial ANOVA, trial-by-cotton interaction nonsignificant, df = 1, 17

[b] Marginal mortality rates of nymphs over two separate trials; factorial ANOVA, trial x cotton interaction nonsignificant, df = 1, 11

DISCUSSION

Non-target studies of the potential impact of transgenic crops under larger scale production are in their infancy. However, the general results of our studies are consistent with findings of similar field studies conducted in transgenic cotton, corn, and potatoes in which no consistent or detrimental non-target effects could be demonstrated (e.g., Orr and Landis, 1997; Riddick and Barbosa, 1998a, 2000; Wilson and Fitt, 2000; Dively and Rose, 2002; Moar *et al.*, 2002). The potential effects of *Bt* toxins on natural enemies can be both direct and indirect. A recent study by Armer *et al.* (2000) found no deleterious effects on four heteropteran predators feeding on *Bt* potato foliage. Results from studies of indirect exposure, which occurs through consumption of intoxicated non-target prey, have been mixed. Hilbeck *et al.* (1998, 1999) showed minor effects on life history traits of *Chrysoperla carnea* Stephens feeding on intoxicated *Spodoptera littoralis* (Boisduval) and *Ostrinia nubilalis* (Hübner). But Dogan *et al.* (1996) reported no effects on *Hippodamia convergens* Guérin-Méneville feeding on intoxicated aphids in potato, and Riddick and Barbosa (1998b) found no effects on *Coleomegilla maculata* (De Geer) feeding on intoxicated *Leptinotarsa decemlineata* (Say).

Field studies integrate both direct and indirect effects, but existing studies have generally been limited to comparative evaluations of abundance. We exemplify an approach here that attempts to evaluate non-target effects in a more holistic fashion by including not only comparisons of species abundance, but also examination of potential changes in community composition and structure, and potential effects on the ecological function of members of that community. The preliminary results presented here suggest that use of transgenic cotton may not have any consistent adverse effects and represents an extremely selective control method that may facilitate the broader use of biological control and IPM in an agricultural system long dominated by the use of broad-spectrum insecticides.

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