Long-Term Assessment of the Effects of Transgenic *Bt* Cotton on the Function of the Natural Enemy Community

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ABSTRACT   Field studies to assess effects of transgenic crops on nontarget organisms primarily have focused on nontarget abundance and diversity, whereas few have examined effects on nontarget community function. A 5-yr field study in Arizona assessed whether transgenic cotton producing the Cry1Ac toxin of *Bacillus thuringiensis* (*Bt*) had an effect on the natural enemy community's impact on three key pests. Predator:prey ratios for *Bemisia tabaci* (Gennadius) and *Lygus hesperus* Knight were similar in unsprayed *Bt* and non-*Bt* cotton. Insecticide applications in positive control plots inconsistently altered ratios for *B. tabaci*. Predation indices based on the known feeding activity of selected predators showed that potential predator impact was unaltered by *Bt* cotton but was consistently depressed with insecticide applications. Sentinel eggs and pupae of *Pectinophora gossypiella* (Saunders) experienced the same rates of predation in both unsprayed *Bt* and non-*Bt* cotton. Cohort-based life tables for *B. tabaci* showed that rates of sucking predation, parasitism, and dislodgement (chewing predation in part) were unchanged by *Bt* cotton. Underlying experimental designs were sufficient to consistently detect changes of <20% in predator:prey ratios and predation indices and changes of <10% in rates of *P. gossypiella* and *B. tabaci* mortality with >80% power (α = 0.05). These long-term studies showed essentially no effects of *Bt* cotton on natural enemy function in Arizona cotton and further showed that minor reductions in density of several predator taxa in *Bt* cotton observed in a companion study may have little ecological meaning relative to natural enemy impact on key pests in the system.

KEY WORDS   *Pectinophora gossypiella*, *Bemisia tabaci*, *Lygus hesperus*, arthropod predators, nontarget effects

A RICH DIVERSITY OF parasitoid and arthropod predator species are known to inhabit cotton fields (Whitcomb and Bell 1964, Van den Bosch and Hagen 1966, Gonzalez et al. 1977), and it is generally recognized that natural enemies play an important role in regulating pest herbivore populations (e.g., Leigh et al. 1966, Eveleens et al. 1973, Stoltz and Stern 1978, Whitcomb 1980, Naranjo and Ellsworth 2005). The use of insecticides with broad toxicity to both pests and their natural enemies is one of the most severe constraints to realizing the potential of biological control in managing pests of field and other crops (Stern et al. 1959, Newsom et al. 1976, Croft, 1990). 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 (Newsom et al. 1976, Hull and Beers 1985). Transgenic crops producing the insecticidal

proteins of *Bacillus thuringiensis* Berliner (*Bt*) have the potential to contribute to natural enemy conservation through both their selective activity and associated reductions in the broad-spectrum insecticides they replace (Edge et al. 2001, Shelton et al. 2002, Federici 2003).

Transgenic *Bt* crops have been commercially available in the United States since 1996, and their adoption continues to expand rapidly in the United States and other parts of the developed and developing world (James 2004). In 2004, it was estimated that *Bt* cotton represented ≈46% of all upland cotton production in the United States (USDA 2004). Use rates are much higher in Arizona where the primary target is the pink bollworm, *Pectinophora gossypiella* (Saunders), a caterpillar that feeds on seeds within the cotton fruit and is difficult to control with conventional insecticides (Henneberry and Naranjo 1998). In Arizona, *Bt* cotton was grown on 81% of the upland cotton acreage in 2003, most of it (74%) in a stacked configuration with transgenes conferring resistance to the herbicide glyphosate (Tronstad et al. 2004). *P. gossypiella* is highly susceptible to *Bt* cotton (Flint et al. 1996, Flint and Parks 1999, Ellsworth et al. 2002), and its wide-

spread cultivation has been associated with dramatic reductions in regional populations of this insect (Carrière et al. 2001).

Along with the potential benefits associated with the use of transgenic crops in agricultural production systems, there are also putative risks, including outcrossing through pollen drift, horizontal transfer of transgenes to other organisms, food safety, loss of susceptibility to *Bt* toxins in target pests, and direct or indirect effects on nontarget organisms including the soil biota (Cannon 2000, Wolfenbarger and Phifer 2000, Marvier 2001, Shelton et al. 2002, Conner et al. 2003). Various studies have examined arthropod nontarget effects in both the laboratory and field (reviewed by Schuler et al. 1999, Glare et al. 2001, Pilson and Prendeville 2004, Lovei and Arpaia 2005, O'Callaghan et al. 2005). Many laboratory studies have focused on defining the effects of direct exposure or indirect exposure through trophic interactions to *Bt* toxins on the biology of nontarget species. Most field studies have largely focused on changes in abundance and diversity of various nontarget taxa. Comparatively less attention has been focused on exploring tri-trophic interactions and their implications on predator/prey or host/parasitoid dynamics, especially in the field. Using "field simulators," Schuler et al. (2001) showed that *Bt* transgenic rape did not effect parasitoid/host interactions between *Myzus persicae* (Sulzer) and its endoparasitoid *Diaeretiella rapae* (McIntosh) or the ability of the parasitoid to suppress aphid populations. Bourguet et al. (2002) found lower levels of parasitism on *Ostrinia nubilalis* (Hübner) by tachinid parasitoids in *Bt* compared with non-*Bt* corn. However, Orr and Landis (1997) found no differences in parasitism or predation of natural eggs or parasitism of sentinel larvae of *O. nubilalis* by two species of parasitic Hymenoptera between *Bt* and non-*Bt* corn. Likewise, Hilbeck et al. (1998) generally found no difference in predation of *Leptinotarsa decemlineata* (Say) eggs on potatoes that had received applications of beetle active *Bt* compared with those left untreated, and Sisterson et al. (2004) reported no differences in rates of predation on sentinel eggs of *P. gossypiella* in *Bt* compared with non-*Bt* cotton. Finally, Schoenly et al. (2003) used food-web analyses to show that *Bt* sprays on rice in the Philippines had no effect on trophic structure. These studies have been relatively short-term in nature and for the most part have examined only a few of the potential interactions that may occur between natural enemies and their prey or hosts in transgenic crops.

A 5-yr field study was conducted within the main cotton-producing region of Arizona to assess the long-term impact of *Bt* cotton producing the Cry1Ac δ-endotoxin on the potential impact of the natural enemy community on prey populations using three approaches. Comparative studies of natural enemy abundance in *Bt* and non-*Bt* cotton are the subject of a companion paper (Naranjo 2005). The objectives here were to (1) compare predator:prey ratios and predation indices for three key pests in western cotton, *P. gossypiella*, *Bemisia tabaci* (Gennadius), and

Lygus hesperus Knight, between *Bt* and non-*Bt* cotton over the entire growing season in multiple years and to contrast any effects relative to conventional production practices using an array of selective and broad-spectrum insecticides, and (2) compare rates of natural enemy–induced mortality on two of these key pests between unsprayed *Bt* and non-*Bt* cotton using sentinel prey and field life-table studies over a 3-yr period.

Materials and Methods

Study Site and Experimental Design

Research plots of cotton, *Gossypium hirsutum L.*, were established within an ≈160-ha area of the University of Arizona, Maricopa Agricultural Center research farm in Maricopa, AZ, between 1999 and 2003. Each year included a comparison between Deltapine NuCOTN 33B, a transgenic cultivar producing the Cry1Ac insecticidal protein of *B. thuringiensis*, and its nontransgenic parent cultivar Deltapine 5415. A randomized complete block design with four replications was used in all years, but plot size and use of split-plots varied over years (Table 1). Studies in 2001 and 2002 included positive control treatments that consisted of split plots of *Bt* and non-*Bt* main plots that were sprayed for *P. gossypiella* and other lepidopteran pests, *B. tabaci*, and *L. hesperus*, based on established action thresholds (Ellsworth et al. 1996, University of California 1996, Ellsworth and Barkley 2001). No sprays for lepidopteran pests were needed in 2001. Low densities of lepidopterans also occurred in 2002, but two sprays were applied for these pests in the treated splits of the non-*Bt* main plots to simulate typical grower practice. In 2003, *Bt* and non-*Bt* main plots were split to include cultivars resistant to glyphosate (Deltapine 5415RR or Deltapine 449RRBt). All plots were planted in early April of each year and grown according to standard agronomic practices for the area.

Arthropod Natural Enemy 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 *B. tabaci* and *L. hesperus* (Naranjo 2005). Most arthropods were sampled using a standard sweep net (38 cm diameter) that was swung perpendicular to a single row in a figure-eight pattern. Two sets of 25 sweeps were collected in each plot using a random starting point. The contents of the net were frozen and later sorted in the laboratory with the aid of a dissecting microscope. Samples were collected weekly on 12–16 dates between early June and mid-September each year. Densities of immature aphelinid parasitoids attacking *B. tabaci* (*Eretmocerus* spp. and *Encarsia* spp.) were estimated by taking leaf samples (20–30 per plot) from the seventh mainstem node below the terminal. Samples were collected weekly from early July through mid-September on 8–11 dates each year. In the lab-

Table 1. Summary of experimental studies conducted between 1999 and 2003 at the University of Arizona, Maricopa Agricultural Center, Maricopa, AZ

Year	Design	Blocks	Plot size	Pesticide use
1999	RCB	4	0.12 ha	None
2000	RCB	4	0.17 ha	None
2001	RCB split-plot	4	0.17-ha main plots 0.085-ha split-plots	Bt and non-*Bt* subplots 12 July: buprofezin (392 g A.I./ha) 20 July: oxamyl (843 g A.I./ha) 2 Aug.: acephate(1121 g A.I./ha)
2002	RCB split-plot	4	0.17-ha main plots 0.085-ha split-plots	Bt and non-*Bt* subplots 25 July: buprofezin (392 g A.I./ha), oxamyl (843 g A.I./ha) 16 Aug.: acephate(1121 g A.I./ha) 28 Aug.: fenpropathrin (224 g/ha), acephate (561 g/ha) Non-*Bt* subplots only 12 July: chlorpyrifos(1121 g A.I./ha) 16 Aug.: cyfluthrin (45 g A.I./ha)
2003	RCB split-plot	4	0.17-ha main plots 0.085-ha split-plots	Glyphosate resistant subplots 23 May: glyphosate (340 g A.I./ha)

RCB, randomized complete block.

oratory, all larval and pupal parasitoids within fourth-instar whitefly nymphs on the entire leaf were counted. Displacement of the host's mycetomes was used to determine the presence of young parasitoid larvae. An index of parasitism was estimated as the proportion of fourth-instar hosts parasitized by both genera combined.

Pest Insect Density

Densities of *B. tabaci* eggs, nymphs, and adults were estimated weekly from early July through mid-September each year. Egg and nymph densities were estimated by the method of Naranjo and Flint (1994), which consists of counting individuals under a dissecting microscope on a 3.88-cm^2 disk taken from the fifth mainstem leaf below the terminal. Adult density was estimated by counting individuals, in situ, on the underside of leaves from the fifth mainstem node below the terminal (Naranjo and Flint 1995). Ten to 30 leaves were randomly selected for immature and adult stages in each plot on each sample date. The relationships described by Naranjo and Flint (1994) were used to estimate the number of eggs and nymphs per leaf from leaf disk counts for the analyses detailed below. The abundance of *L. hesperus* was estimated from the sweep net samples. Densities of *P. gossypiella* larvae were estimated as part of a companion study (Naranjo 2005), but because these larvae reside inside the cotton fruit and are thus largely invulnerable to natural enemies, they were not considered in analyses here. Larvae collected from bolls during the sampling process and subsequently maintained on artificial diet until pupation did not reveal any parasitism.

Functional Studies

Three approaches were used to examine effects of *Bt* cotton on the functional activity of the natural enemy complex against key pests in the system. The first two approaches focused on examining relation-ships between predator and prey based on abundance and the known feeding activity of various natural enemy species. A third approach measured rates of natural-enemy induced mortality on sentinel and natural prey.

Predator:Prey Ratios and Predation Indices. Predator to prey ratios were estimated for *B. tabaci* and *L. hesperus* based on known predator/prey associations (Table 2). A weighted predator:prey ratio for *B. tabaci* was estimated by

$$\Sigma S_i / (a_i Egg + b_i Nymph + c_i Adult) \quad [1]$$

where S_i is the density of predator taxon i per 50 sweeps, and a_i, b_i, and c_i are proportionality coefficients describing the preference of species i for the three life stages of *B. tabaci* (Table 2). Densities of the various *B. tabaci* life stage were measured as numbers per leaf. Because many predator taxa are known to prey on *B. tabaci* (Gerling et al. 2001, Naranjo 2001), an unweighted predator:prey ratio also was estimated based on the summed density of all predator taxa sampled to all life stages of *B. tabaci* combined per leaf. An unweighted predator:prey ratio also was estimated for *L. hesperus* based on the density of five predator taxa including *Geocoris punctipes* (Say), *Orius tristicolor* (White), *Nabis alternatus* Parshley, *Zelus renardii* Kolenati, and spiders as a group, all of which are known predators of this insect (Hagler et al. 1992, Ruberson and Williams 2000). The ratios estimated here were based on the abundance of selected predators and prey rather than species richness, which is more typical of ecological food-web analyses that examine the entire community (Lockwood et al. 1990). Furthermore, ratios were estimated for individual prey species rather than all prey species pooled. The goal was to qualitatively measure the relationship between individual prey species and the predator taxa that are likely to attack these prey in the field, and by inference, measure relative changes in biological control potential.

Table 2. Parameter values used for estimating predator:prey ratios and predation indices for three key pest species

Predator	Parameters for *B. tabaci* predator:prey ratio[a]			Parameters for predation index[b]		
	Eggs (a_i)	Nymphs (b_i)	Adults (c_i)	*B. tabaci*	*P. gossypiella*	*L. hesperus*
Misumenops celer	—	—	—	0.308	—	—
Collops vittatus	0.537	0.149	0.314	0.533	0.154	—
Hippodamia convergens	0.536	0.182	0.282	0.277	0.041	—
Geocoris punctipes	0.020	0.225	0.755	0.320	0.136	0.197
Geocoris pallens	—	—	—	0.330	0.103	—
Orius tristicolor	0.036	0.214	0.750	0.420	0.080	0.063
Nabis alternatus	—	—	—	0.033	0.007	0.350
Zelus renardii	—	—	—	0.165	0.119	0.267
Lygus hesperus	0.074	0.889	0.037	0.257	0.301	—
Pseudatomoscelis seriatus	—	—	—	0.463	—	—
Spanogonicus albofasciatus	—	—	—	0.320	—	—
Drapetis nr. *divergens*	0.000	0.000	1.000	0.444	—	—

[a] Proportionality parameters derived from Hagler et al. (2004).
[b] Frequency of predation on indicated species based on field gut content analyses (Hagler and Naranjo 1994a, b, Naranjo and Hagler 1998, Hagler and Naranjo 2005).

A final set of indices was calculated for *B. tabaci*, *L. hesperus*, and *P. gossypiella* according to known patterns of feeding activity by various predator taxa in the field based on gut content analyses. The index is given by

$$\Sigma W_i F_i \qquad [2]$$

where W_i is the density of species i, and F_i is the frequency of predation on *B. tabaci*, *P. gossypiella*, or *L. hesperus* for species i. This frequency was derived from results of gut content immunoassays (Hagler and Naranjo 1994a, b, Naranjo and Hagler 1998, Hagler and Naranjo 2005) and estimated as the mean proportion of sampled predators scoring positive for prey remains by enzyme-linked immunosorbent assay (ELISA) over the season in these field studies. The frequency of predation by any one species generally varied without pattern over the season, and was unrelated to either predator or prey density (Hagler and Naranjo 1994a, b). Thus, mean frequency values were used here. This frequency, and thus the index, is qualitative because no assumptions are made about the number of prey consumed by a predator. In addition, the index is independent of multiple predation events on different prey species because predation frequency is measured within the matrix of multiple species interactions in the field. Finally, although intraguild predation is likely among the predators composing the index, the ELISA used has been shown to be insensitive to detecting the movement of prey proteins through the food chain (S. E. Naranjo and J. R. Hagler, unpublished data). Parameter values are listed in Table 2. Analyses were conducted based on index values for each treatment plot, but results are presented as a quotient of the index for *Bt* or sprayed cotton over non-*Bt* or unsprayed cotton, respectively. A quotient <1 indicates a reduction in potential predator function in *Bt* or sprayed cotton, and a value >1 indicates an increase in function. All ratios and indices were estimated for all sampling dates in each year.

Pest Mortality Studies. A final set of studies compared rates of natural enemy–induced mortality in *P. gossypiella* and *B. tabaci* in unsprayed plots of *Bt* and non-*Bt* cotton from 2001 to 2003. To examine mortality of *P. gossypiella* eggs, small cards containing 20 eggs (1 d old) obtained from the USDA-ARS, Western Cotton Research Laboratory rearing facility in Phoenix, AZ, were pinned under the bracts of ≈20-d-old cotton bolls. This placement simulated the oviposition behavior of female moths. Twenty cards (one per plant) were placed along two diagonal transects in each plot and left exposed for 24 h. Green flagging tape was tied around the mainstem of the plant to facilitate relocation. Cards were collected and examined under magnification in the laboratory for evidence of predation. Chewing predators completely removed the egg (often with scarring of the underlying paper) while sucking predators left behind a deflated and transparent egg chorion. The remaining intact eggs were held at 27°C for an additional 6 d to evaluate parasitism. Controls consisted of egg cards (20 total) that were carried to the field and handled similar to those pinned to the plant but were returned to the laboratory without exposure. Damaged or missing eggs were noted and used to correct the calculation of proportional mortality of field-exposed eggs. The experiment was repeated three, four, and three times in 2001–2003, respectively, between mid-July and mid-September.

The mortality of *P. gossypiella* pupae (obtained from the rearing facility) were studied on four dates between mid-July and mid-September in 2002 and 2003. Individual pupae were placed at the base of 20 cotton plants along two diagonal transects in each plot and exposed for 24 h. This simulated the pupation site used by larvae exiting bolls. A small green plastic pot stake was placed near each pupa to facilitate relocation. Pupae that remained were returned to the laboratory and examined under magnification for evidence of predation (e.g., chewing marks or necrosis around punctures). Intact pupae were held for 3 wk to evaluate parasitism. As a control, a set of 20 pupae were exposed in the field during each trial (one set per plot) within a small paper carton covered with fine mesh cloth.

To examine morality of *B. tabaci* nymphs, an in situ life-table approach was used (Naranjo and Ellsworth 2005). Briefly, the method involved marking the location of individual settled first-instar nymphs ($\approx$6–12 h old; $\geq$50 per plot) with a nontoxic felt pen on the underside of leaves, followed by repeated observations every 2–3 d until death or adult emergence. No more than four nymphs were marked on an individual plant. Mortality caused by dislodgment, predation, parasitism, and unknown causes was recorded for each of the four nymphal instars. Dislodged nymphs were those that were simply lost from the leaf surface as a result of weather (wind and rain) or the activity of chewing predators (Naranjo and Ellsworth 2005). The instar of dislodged nymphs was estimated by the average instar of other dead nymphs on the same observation date. Predation was mortality primarily caused by sucking predators that had evacuated the contents of the whitefly body leaving a deflated and transparent nymphal cuticle on the leaf. Parasitism could only be observed in fourth-instar nymphs by the displacement of mycetomes or the presence of parasitoid larvae or pupae within the host. Life-table studies were repeated twice each year between mid-July and early September in 2001–2003.

Statistical Analyses

Mixed-model, repeated-measures analysis of variance (ANOVA) (Littell et al. 1996) was used to test for treatment differences in predator:prey ratios and predation indices over the season each year. The block variable and associated interaction terms were entered as random effects, and the Kenward/Roger option was used to estimate corrected degrees of freedom for *F* tests. The first-order autoregressive or heterogeneous autoregressive option (AR1 or ARH1 in SAS Proc Mixed) was used to estimate the repeated measures covariance structure, because these consistently maximized Akaike's Information and Schwarz' Bayesian Criteria (Littell et al. 1996). Multi-year analyses were conducted by calculating seasonal mean values for ratios and indices for each plot in each year and entering block and year as random effects. Data were log-transformed to achieve normality and homoscedasticity before analyses; untransformed means are presented. For mortality studies involving *P. gossypiella* eggs and pupae, the response variable was the proportion of eggs or pupae missing, eaten, or parasitized. Marginal mortality rates were calculated from *B. tabaci* life-table data based on apparent mortality using the methods outlined by Buonaccorsi and Elkinton (1990) and Elkinton et al. (1992) (see Naranjo and Ellsworth 2005 for details). Marginal rates corrected for mortality caused by a given cause that might be obscured by the action of other 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 also was used for multi-year analyses, with block, year, and trial within year entered as random effects. Proportional and marginal mortality values were arc-

sine$\sqrt{}$-transformed before analyses; untransformed means are presented.

Statistical power curves were estimated for all multi-year analyses using the PASS software program (NCSS, Kaysville, UT). Significance level for all analyses was based on $\alpha = 0.05$.

Results

Predator:Prey Ratios

Weighted predator:prey ratios based on the preference of six predator taxa to the three life stages of *B. tabaci* varied significantly ($F > 14.8$; df = >8,>33.3; $P < 0.0001$) over the season in each year with changing densities of predators and prey. However, there were no significant treatment effects when comparing unsprayed *Bt* and non-*Bt* cotton, and there were no date by treatment interactions (Fig. 1A). These ratios also varied over years of the study but again there was no significant treatment effect based on a combined multi-year analysis. Unweighted predator:prey ratios based on all predator taxa observed and all *B. tabaci* life stages were similar in pattern to weighted ratios, and there were no significant treatment effects for any individual year or all years combined (Fig. 1B). The application of insecticides inconsistently affected predator:prey ratios. In 2001, unsprayed plots had significantly higher weighted ($F = 8.88.6$; df = 1,11.6; $P = 0.011$) and unweighted ($F = 11.1$; df = 1,5.9; $P < 0.021$) predator:prey ratios for *B. tabaci*, but this pattern was reversed in 2002 for weighted ratios ($F = 8.88$; df = 1,11.6; $P < 0.011$; Fig. 1C and D). Neither ratio differed significantly in multi-year analyses.

Unweighted predator:prey ratios for *L. hesperus* varied over the season each year ($F > 15.2$; df = >9,>35.6; $P < 0.0001$) and between years, but these ratios were not affected by the use of *Bt* cotton or insecticides compared with non-*Bt* or unsprayed cotton, respectively (Fig. 2). Date by treatment interactions were not significant in any year. There also were no differences in ratios for *L. hesperus* or *B. tabaci* with respect to transgenes conferring glyphosate resistance in 2003.

The statistical power of multi-year analyses was relatively high for all predator:prey ratios examined (Fig. 3). The underlying experimental design was capable of detecting changes in all ratios of <20% with >80% power.

Predation Indices

As with predator:prey ratios, predation indices based on the feeding behavior of various predator taxa varied significantly over the season in each year ($F > 9.9$; df = >11,> 38.1; $P < 0.0001$), but not as a function of the use of *Bt* cotton ($P > 0.05$; Fig. 4A–C). Indices were <1 in the majority of years for each pest species, but only for *L. hesperus* was the index based on all years combined significantly <1 ($F = 14.4$; df = 1,15; $P < 0.0018$), indicating a reduction in predator function for this pest species in *Bt* cotton. In contrast,
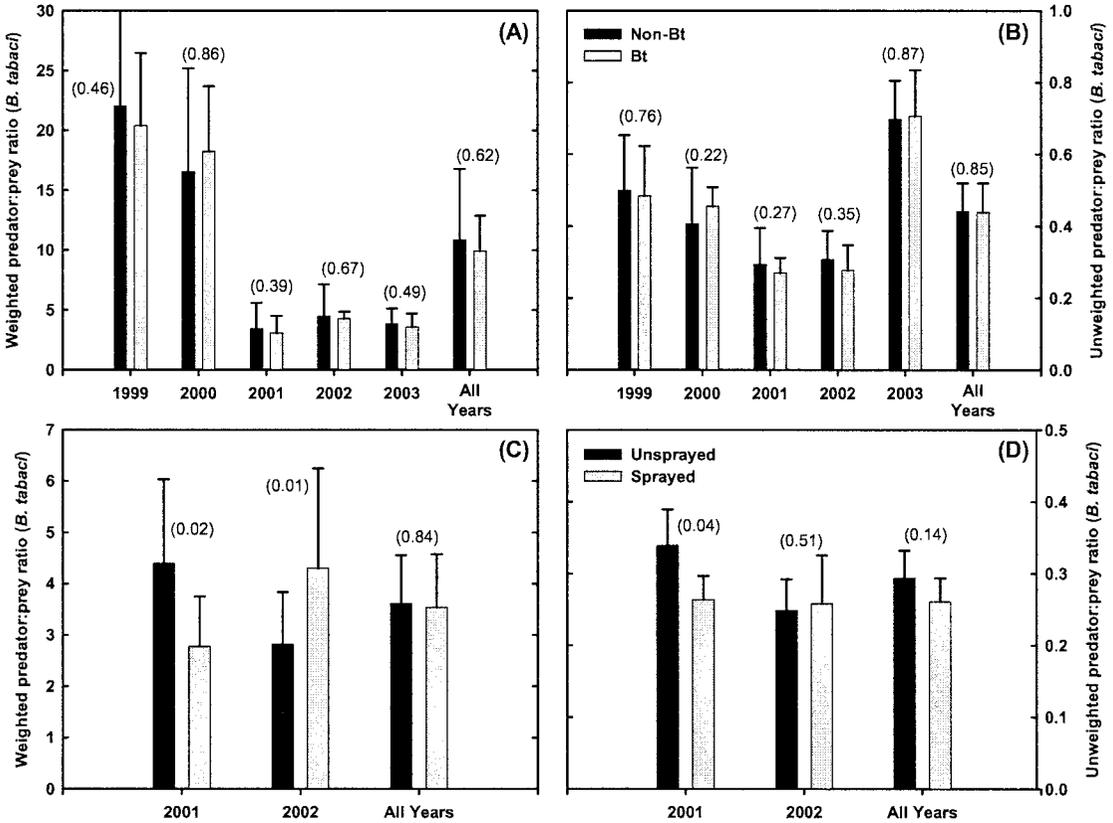
Fig. 1. Comparison of weighted and unweighted predator:prey ratios for *B. tabaci* between unsprayed *Bt* and non-*Bt* cottons (A and B) and between sprayed (C and D) and unsprayed cotton over a 5-yr period. Numbers above paired bars are *P* values for ANOVAs of each individual year or all years combined. Error bars are 95% confidence intervals (CIs).

the use of insecticides had a marked effect on predator function, significantly reducing the potential impact of predators on all three pest species in each year alone ($F > 10.1$; df = 1,> 6; $P < 0.018$) and both years combined ($F = 31.6$; df = 1,>20; $P < 0.0001$; Fig. 4D–F). There were no differences in indices relative to transgenes conferring glyphosate resistance in 2003.
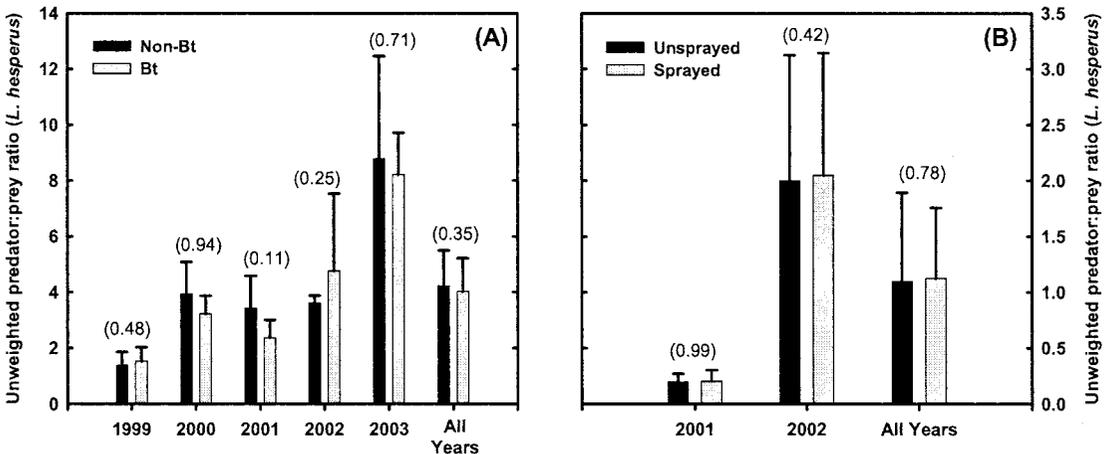

Fig. 2. Comparison of unweighted predator:prey ratios for *L. hesperus* between unsprayed *Bt* and non-*Bt* cottons (A) and between sprayed and unsprayed cotton (B) over a 5-yr period. Numbers above paired bars are *P* values for ANOVAs of each individual year or all years combined. Error bars are 95% CIs.
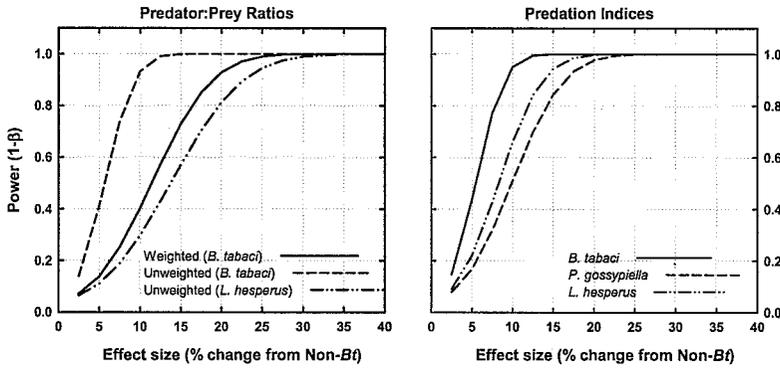
Fig. 3. Power curves as a function of effect size for predator:prey ratios and predation indices based on the underlying experimental design of multi-year analyses. Type I error was set to 0.05.

The statistical power of multi-year analyses was relatively high for all predation indices (Fig. 3). The underlying experimental design was capable of detecting changes in all indices of <15% with >80% power.

Mortality of Pest Insects

Eggs of *P. gossypiella* were readily preyed on in both *Bt* and non-*Bt* cotton with no significant differences in rates of predation in each of 3 individual yr or all years combined (Fig. 5). Predation rates varied from around

53% in 2001 to ≈35% in 2003. No parasitism was detected in eggs that survived predation in any year. Partitioning egg mortality, there also was no significant difference in rates of chewing or sucking predation between *Bt* and non-*Bt* cotton (data not shown). On average, chewing and sucking predators were each responsible for ≈50% of the total mortality.

Pupae of *P. gossypiella* placed on the soil surface beneath plants also experienced high rates of mortality ranging from around 40% in 2002 to 64% in 2002. There was no significant difference in rates of pupal mortality between *Bt* and non-*Bt* cotton in either year or both
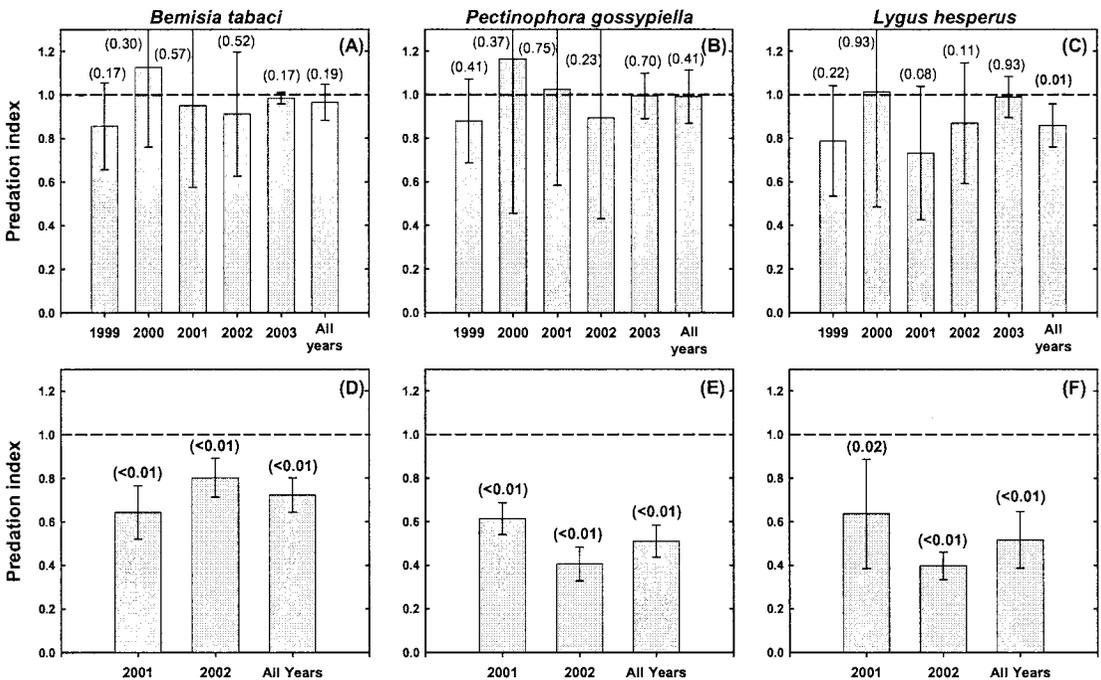

Fig. 4. Predation indices for *B. tabaci*, *P. gossypiella*, and *L. hesperus* comparing unsprayed *Bt* and non-*Bt* cottons (A–C) and sprayed and unsprayed cotton (D–F) over a 5-yr period. Indices are based on known frequencies of predation by various arthropod predators derived from field gut-content analyses. An index <1 indicates a reduction in potential predator function in *Bt* or sprayed cotton, and a value >1 indicates an increase in function. Numbers above paired bars are *P* values for ANOVAs of each individual year or all years combined. Error bars are 95% CIs.
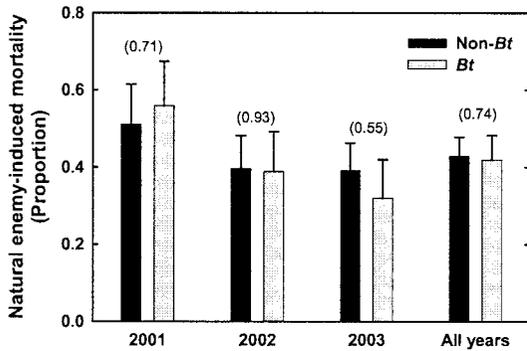
Fig. 5. Comparison of natural enemy–induced mortality of sentinel *P. gossypiella* eggs between unsprayed *Bt* and non-*Bt* cottons over a 3-yr period. Numbers above paired bars are *P* values for ANOVAs of each individual year or all years combined. Error bars are 95% CIs. Results for each individual year are based on three to four separate experiments.

years combined (Fig. 6). 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. Adult moths emerged from these intact pupae at the same rates as control pupae that were placed in the field but were protected from natural enemies.

Marginal rates of predation on *B. tabaci* nymphs varied from ≈52% in 2001 to 63% in 2002 and did not differ significantly between *Bt* and non-*Bt* cotton in any year or all years combined (Fig. 7). Rates of dislodgement, which included the effects of chewing predation and weather (Naranjo and Ellsworth 2005), varied over years (12–23%), but not as a result of the use of *Bt* cotton. Parasitism ranged from around 5% in 2001 to ≈23% in 2002, but again there was no difference in rates of parasitism between the two cottons. Predation and dislodgement were further partitioned among the four nymphal instars, but no significant differences were detected between *Bt* and non-*Bt*
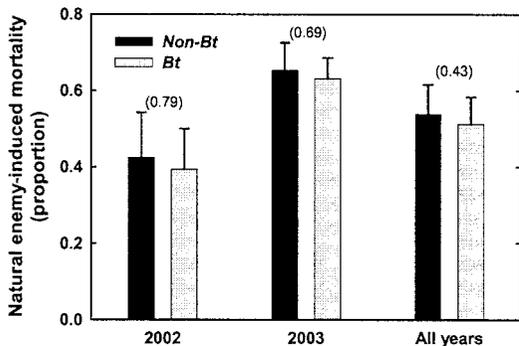

Fig. 6. Comparison of natural enemy–induced mortality of sentinel *P. gossypiella* pupae between unsprayed *Bt* and non-*Bt* cottons over a 2-yr period. Numbers above paired bars are *P* values for ANOVAs of each individual year or all years combined. Error bars are 95% CIs. Results for each individual year are based on four separate experiments.

cotton for any stage (data not shown). Rates of mortality were always highest during the fourth stadium.

Parasitism of *B. tabaci* nymphs by aphelinid parasitoids based on leaf samples was variable over years, ranging from 3 to 25% (Table 3). The effects of *Bt* cotton were inconsistent with slightly higher rates of parasitism observed in *Bt* cotton in 2001 ($F = 11.6$; df = 1,17.6; $P = 0.003$) and slightly lower rates observed in *Bt* cotton in 2003 ($F = 4.79$; df = 1,15.4; $P = 0.045$). There was no significant effect over all years combined and no effect of insecticide applications in 2001.

The statistical power of multi-year analyses was high for both sentinel prey studies of *P. gossypiella* and life-table studies of *B. tabaci* (Fig. 8). The underlying experimental design was capable of detecting changes in rates of mortality from predation, dislodgement, and parasitism of <7% with >80% power. Power was even greater for detecting very small changes (≈2%) in rates of parasitism determined from leaf samples.

Discussion

Many field studies evaluating nontarget effects of transgenic crops on the natural enemy community have focused on abundance, species richness, and diversity. Relatively few studies have examined effects on the activity and ecological function of this community. From the perspective of biological control, changes in the impact of natural enemies on both target and nontarget pest populations caused by production practices could have important consequences for pest management. This has been repeatedly shown in the cotton system where the use of broad-spectrum insecticides has disrupted natural biological control of pest herbivores (Leigh et al. 1966, Eveleens et al. 1973, Stoltz and Stern 1978, Abdelrahmin and Munir 1989, Devine et al. 1998). Thus, changes in natural enemy impact that may arise from use of *Bt* transgenic plants provide an important measure of nontarget effects in addition to the typical assessment of differences in nontarget abundance and diversity. Long-term studies here showed that there were essentially no effects of *Bt* cotton on natural enemy function in Arizona cotton as measured by three increasingly quantitative approaches: predator:prey ratios, predation indices, and rates of natural enemy–induced mortality on three key pest species. Moreover, the high statistical power of the analyses provides confidence that the chances of detecting true changes in function also were high. Overall, the results suggest that the minor reductions in population density observed for five predator taxa in *Bt* cotton (Naranjo 2005) may hold little ecological relevance, at least with respect to natural biological control of key pests in the western cotton system.

Predator:prey ratios provided a qualitative measure of changes in biological control potential. These ratios based on *B. tabaci* and *L. hesperus* were highly variable among the 5 yr of study as a result of variable densities of predators and prey over time (Naranjo 2005). However, in no case was the ratio altered by the use of *Bt* compared with non-*Bt* cotton. Weighted ratios were used for *B. tabaci* because data were available on the
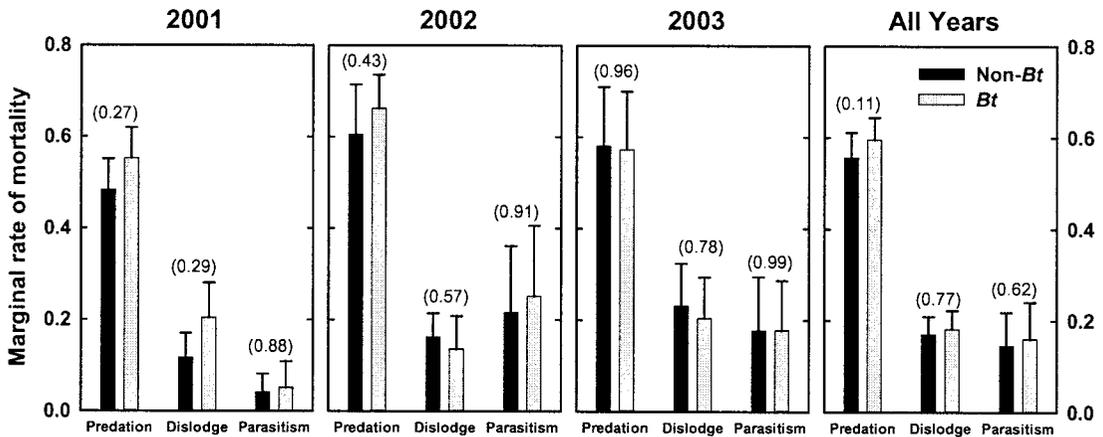
Fig. 7. Comparison of marginal rates of natural enemy–induced mortality of natural cohorts of *B. tabaci* nymphs between unsprayed *Bt* and non-*Bt* cottons over a 3-yr period. Numbers above paired bars are *P* values for ANOVAs of each individual year or all years combined. Error bars are 95% CIs. Results for each individual year are based on two separate experiments. Dislodged nymphs disappeared from the leaf surface as a result of weather or chewing predation; predation indicates mortality by predators with sucking mouthparts.

stage preference of various species attacking this pest. However, unweighted ratios provided similar patterns and conclusions, suggesting that cruder measures of the predator:prey ratio may be sufficient as one measure of the potential impact of *Bt* cotton. Insecticides had inconsistent effects on ratios for *B. tabaci* and no effect on ratios based on *L. hesperus.* These results are largely consistent with expectations from using nonselective insecticides. Populations of *L. herperus* were impacted equally as those of the five predator species making up the ratio while the higher ratio for *B. tabaci* in 2001 reflects the use of selective materials for its control and a generally lower use of broader-spectrum materials.

Predation indices further weighted the relative value of various predator species based on their known feeding activity on three pest species in the field and provided a more meaningful measure of potential negative impacts (Naranjo et al. 2002). Declines in the predation index for *L. hesperus* in *Bt* cotton plots follow directly from the fact that declines in density were observed in two of the most frequent predators of *L. hesperus*, *G. punctipes*, and *N. alternatus* (Naranjo 2005). These predators also feed on *B. tabaci* and *P. gossypiella*, but negative effects were dampened because the indices for these species involved a greater

number of predator species and because the two predator species that declined were relatively less important on the basis of predation frequency. Relatively little is known about the activity of predators of *L. hesperus* in the field (Hagler et al. 1992, Ruberson and Williams 2000), and additional information on other potential predator species could alter the findings reported here. Regardless, densities of *L. hesperus* were similar in *Bt* and non-*Bt* cotton (Naranjo 2005), indicating a lack of resurgence as a result of negative effects of *Bt* cotton on some predator populations. In contrast, the effects of insecticide use led to marked significant declines in potential predation for all three prey species. This predation index has limitations in predicting predator impact because it contains no information about quantitative levels of predation by a predator species. The number of prey consumed by a given predator will depend on many things, including density of target and alternate prey, interactions among predators, and environmental factors.

The most quantitative and direct measure of natural enemy function further showed no effect of *Bt* cotton on rates of predation and parasitism of two prey species. The eggs and pupae of *P. gossypiella* are the stages most vulnerable to natural enemies (Henneberry and Naranjo 1998). Predators readily attacked sentinel

Table 3. Seasonal mean proportional parasitism of *B. tabaci* by two genera of aphelinid parasitoids in non-*Bt* and *Bt* and unsprayed and sprayed cotton, Maricopa, AZ, 1999–2003

Year	Non-*Bt*	Bt	*P*[a]	Unsprayed	Sprayed	*P*[a]
1999	0.04 ± 0.01	0.03 ± 0.01	0.46	—	—	—
2000	0.25 ± 0.02	0.22 ± 0.01	0.57	—	—	—
2001	0.03 ± 0.01	0.06 ± 0.01	0.003	0.04 ± 0.01	0.05 ± 0.01	0.43
2003	0.12 ± 0.01	0.09 ± 0.02	0.04	—	—	—
All years	0.11 ± 0.02	0.10 ± 0.02	0.53	—	—	—

Values are seasonal means (±SE) based on 8–11 dates each year within four replicate plots each year.

[a] *P* values from repeated-measures ANOVA (individual years) or multi-year ANOVA based on seasonal means using Proc Mixed (Littell et al. 1996).
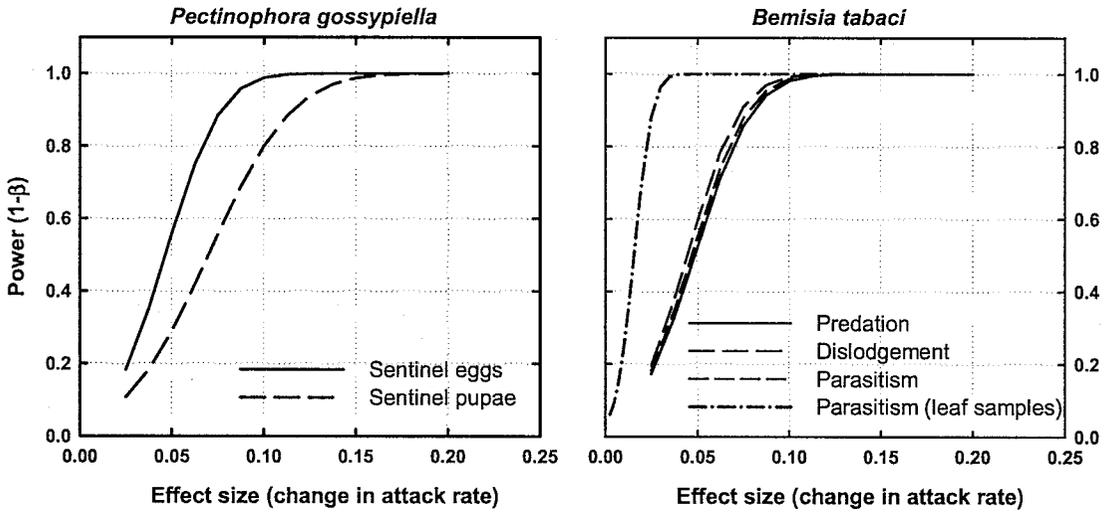
Fig. 8. Power curves as a function of effect size for studies examining predation and parasitism of sentinel *P. gossypiella* eggs and pupae and natural *B. tabaci* nymphs based on the underlying experimental design of multi-year analyses. Type I error was set to 0.05. For *B. tabaci*, dislodged nymphs disappeared from the leaf surface as a result of weather or chewing predation; predation indicates mortality by predators with sucking mouthparts.

eggs placed beneath the bracts of developing cotton bolls on both *Bt* and non-*Bt* cotton. There was no evidence of parasitism, which is consistent with the uncertain establishment of an exotic trichogrammatid introduced into Arizona in the mid-1980s (Hutchison et al. 1990). Most pupal mortality resulted from disappearance, presumably because of predation by various spiders, carabid beetles, ants, and earwigs, which were frequently caught in pitfall traps during 1 yr of this study (unpublished data). Some predation also may have resulted from lizards, birds, and small rodents. Thus, the sole impact of arthropods cannot be quantified for this particular prey stage. No pupal parasitism was observed in *Bt* or non-*Bt* cotton, which is consistent with the uncertain establishment of several pupal parasitoids introduced in the western United States in the 1970s and 1980s (Legner and Medved 1979, Gordh and Medved 1986). *Bt* cotton had no effect on rates of mortality of *B. tabaci* nymphs, and rates and patterns of mortality are consistent with prior studies in unsprayed cotton at the same site, which also showed predation to be the key factor (Naranjo and Ellsworth 2005). The similar rates of mortality on *B. tabaci* are consistent with the lack of differences in densities of this pest between *Bt* and non-*Bt* cotton (Naranjo 2005).

The significant, but minor, reductions in density of five predator taxa in unsprayed *Bt* cotton (Naranjo 2005) were not associated with significant changes in the function of the overall natural enemy community. This result may be explained by the general feeding behavior of many of the natural enemies in the cotton system, especially predators. Reductions in the density and associated activity of any one species in the complex are offset by other members of the community. Life-table studies with *B. tabaci* revealed that most of the mortality from any one source, be it pre-

dation, parasitism, dislodgement, egg inviability, or unknown causes, is replaceable by other sources (Naranjo and Ellsworth 2005). Thus, a reduction in predation by, say, *G. punctipes* or *N. alternatus* could be replaced easily by *O. tristicolor* or *Z. renardii.* However, there are limits to the amount of mortality that can be replaced by the natural enemy community. Life-table studies with *B. tabaci* also showed that the use of broad-spectrum insecticides, which cause large reductions in natural enemy density, significantly reduces the contribution of these natural enemies to pest mortality (Naranjo 2001, S. E. Naranjo and P. C. Ellsworth, unpublished data). This leads to a situation where the continued use of insecticides is required for pest suppression. In contrast, the use of selective insecticides for *B. tabaci* only slightly reduces the abundance of various natural enemies (Naranjo et al. 2004) but allows the complex to continue inflicting significant mortality, enabling long-term pest suppression in the absence of additional insecticides (Naranjo 2001, S. E. Naranjo and P. C. Ellsworth, unpublished data). This pattern is supported in other systems. For example, Head et al. (2005) showed that rates of predation on sentinel eggs and larvae of *Helicoverpa zea* (Boddie) was significantly reduced in commercial non-*Bt* cotton fields treated with broad-spectrum insecticides compared with *Bt* cotton fields in which such applications were reduced or unnecessary. Further field studies will be needed to more clearly define the magnitude of change in natural enemy density that might be associated with significant changes in biological control.

It has been shown that plot size can be an important factor in measuring population level toxicological effects (Jepson and Thacker 1990, Sherratt and Jepson 1993, Prasifka et al. 2005), and the relatively small plot size used here may have dampened responses to cul-

tivar treatments. However, Naranjo (2005) showed that changes in density of natural enemy populations in response to *Bt* cotton were similar in the small plots used here and in plots an order of magnitude larger (≈2 ha). In addition, predation indices in positive control treatments that received insecticide applications were significantly and consistently lower than unsprayed plots, showing that real effects can be measured in these smaller plots. While a commercial production scale may be desirable for nontarget evaluations, it may not always be feasible, economically viable, or even necessary to make reasonable assessments of impact.

Transgenic *Bt* cotton provide a highly effective and selective technology for lepidopteran pest control. *Bt* cotton and the coincident introduction and adoption of selective insect growth regulators for suppression of *B. tabaci* in Arizona have played a major role in reducing insecticide use in cotton from a high of 12.5 applications per acre in 1995 to 1.9 in 1999 (Ellsworth and Jones 2001). The long-term studies described here and in Naranjo (2005) suggest that negative effects of *Bt* cotton on nontarget arthropods, particularly natural enemies, are minimal and that even minor declines in density of some taxa do not seem to be associated with any meaningful changes in the function of the natural enemy community. The use of *Bt* cotton and other selective methods of pest control will continue to advance the important role of biological control in cotton integrated pest management in the western United States and elsewhere.

Acknowledgments

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