Long-Term Assessment of the Effects of Transgenic *Bt* Cotton on the Abundance of Nontarget Arthropod Natural Enemies

STEVEN E. NARANJO[1]

USDA–ARS, Western Cotton Research Laboratory, 4135 East Broadway Rd., Phoenix, AZ 85040

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ABSTRACT A 6-yr field study assessed the long-term impact of *Bt* cotton producing the Cry1Ac δ-endotoxin on 22 taxa of foliar-dwelling arthropod natural enemies in Arizona. No chronic, long-term effects of *Bt* cotton were observed over multiple generations of nontarget taxa. Zero-2 taxa declined significantly in unsprayed *Bt* compared with non-*Bt* cotton each year. In contrast, positive control studies showed that insecticide applications for caterpillars and other pests in both non-*Bt* and *Bt* cotton had much greater negative effects on 10 taxa. Multivariate principal response curves supported the findings of univariate analyses for the entire natural enemy community, showing no effect of *Bt* cotton but large and long-lasting negative effects from the use of insecticides. Multi-year analyses provided greater statistical power and indicated significant reductions that averaged 19% in five arthropod predator taxa in unsprayed *Bt* compared with non-*Bt* cotton. Most of these reductions were likely associated with reductions in lepidopteran prey. However, results of a companion study examining natural enemy function suggest that these minor reductions in *Bt* cotton have little ecological meaning. Multi-year analyses showed an average significant reduction of 48% in 13 taxa for plots receiving insecticide applications. On average, a 3-yr study with four replicates per year was sufficient to discern changes of ≈20%, with 80% power in unsprayed cotton. This long-term study indicates that the effects of *Bt* cotton on a representative nontarget community are minor, especially in comparison with the alternative use of broad-spectrum insecticides. Guidelines for improving nontarget field studies are discussed.

KEY WORDS *Pectinophora gossypiella*, arthropod predators, parasitic Hymenoptera, principal response curves, statistical power analysis

THE ADOPTION OF TRANSGENIC crops producing insecticidal proteins of *Bacillus thuringiensis* Berliner (*Bt*) continues to expand rapidly worldwide, with an 11% increase in the United States from 2003 to 2004 and much greater growth in certain countries such as China (James 2004). Transgenic *Bt* cottons have been available commercially in the United States since 1996, and it is estimated that ≈46% of all upland cotton grown in the Unitd States in 2004 was *Bt* cotton (USDA 2004). 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 glyphosate resistance (Tronstad et al. 2004). The primary target of *Bt* cotton in Arizona and southern California is the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), a seed-feeding caterpillar that is difficult to control with conventional insecticides because of its cryptic feeding habits (Henneberry and Naranjo 1998). *Bt* cotton is highly effective against *P. gossypiella* (Flint et al. 1996, Flint and Parks 1999, Ellsworth et al. 2002), and its widespread cultivation has had dramatic impacts on regional populations of this insect in Arizona (Carrière et al. 2001).

As with any new technology, both benefits and risks are associated with the use of transgenic crops in agricultural production systems. Among the potential benefits are significant reductions in conventional, broad-spectrum insecticide use, 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, Shelton et al. 2002, Federici 2003). 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, disruption of ecosystem processes, and direct or indirect effects on nontarget organisms and biodiversity (Cannon 2000, Wolfenbarger and Phifer 2000, Marvier 2001, Shelton et al. 2002, Conner et al. 2003). Despite the long history of safety associated with the topical use of *Bt*

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[1] Corresponding author, e-mail: snaranjo@wcrl.ars.usda.gov.

endotoxins (Glare and O'Callaghan 2000, Federici 2003, Benedict and Ring 2004), the season-long production of these toxins in crop plants through genetic transformation has prompted research to address ecological concerns such as effects on nontarget organisms, particularly arthropods.

A growing number of studies has examined nontarget effects in both the laboratory and field (see reviews by Schuler et al. 1999, Glare et al. 2001, Pilson and Prendeville 2004, Lovei and Arpaia 2005, O'Callaghan et al. 2005) and most have supported the findings from research on sprayable products that *Bt* is highly selective. For example, Pilcher et al. (1997) found no effects on survival or development of *Orius insidiosus* (Say), *Chrysoperla carnea* Stephens, and *Coleomegilla maculata* (DeGeer) feeding on *Bt* corn pollen; Riddick and Barbosa (1998a) found no effects on *C. maculata* feeding on *Leptinotarsa decemlineata* (Say) that had fed on *Bt* potato, and Armer et al. (2000) showed no deleterious effects of plant feeding by a number of heteropteran predators on *Bt* potato foliage. Comparative field studies also generally have failed to show significant effects of *Bt* potato (Riddick and Barbosa 1998b, Reed et al. 2001), *Bt* corn (Orr and Landis 1997, Lozzia 1999, Wold et al. 2001, Bourguet et al. 2002, Al-Deeb and Wilde 2003, Musser and Shelton 2003, Candolfi et al. 2004), or *Bt* cotton (Flint et al. 1995, Men et al. 2003, Sisterson et al. 2004, Hagerty et al. 2005) on populations of various nontarget arthropod taxa. Although studies initially reported negative effects of *Bt* on the growth and survival of insects such as the monarch butterfly (*Danaus plexippus* L.) (Losey et al. 1999, Hansen and Obrycki 2000) and *C. carnea* (Hilbeck et al. 1998, 1999), subsequent studies have found that the result were caused by prey quality factors rather than *Bt* toxins per se (Romeis et al. 2004) or that *Bt* crops pose negligible risks to these insects in the field (Sears et al. 2001).

There is a rich diversity of parasitoid and arthropod predator species that naturally inhabit cotton fields in the western United States (Van den Bosch and Hagen 1966, Gonzalez et al. 1977), and it is generally recognized that they play an important role in regulating pest populations (e.g., Leigh et al. 1966, Eveleens et al. 1973, Stoltz and Stern 1978, Naranjo and Ellsworth 2005). Although several field studies have examined the effects of *Bt* cotton on the abundance and diversity of natural enemies, they have either examined only a few arthropod natural enemy taxa (Flint et al. 1995, Luttrell et al. 1995, Deng et al. 2003, Hagerty et al. 2005) or have tended to focus analyses on higher taxonomic groupings or pooled species groups (Men et al. 2003, Sisterson et al. 2004). In addition, several of these studies were of relatively short duration, both in the number of seasons that were examined and in the intensity of sampling within seasons. Current evidence suggests that the *Bt* endotoxins produced in commercially available transgenic crops are not acutely toxic and that the effects, if any, are likely to be the result of subtle sublethal and indirect factors (Schuler et al. 1999, Groot and Dicke 2002, Conner et al. 2003). Such potential effects are unlikely to be resolved without taxonomically broader and more intensive long-term field studies.

A 6-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 populations of 22 taxa of foliar-dwelling arthropod natural enemies, primarily predators, commonly found in cotton. The overall objectives of this study were to compare populations of these nontarget natural enemy taxa and several key target and nontarget pests between *Bt* and non-*Bt* cottons over the entire growing season in multiple years, and to contrast any potential effects relative to conventional production practices using an array of currently available selective and broad-spectrum insecticides. Additional goals were to examine the influence of plot size and sampling method and to analyze the statistical power of underlying experimental designs as a means of providing some guidance for the conduct of future nontarget evaluations in transgenic crops.

Materials and Methods

Study Site and Experimental Design

All cotton, *Gossypium hirsutum* L., research plots were established at the University of Arizona, Maricopa Agricultural Center, Maricopa, AZ, in 1996 and between 1999 and 2003. In each year, Deltapine NuCOTN 33B, a transgenic cultivar producing the Cry1Ac insecticidal protein of *B. thuringiensis*, was compared with its nontransgenic parent cultivar, Deltapine 5415. The underlying experimental design in all years was a randomized complete block although plot size, replication, and use of split-plots varied over years (Table 1). In 1996, a pilot study was conducted as part of a large commercial-scale experiment to evaluate management regimens for *Bemisia tabaci* (Gennadius) (Naranjo et al. 2003). Individual plots were 1.2–2.0 ha in size and replicated in three blocks that each consisted of separate fields within a 72-ha area of the Maricopa Agricultural Center demonstration farm. All plots were treated with two applications of the selective insect growth regulators buprofezin and pyriproxyfen (Naranjo et al. 2004) for control of *B. tabaci* and one application of oxamyl to suppress an outbreak of *Lygus hesperus* Knight. Beginning in 1999, all other studies were conducted in smaller plots (0.12–0.17 ha) and replicated in four blocks within the ≈160-ha area designated as the Maricopa Agricultural Center research farm. Plots did not occupy the same ground in each year. Studies in 2001 and 2002 included positive control treatments, which 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

Table 1. Summary of experimental studies conducted between 1996 and 2003 at the University of Arizona, Maricopa Agricultural Center, Maricopa, AZ

Year	Design	Blocks	Plot size	Pesticide use
1996	RCB	3	1.2–2.0 ha	3–5 July: buprofezin (392 g A.I./ha) 17–22 July: pyriproxyfen (60 g A.I./ha) 1 Aug.: oxamyl (843 g A.I./ha)
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* sub-plots 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* sub-plots 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* sub-plots 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 sub-plots 23 May: glyphosate (340 g A.I./ha)

RCB, randomized complete block.

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 producing resistance to the herbicide 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 foliage-dwelling arthropod natural enemies, primarily predators (see Table 2). *L. hesperus*, *Pseudatomoscelis seriatus* (Reuter), *Spanogonicus albofasciatus* (Reuter), and *Rhinacloa forticornis* Reuter were included because these species exhibit omnivorous feeding habits (Butler 1965, Agnew et al. 1982, Hagler and Naranjo 1994, 2005). Most of these 22 taxa are generalist predators or omnivores that prey on a wide range of herbivores, including those that may ingest toxins from the cotton plant through their feeding activities (e.g., caterpillars, mites, thrips, plant bugs). In addition, all the omnivorous Heteroptera and most of the predaceous Heteroptera also are phytophagous (Naranjo and Gibson 1996) and are thus potentially exposed to toxins directly from the plant.

In the 1996 pilot study, two sampling methods were used to estimate densities of arthropod natural enemies. Twenty individual whole plant samples were randomly collected on seven dates in each plot between 5 June and 19 August. To sample a plant, a muslin cotton tube with draw strings on each end was placed over the plant with minimal disturbance. The bottom draw string was tightened around the main stem at ground level while the top end was left open and gathered around the base of the plant. Twenty-four hours later the bag was pulled rapidly over the top of the plant and the top draw string was pulled closed. The stem was cut at ground level, and the bagged plant was returned to the laboratory, chilled for several hours at 4°C, and searched. Arthropods on the plant and in the bag were counted and recorded. The second sampling method consisted of a standard sweep net (38 cm diameter) that was swung perpendicular to a single row in a figure-eight pattern. Four sets of 25 sweeps each 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 13 dates between 5 June and 10 September.

Results from 1996 (see below) showed that whole plant and sweep net samples provided similar information on the relative effect of transgenic cotton on the arthropod natural enemy complex examined. Thus, because of the high cost of whole plant sampling, sweep nets were used throughout the remaining years as the primary method of estimating densities of foliage-inhabiting arthropods. The only change was that two sets of 25 sweeps each were collected per plot from 1999 to 2003 because of the smaller size of the main plots and subplots. In total, sweep net samples were collected weekly from early June to mid-September on a total of 16, 14, 14, 15, and 12 dates in 1999–2003, respectively. 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 laboratory, all larval and pupal parasitoids within fourth-instar whitefly nymphs on the entire leaf were counted. Displacement of the host's

Table 2. Change in mean densities of arthropods in *Bt* relative to non-*Bt* cotton based on two sampling methods, Maricopa, AZ, 1996

Taxon	No. per 100 sweeps[a]			No. per 20 plants[b]		
	Non-*Bt* density	Prop. Δ (*P*)	Power[c] (50% effect)	Non-*Bt* density	Prop. Δ (*P*)	Power[c] (50% effect)
Dictyna reticulata (Araneida: Dictynidae)	0.23 ± 0.04	0.750 (0.03)*	0.32 (8)	0.38 ± 0.06	0.800 (0.02)*	0.50 (5)
Misumenops celer (Araneida: Thomisidae)	2.81 ± 0.44	−0.114 (0.74)	0.79 (4)	0.72 ± 0.17	−0.077 (0.69)	0.46 (6)
Salticidae (Araneida: Salticidae)	0.19 ± 0.04	−0.050 (0.69)	0.41 (6)	0.17 ± 0.06	−0.133 (0.73)	0.28 (9)
Other Araneida (Araneida)	0.10 ± 0.05	−0.667 (0.53)	0.31 (8)	0.33 ± 0.10	−0.450 (0.80)	0.46 (6)
Collops vittatus (Coleoptera: Melyridae)	0.78 ± 0.12	0.425 (0.38)	0.25 (10)	0.33 ± 0.17	0.333 (0.53)	0.29 (9)
Hippodamia convergens (Coleoptera: Coccinellidae)	3.77 ± 1.03	−0.543 (0.51)	0.31 (8)	1.07 ± 0.17	−0.302 (0.58)	0.14 (20)
Anthicidae (Coleoptera)	0.14 ± 0.10	0.133 (0.62)	0.18 (14)	3.28 ± 0.35	0.373 (0.41)	0.82 (3)
Other Coccinellidae (Coleoptera: Coccinellidae)	0.19 ± 0.07	1.267 (0.23)	0.30 (8)	—[e]	—	—
Geocoris punctipes (Heteroptera: Lygaeidae)	0.86 ± 0.14	−0.054 (0.63)	0.30 (8)	0.5 ± 0.19	0.167 (0.31)	0.14 (20)
Geocoris pallens (Heteroptera: Lygaeidae)	8.96 ± 0.82	−0.392 (0.28)	0.47 (6)	2.94 ± 1.33	−0.340 (0.53)	0.28 (9)
Orius tristicolor (Heteroptera: Anthocoridae)	14.5 ± 2.57	−0.066 (0.97)	0.13 (23)	11.94 ± 2.61	−0.042 (0.93)	0.19 (14)
Nabis alternatus (Heteroptera: Nabidae)	2.24 ± 0.32	−0.330 (0.46)	0.39 (6)	0.94 ± 0.38	−0.118 (0.83)	0.23 (11)
Zelus renardii (Heteroptera: Reduviidae)	0.41 ± 0.18	0.067 (0.53)	0.47 (6)	—	—	—
Sinea spp. (Heteroptera: Reduviidae)	0.05 ± 0.05	0.400 (0.61)	0.20 (13)	—	—	—
Lygus hesperus (Heteroptera: Miridae)	16.9 ± 3.22	−0.175 (0.39)	0.68 (4)	1.89 ± 0.53	−0.153 (0.70)	0.08 (67)
Pseudatomoscelis seriatus (Heteroptera: Miridae)	3.41 ± 1.02	0.151 (0.52)	0.24 (10)	1.05 ± 0.53	1.158 (0.13)	0.33 (8)
Spanogonicus albofasciatus (Heteroptera: Miridae)	20.9 ± 4.30	−0.246 (0.84)	0.19 (14)	33.8 ± 7.60	−0.207 (0.76)	0.83 (3)
Rhinacloa forticornis (Heteroptera: Miridae)	0.03 ± 0.03	0.800 (0.49)	0.28 (9)	—	—	—
Chrysoperla carnea s.l. (Neuroptera: Chrysopidae)	4.91 ± 0.94	−0.015 (0.92)	0.46 (6)	5.67 ± 1.64	−0.107 (0.62)	0.36 (7)
Drapetis nr. *divergens* (Diptera: Empididae)	14.8 ± 1.29	−0.340 (0.04)*	0.89 (4)	0.28 ± 0.16	−0.600 (0.23)	0.32 (8)
Aphelinid parasitoids[f] (Hymenoptera: Aphelinidae)	0.70 ± 0.47	−0.315 (0.69)	0.25 (10)	NA	NA	NA
Other Hymenoptera (Hymenoptera)	3.66 ± 0.75	−0.052 (0.69)	0.31 (8)	0.23 ± 0.11	0.175 (0.39)	0.12 (27)
Araneida	3.33 ± 0.52	−0.082 (0.90)	0.92 (3)	3.22 ± 0.59	0.071 (0.33)	0.55 (5)
Predaceous Coleoptera	4.88 ± 1.27	−0.009 (0.64)	0.43 (6)	4.68 ± 0.72	0.306 (0.49)	0.24 (10)
Predaceous Heteroptera	27.0 ± 3.29	−0.160 (0.39)	0.83 (3)	16.3 ± 4.85	−0.095 (0.69)	0.44 (6)
Omnivorous Heteroptera	41.2 ± 7.48	−0.158 (0.41)	0.62 (4)	36.7 ± 5.06	−0.139 (0.98)	0.82 (3)
All taxa	99.8 ± 10.7	−0.178 (0.23)	0.80 (3)	73.5 ± 4.39	−0.061 (0.27)	0.79 (3)

All *Bt* and non-*Bt* plots received single applications of buprofezin and pyriproxyfen for control of *B. tabaci* and a single application of oxamyl for control of *L. hesperus* (see Table 1).

[a] Seasonal means (± SE) based on 13 sample dates between 5 June and 10 Sept. in three replicate plots. Prop. Δ is the proportional change in density in *Bt* cotton relative to non-*Bt* cotton. Numbers in parentheses following prop. Δ are *P* values of repeated-measures ANOVA on arthropod density using Proc Mixed (Littell et al. 1996); * $P < 0.05$.

[b] Seasonal means based on seven sample dates between 5 June and 19 Aug. in three replicate plots. Prop. Δ is the proportional change in density in *Bt* cotton relative to non-*Bt* cotton. Numbers in parentheses following prop. Δ are *P* values of repeated-measures ANOVA on arthropod density using Proc Mixed (Littell et al. 1996); * $P < 0.05$.

[c] Power $(1 - \beta)$ to detect a 50% change in density (effect size) from the non-*Bt* cotton given the experimental design and $\alpha = 0.05$; no. in parentheses indicates the sample size (replicate blocks) needed to detect a 50% change with 80% power given the observed no. of repeated measures (sampling dates).

[e] Insufficient density for analysis.

[f] Immature parasitoids (no. per seventh node leaf) of the genera *Eretmocerus* and *Encarsia* attacking *Bemisia tabaci* hosts.

NA, not available.

mycetomes was used to determine the presence of young parasitoid larvae.

Immature and adult stages of most predator taxa were pooled for analyses. Only larval stages of the green lacewing were counted, and following Tauber et al. (2000), the designation *C. carnea* sensu lato was used for this species. All parasitic Hymenoptera collected by sweep net were pooled into a single taxon for further analyses. Voucher specimens reside at the Department of Entomology, University of Arizona, Tucson, AZ.

Pest Insect Density

In addition to nontarget natural enemies, densities of various key pests including *P. gossypiella*, *L. hesperus*, and *B. tabaci* also were monitored. Densities of *P. gossypiella* larvae were estimated by counting all larvae inside 100 randomly collected green bolls (14–20 d old) per plot. Boll samples were not collected in 1996, and these samples were collected on only a single date in 1999 at the end of the season. From 2000 to 2003, boll samples were collected on a total of four to seven dates between early July and mid-September. The abundance of other larval Lepidoptera as well as *L. hesperus* were estimated from the sweep net samples. Densities of *B. tabaci* nymphs and adults were estimated weekly from early July through mid-September each year. 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. The densities of adults were 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.

Statistical Analyses

Mixed-model, repeated-measures analysis of variance (ANOVA) (Littell et al. 1996) was used to test for treatment differences in each arthropod taxon 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, as these consistently maximized Akaike's Information and Schwarz' Bayesian Criteria (Littell et al. 1996). Multi-year analyses were conducted by calculating seasonal mean densities for all arthropod natural enemy taxa for each plot in each year and entering block and year as random effects. Further repeated-measure and multi-year analyses were conducted on broader taxonomic groupings including Araneida, predaceous Coleoptera, predaceous Heteroptera, omnivorous Heteroptera, and all taxa combined. Arthropod counts were transformed by $(x + 0.5)^{0.5}$ or $\ln(x + 1)$ throughout, as necessary, to achieve normality and homoscedasticity; untransformed means are presented.

Statistical power analyses were conducted for all ANOVAs using the PASS software program (NCSS, Kaysville, UT). For repeated-measure analyses, the statistical power ($1-\beta$; where β is the type II error rate) to detect a 50% change in density (effect size) from non-*Bt* cotton or unsprayed cotton was estimated based on the underlying experimental design. The number of replicate blocks (sample size) needed to detect a 50% change with 80% power, given the observed number of repeated measures (sampling dates), also was estimated. Sensitivity analyses were conducted for three species [*Collops vittatus* Say, *Geocoris punctipes* (Say), and the empidid fly *Drapetis* nr *divergens*] that occurred at low, moderate, and high densities, respectively, over the years of study and represent the range of variation observed in all taxa. These analyses estimated power as a function of the number of replicate blocks for a fixed number of sample dates and as a function of the number of sample dates (repeated measures) for a fixed number of blocks. For multi-year analyses, the statistical power to detect a 20% change in density (effect size) from non-*Bt* cotton or unsprayed cotton was estimated based on the underlying experimental design. Power curves were constructed for selected individual taxa and taxonomic groups as a function of effect size. Finally, multi-year sensitivity analyses were conducted by estimating power curves as a function of effect size for *C. vittatus*, *G. punctipes*, and *D.* nr. *divergens* based on varying numbers of replicate blocks per year and varying numbers of years. Here, the SDs for 1 yr were based on the average SD over 5 individual yr; results for 2, 3, and 4 yr were based on the average SD over all possible combinations of 2, 3, and 4 yr, respectively. In all analyses, the type I error rate, α, was 0.05.

To further examine seasonal treatment effects on arthropod populations, a time-dependent, multivariate analysis called principal response curves (PRCs) (Van den Brink and Ter Braak, 1998, 1999) was conducted. PRC is based on an ordination method known as partial redundancy analysis, a type of principal component analysis in which information is extracted only from the variance explained by treatment effects. PRCs provide a simple means of visualizing and testing the overall response of a biological community to environmental stress by determining treatment effects relative to a standard control, here unsprayed non-*Bt* cotton. The program CANOCO 4 (Ter Braak and Smilauer 1998) was used to perform the partial redundancy analyses, construct the PRCs, and test for treatment differences in community composition using a distribution-free *F*-type test based on sample permutation. In CANOCO, the analyses can be structured to account for blocking and split-plot effects and to allow statistical inference on paired treatment contrasts. Arthropod count data were transformed by $\ln(x + 1)$ before analysis.

Results

Natural Enemy Abundance

Univariate Analyses and Power for Individual Years. Densities of two predator taxa differed significantly between non-*Bt* and *Bt* cottons in 1996 based on sweep net samples (Table 2). Densities of the spider *Dictyna reticulata* Gertsch and Ivie increased on average over the season in *Bt* compared with non-*Bt* by 75% ($F = 6.44$; df = 1,10.7; $P = 0.028$), whereas those of *D.* nr. *divergens* declined by 34% ($F = 4.81$;

Table 3. Change in mean densities of arthropods in unsprayed *Bt* cotton relative to non-*Bt* cotton, Maricopa, AZ, 1999–2003

Taxa	1999			2000			2001			2002			2003		
	Non-*Bt* density	Prop. Δ[a]	Power[b]	Non-*Bt* density	Prop. Δ	Power	Non-*Bt* density	Prop. Δ	Power	Non-*Bt* density	Prop. Δ	Power	Non-*Bt* density	Prop. Δ	Power
Dictyna reticulata	0.59 ± 0.07	0.263 (0.54)	0.66 (6)	0.59 ± 0.09	0.212 (0.28)	0.63 (6)	0.50 ± 0.12	−0.107 (0.67)	0.39 (9)	0.65 ± 0.12	−0.026 (0.90)	0.41 (9)	0.78 ± 0.10	0.213 (0.31)	0.80 (4)
Misumenops celer	3.92 ± 0.50	−0.032 (0.63)	0.62 (6)	3.18 ± 0.24	−0.073 (0.92)	0.77 (5)	1.93 ± 0.17	−0.074 (0.66)	0.60 (6)	0.72 ± 0.13	−0.140 (0.56)	0.63 (6)	3.19 ± 0.22	0.033 (0.96)	0.99 (3)
Salticidae	0.88 ± 0.10	−0.304 (0.22)	0.81 (4)	0.32 ± 0.09	−0.278 (0.57)	0.45 (8)	0.04 ± 0.02	0.000 (0.94)	0.59 (6)	0.15 ± 0.02	−0.222 (0.60)	0.61 (6)	0.26 ± 0.04	−0.200 (0.49)	0.70 (5)
Other Araneida	1.72 ± 0.15	−0.364 (0.03)*	0.93 (4)	0.41 ± 0.11	0.130 (0.78)	0.48 (8)	0.04 ± 0.04	0.000 (0.93)	0.42 (9)	0.30 ± 0.03	−0.444 (0.35)	0.56 (7)	0.69 ± 0.08	−0.045 (0.77)	0.84 (4)
Collops vittatus	2.28 ± 0.21	−0.151 (0.39)	0.98 (3)	0.79 ± 0.13	0.386 (0.34)	0.49 (8)	0.32 ± 0.07	−0.167 (0.86)	0.16 (26)	1.20 ± 0.17	−0.125 (0.78)	0.79 (5)	3.65 ± 0.36	−0.074 (0.39)	0.97 (3)
Hippodamia convergens	1.91 ± 0.12	−0.082 (0.45)	0.95 (3)	1.16 ± 0.33	−0.016 (0.62)	0.48 (8)	1.09 ± 0.19	−0.148 (0.48)	0.61 (6)	1.17 ± 0.25	−0.500 (0.04)*	0.87 (4)	0.68 ± 0.12	−0.323 (0.04)*	0.87 (4)
Anthicidae	1.91 ± 0.84	−0.082 (0.95)	0.11 (53)	0.93 ± 0.17	−0.442 (0.15)	0.80 (4)	1.14 ± 0.17	0.484 (0.26)	0.89 (4)	0.95 ± 0.40	−0.421 (0.15)	0.70 (5)	2.49 ± 0.42	−0.117 (0.94)	0.34 (11)
Other Coccinellidae	2.09 ± 0.31	−0.254 (0.16)	0.81 (4)	0.11 ± 0.02	−0.333 (0.60)	0.87 (4)	0.20 ± 0.08	0.727 (0.23)	0.53 (7)	0.35 ± 0.09	0.000 (0.82)	0.51 (7)	0.22 ± 0.03	0.143 (0.69)	0.48 (8)
Geocoris punctipes	19.4 ± 4.50	−0.243 (0.40)	0.14 (33)	5.05 ± 0.83	−0.088 (0.72)	0.71 (5)	1.27 ± 0.26	−0.563 (0.49)	0.19 (21)	7.75 ± 0.37	−0.099 (0.31)	0.96 (4)	3.04 ± 0.28	0.075 (0.64)	0.85 (4)
Geocoris pallens	2.38 ± 0.36	−0.059 (0.51)	0.81 (4)	1.63 ± 0.27	0.385 (0.09)	0.78 (5)	2.82 ± 0.33	−0.089 (0.73)	0.29 (13)	3.72 ± 0.29	0.063 (0.94)	0.51 (7)	10.9 ± 0.49	0.071 (0.56)	0.92 (4)
Orius tristicolor	6.52 ± 0.96	0.113 (0.45)	0.50 (7)	3.39 ± 0.42	0.205 (0.54)	0.78 (5)	0.49 ± 0.09	1.500 (0.09)	0.28 (13)	6.32 ± 0.49	−0.018 (0.65)	0.94 (4)	7.95 ± 0.60	−0.052 (0.84)	0.98 (3)
Nabis alternatus	3.97 ± 0.74	−0.382 (0.14)	0.31 (12)	1.68 ± 0.17	−0.053 (0.73)	0.83 (4)	1.61 ± 0.19	−0.278 (0.59)	0.19 (21)	2.53 ± 0.22	−0.257 (0.03)*	0.96 (3)	2.85 ± 0.09	−0.109 (0.55)	0.87 (4)
Zelus renardii	0.92 ± 0.36	−0.407 (0.25)	0.14 (31)	0.18 ± 0.06	1.000 (0.14)	0.44 (8)	0.12 ± 0.04	−0.429 (0.52)	0.37 (10)	0.05 ± 0.03	−0.333 (0.73)	0.41 (9)	2.26 ± 0.15	0.101 (0.39)	0.98 (3)
Sinea spp.	0.02 ± 0.02	−0.680 (0.33)	0.82 (4)	0.02 ± 0.02	2.000 (0.38)	0.23 (16)	—[c]	—	—	—	—	—	—	—	—
Lygus hesperus	33.1 ± 0.99	−0.094 (0.20)	0.99 (3)	7.89 ± 1.33	0.061 (0.42)	0.90 (4)	18.9 ± 1.61	0.017 (0.62)	0.97 (3)	23.6 ± 3.49	−0.175 (0.35)	0.71 (5)	10.4 ± 1.03	−0.038 (0.83)	0.98 (3)
Pseudatomoscelis seriatus	3.70 ± 0.80	−0.308 (0.06)	0.16 (25)	21.5 ± 1.69	0.192 (0.25)	0.99 (2)	0.38 ± 0.07	0.381 (0.17)	0.92 (4)	2.32 ± 0.52	−0.216 (0.14)	0.79 (5)	23.5 ± 0.42	−0.016 (0.62)	0.99 (2)
Spanogonicus albofasciatus	3.92 ± 0.84	−0.096 (0.37)	0.14 (32)	2.21 ± 0.89	0.065 (0.71)	0.47 (8)	0.68 ± 0.14	0.158 (0.18)	0.99 (2)	1.67 ± 0.15	0.290 (0.32)	0.60 (6)	6.47 ± 0.71	0.064 (0.62)	0.99 (3)
Rhinacloa forticornis	0.75 ± 0.16	−0.063 (0.82)	0.79 (5)	0.09 ± 0.03	−0.600 (0.20)	0.75 (5)	—	—	—	0.08 ± 0.02	−0.400 (0.56)	0.46 (8)	0.39 ± 0.01	−0.243 (0.44)	0.81 (4)
Chrysoperla carnea s.l.	2.41 ± 0.16	−0.175 (0.42)	0.93 (4)	1.98 ± 0.15	0.234 (0.28)	0.57 (6)	3.75 ± 0.41	−0.100 (0.45)	0.93 (3)	1.67 ± 0.16	−0.120 (0.42)	0.86 (4)	1.54 ± 0.19	0.034 (0.62)	0.88 (4)
Drapetis nr. *divergens*	54.7 ± 6.93	−0.158 (0.28)	0.99 (2)	14.6 ± 1.28	0.009 (0.98)	0.63 (6)	7.46 ± 0.97	−0.182 (0.68)	0.86 (4)	11.5 ± 0.92	−0.061 (0.43)	0.77 (5)	8.34 ± 2.37	−0.096 (0.48)	0.81 (4)
Aphelinid parasitoids[d]	6.24 ± 2.70	−0.346 (0.81)	0.10 (55)	11.03 ± 3.31	−0.351 (0.30)	0.69 (5)	3.24 ± 0.88	0.201 (0.23)	0.98 (3)	—	—	—	4.22 ± 0.77	−0.325 (0.35)	0.53 (7)
Other Hymenoptera	2.72 ± 0.17	−0.115 (0.59)	0.38 (10)	0.84 ± 0.23	0.213 (0.53)	0.41 (9)	0.98 ± 0.29	0.018 (0.64)	0.42 (9)	1.58 ± 0.14	0.158 (0.65)	0.84 (4)	2.46 ± 0.25	0.081 (0.59)	0.83 (4)
Araneida	7.11 ± 0.76	−0.121 (0.52)	0.74 (5)	4.50 ± 0.29	−0.032 (0.84)	0.93 (4)	2.50 ± 0.12	−0.079 (0.66)	0.58 (6)	2.50 ± 0.20	−0.147 (0.36)	0.66 (6)	4.92 ± 0.30	0.038 (0.85)	0.85 (4)
Predaceous Coleoptera	8.19 ± 0.66	−0.145 (0.12)	0.99 (3)	2.98 ± 0.46	−0.054 (0.77)	0.75 (5)	2.75 ± 0.21	0.175 (0.64)	0.83 (4)	3.67 ± 0.63	−0.309 (0.07)	0.84 (4)	7.03 ± 0.81	−0.107 (0.43)	0.99 (3)
Predaceous Heteroptera	33.2 ± 4.63	−0.182 (0.30)	0.29 (13)	11.9 ± 0.83	0.084 (0.60)	0.80 (4)	6.11 ± 0.71	−0.170 (0.95)	0.26 (15)	20.4 ± 0.95	−0.065 (0.33)	0.99 (2)	27.1 ± 0.17	0.019 (0.68)	0.99 (2)
Omnivorous Heteroptera	41.4 ± 2.08	−0.112 (0.06)	0.93 (4)	31.6 ± 2.92	0.148 (0.23)	0.98 (3)	19.9 ± 1.71	0.030 (0.11)	0.95 (3)	27.6 ± 3.86	−0.151 (0.38)	0.76 (5)	40.7 ± 1.39	−0.011 (0.84)	0.99 (2)
All taxa	149.7 ± 9.06	−0.148 (0.11)	0.99 (3)	68.5 ± 3.94	0.088 (0.29)	0.99 (3)	43.5 ± 2.55	−0.045 (0.83)	0.49 (8)	68.9 ± 3.03	−0.117 (0.08)	0.99 (3)	94.6 ± 2.36	−0.012 (0.61)	0.99 (2)

[a] Prop. Δ is the proportional change in mean seasonal densities (per 50 sweeps) in *Bt* cotton relative to non-*Bt* cotton (both unsprayed). Seasonal means based on 16, 14, 14,15, and 12 sample dates in 1999 through 2003, respectively, with four replicate plots each year. Numbers in parentheses following Prop. Δ are *P* values of repeated-measures ANOVA on arthropod density using Proc Mixed (Littell et al. 1996); * $P < 0.05$.

[b] Power $(1 - \beta)$ to detect a 50% change in density (effect size) in non-*Bt* cotton given the variance and replication associated with the experimental design each year ($\alpha = 0.05$); no. in parentheses indicates the sample size needed to detect a 50% change with 80% power given the observed no. of repeated measures (sampling dates).

[c] Insufficient density for analysis.

[d] Immature parasitoids (no. per seventh node leaf) of the genera *Eretmocerus* and *Encarsia* attacking *Bemisia tabaci* hosts; samples from 2002 lost due to freezer malfunction.

df = 1,14.5; $P = 0.045$). The statistical power to detect a 50% change in abundance, given the experimental design, was generally poor, with analyses of only a few taxa having power near 80%. On average, a sample size of nearly nine replicate blocks would have been necessary to detect a 50% change in density of any one taxon from non-*Bt* to *Bt* cotton. Broader taxonomic groups showed a generally smaller numerical decline in *Bt* cotton but pooling taxa increased power and improved the likelihood of discerning a 50% change in density (Table 2). Results from whole plant samples were generally similar to those seen from sweep net samples, although whole plant samples were collected over a shorter time period. *D. reticulata* again showed a significant positive increase in *Bt* compared with non-*Bt* cotton ($F = 6.23$; df = 1,19.3; $P = 0.021$), but statistical power was generally poor for all taxa. On average, a sample size of 14 replicate blocks would have been necessary to detect a 50% change in arthropod density between cultivars with 80% power. Whole plant samples also failed to detect four taxa found in sweep net samples, and considerably more time and effort was required to use this sampling method.

Based on a consistent experimental design and plot size from 1999 to 2003, there were very few statistically significant differences in densities of arthropod natural enemies between unsprayed *Bt* and non-*Bt* cotton (Table 3). A single taxon (other Araneida) declined significantly ($F = 5.57$; df = 1,21; $P = 0.028$) by ≈36% in *Bt* compared with non-*Bt* cotton in 1999, *Hippodamia convergens* Guérin-Méneville and *Nabis alternatus* Parshley declined 50% ($F = 4.61$; df = 1,19.5; $P = 0.044$) and 26% ($F = 5.14$; df = 1,32.8; $P = 0.030$), respectively, in 2002, and *H. convergens* declined by ≈32% in 2003 ($F = 4.36$; df = 1,43.4; $P = 0.042$). There were no significant ($P > 0.05$) changes in density of broader taxonomic groups in any year (Table 3). There also were no significant changes in densities of any taxon or taxonomic group with the use of glyphosate-resistant cultivars in 2003 (data not shown). Overall, the number of significant differences is less than what would be expected from applying a type I error rate of 0.05 to multiple comparisons.

Average densities of the various taxa varied over years of the study and were generally highest in 1999 (Table 3). However, there was no evidence of any consistent pattern of decline in density of any taxa over years that might indicate a chronic impact from exposure of multiple generations of natural enemy populations to *Bt* toxins within the defined area of the research center where studies were conducted.

The statistical power of the underlying experimental design to detect a 50% change in density was relatively good, approaching or exceeding 80% for some individual taxa in all years. On average, the sample size (number of replicate blocks) needed to detect a 50% change in density with 80% power was 9.2, 6.4, 9.0, 5.7, and 4.4 in 1999–2003, respectively. Pooling individual taxa into four broad taxonomic groupings increased the power of the analyses and reduced the average number of replicates needed to detect a 50% change with 80% power to between three and seven over the 5 yr (Table 3).

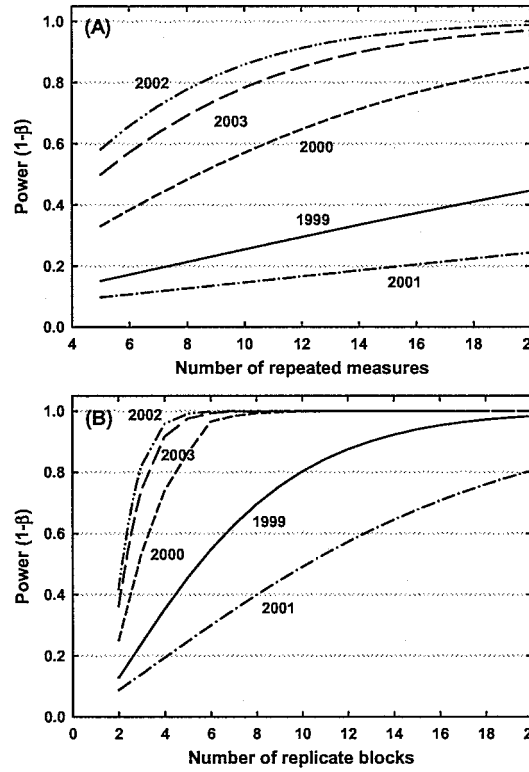


Fig. 1. Statistical power to discern a 50% change in abundance of *G. punctipes* in *Bt* relative to non-*Bt* cotton as a function of sample size for each of 5 yr. (A) Effect of the number of sampling dates (repeated measures) with four replicate treatment blocks. (B) Effect of the number of treatment blocks with 15 sampling dates. The mean density (per 50 sweeps) of *G. punctipes* was 19.4, 5.1, 1.3, 7.8, and 3.0 from 1999 to 2003, respectively.

Further analyses were conducted to examine the relative contribution of sample size (replicate blocks) and the number of sampling dates (repeated-measures) on the power to discern a 50% change in density of three representative predator species. Only the results for *G. punctipes* will be presented because they were representative of the three taxa examined. There was large variation in the relationship between sample size and power across years of the study (Fig. 1). However, regardless of year, power was relatively insensitive to increases in the number of repeated measures relative to increasing the number of replicate blocks. The power to detect a 50% change in density was >80% in most years of the study for *G. punctipes* with four replicates and between 12 and 16 sampling dates (see Table 3). Results from all three species consistently showed that increasing the number of replicate blocks was a more efficient means of increasing power compared with increasing the number of sampling dates.

In contrast to the relatively small and inconsistent change in the density of arthropod natural enemies in

Table 4. Change in mean densities of arthropods (per 50 sweeps) in sprayed relative to unsprayed *Bt* and non-*Bt* cotton, Maricopa, AZ, 2001–2002

Taxa	2001			2002		
	Density (unsprayed)[a]	Prop. Δ[b]	Power[c] (50% effect)	Density (unsprayed)	Prop. Δ	Power (50% effect)
Dictyna reticulata	0.55 ± 0.09	−0.290 (0.15)	0.60 (6)	0.67 ± 0.11	−0.542 (<0.01)*	0.85 (4)
Misumenops celer	1.95 ± 0.27	−0.294 (0.08)	0.86 (4)	0.79 ± 0.09	−0.632 (<0.01)*	0.93 (3)
Salticidae	0.05 ± 0.03	0.333 (0.81)	0.46 (8)	0.15 ± 0.04	−0.273 (0.49)*	0.83 (4)
Other Araneida	—[d]	—	—	0.18 ± 0.08	0.077 (0.98)	0.36 (12)
Collops vittatus	0.13 ± 0.04	−0.286 (0.63)	0.64 (6)	1.10 ± 0.16	−0.354 (0.07)	0.93 (3)
Hippodamia convergens	0.75 ± 0.28	−0.643 (0.03)*	0.73 (5)	0.28 ± 0.08	−0.550 (0.15)	0.82 (4)
Anthicidae	0.36 ± 0.16	−0.500 (0.46)	0.40 (10)	0.29 ± 0.09	−0.381 (0.33)	0.72 (5)
Other Coccinellidae	0.05 ± 0.02	−0.667 (0.28)	0.37 (11)	0.35 ± 0.07	−0.640 (0.02)*	0.97 (3)
Geocoris punctipes	0.48 ± 0.07	−0.778 (<0.01)*	0.96 (3)	10.7 ± 0.62	−0.782 (<0.01)*	0.99 (2)
Geocoris pallens	0.36 ± 0.11	−0.700 (0.11)	0.64 (6)	3.42 ± 0.42	−0.675 (<0.01)*	0.74 (5)
Orius tristicolor	0.61 ± 0.17	0.471 (0.29)	0.38 (10)	9.11 ± 0.46	0.259 (0.23)	0.99 (2)
Nabis alternatus	0.07 ± 0.03	−0.750 (0.14)	0.88 (4)	1.74 ± 0.29	−0.840 (<0.01)*	0.95 (3)
Zelus renardii	0.05 ± 0.03	−0.333 (0.63)	0.71 (5)	0.06 ± 0.03	−0.600 (0.04)	0.98 (3)
Lygus hesperus	34.5 ± 2.25	−0.439 (<0.01)*	0.99 (3)	34.6 ± 3.66	−0.648 (<0.01)*	0.97 (3)
Pseudatomoscelis seriatus	0.39 ± 0.09	0.273 (0.34)	0.66 (5)	2.90 ± 0.41	−0.469 (<0.01)*	0.93 (3)
Spanogonicus albofasciatus	0.16 ± 0.07	0.111 (0.94)	0.52 (8)	1.18 ± 0.19	0.247 (0.93)	0.53 (8)
Rhinacloa forticornis	—[d]	—	—	0.06 ± 0.03	0.000 (1.00)	0.56 (7)
Chrysoperla carnea s.l.	6.14 ± 0.53	−0.110 (0.36)	0.93 (3)	2.47 ± 0.23	−0.096 (0.27)	0.97 (3)
Drapetis nr. *divergens*	12.4 ± 1.65	−0.452 (0.02)*	0.80 (4)	23.3 ± 1.23	−0.543 (0.02)*	0.98 (3)
Aphelinid parasitoids[e]	3.64 ± 0.63	−0.317 (0.08)	0.72 (5)	—	—	—
Other Hymenoptera	1.41 ± 0.31	−0.430 (0.17)	0.66 (6)	1.24 ± 0.13	−0.011 (0.69)	0.93 (3)
Araneida	2.55 ± 0.29	−0.273 (0.09)	0.86 (4)	2.57 ± 0.18	−0.541 (<0.01)*	0.89 (4)
Predaceous Coleoptera	1.25 ± 0.27	−0.529 (0.14)	0.61 (6)	2.01 ± 0.22	−0.434 (<0.01)*	0.95 (3)
Predaceous Heteroptera	1.57 ± 0.18	−0.261 (0.13)	0.78 (5)	25.0 ± 1.29	−0.392 (<0.01)*	0.98 (3)
Omnivorous Heteroptera	35.0 ± 2.31	−0.428 (<0.01)*	0.99 (2)	38.7 ± 3.75	−0.607 (<0.01)*	0.99 (3)
All taxa	60.4 ± 3.83	−0.391 (<0.01)*	0.99 (2)	86.5 ± 5.13	−0.321 (<0.01)*	0.99 (2)

In 2001, split-plots of *Bt* and non-*Bt* cotton were sprayed with single applications of buprofezin for *B. tabaci* control and single applications of oxamyl and acephate for *L. hesperus* control. In 2002, split plots of *Bt* and non-*Bt* cotton were sprayed with single applications of buprofezin and acephate + fenpropathrin for *B. tabaci* control and a single application of acephate for *L. hesperus* control; non-*Bt* split plots also received single sprays of chlorpyrifos and cyfluthrin for caterpillar control (see Table 1).

[a] Seasonal means (±SE) based on seven and nine postspray sample dates between late July to early Sept. in four replicate plots in 2001 and 2002, respectively.

[b] Prop. Δ is the proportional change in density in sprayed cotton relative to unsprayed cotton. Numbers in parentheses following Prop. Δ are *P* values of repeated-measures ANOVA on arthropod density using Proc Mixed (Littell et al. 1996); * $P < 0.05$.

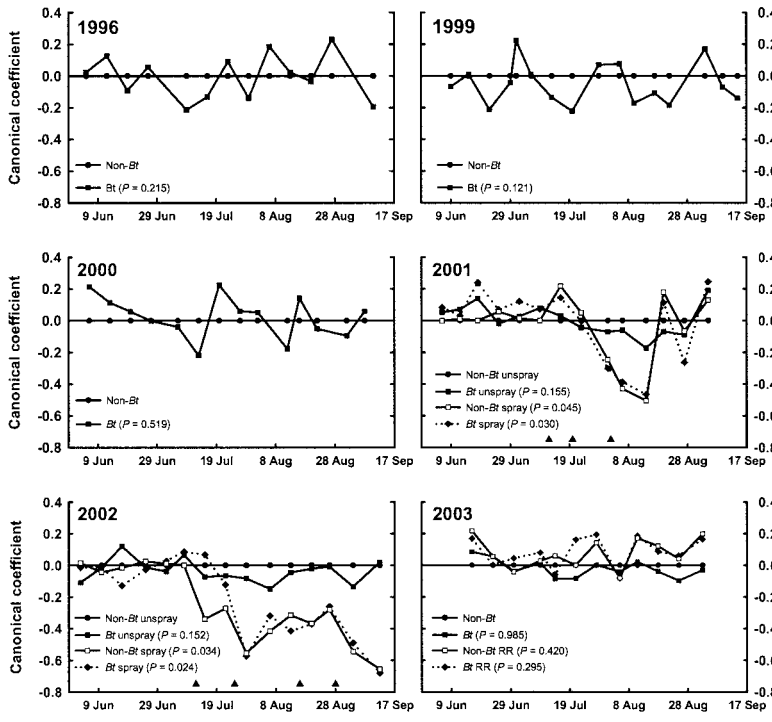
[c] Power $(1 - \beta)$ to detect a 50% change in density (effect size) in unsprayed cotton given the variance and replication associated with the experimental design each year ($\alpha = 0.05$); no. in parentheses indicates the sample size (no. of main-plot replicates) needed to detect a 50% change with 80% power given the observed no. of repeated measures (sampling dates).

[d] Insufficient density for analysis.

[e] Immature parasitoids (no. per seventh node leaf) of the genera *Eretmocerus* and *Encarsia* attacking *Bemisia tabaci* hosts; samples from 2002 lost because of freezer malfunction.

Bt cotton, the application of insecticides to both *Bt* and non-*Bt* cotton had a marked negative impact on these taxa (Table 4). In 2001, the application of insecticides for *B. tabaci*, and especially those for *L. hesperus*, significantly reduced the densities of four taxa including the target *L. hesperus* ($F > 5.53$; df = 1,>11.9; $P < 0.026$). These reductions ranged from 43 to 78% relative to unsprayed cotton. Reductions were also significant for omnivorous bugs as a group ($F = 35.5$; df = 1,12.6; $P < 0.0001$) and for all taxa combined ($F = 28.7$; df = 1,12.1; $P = 0.0002$). The greater number of insecticide sprays made in 2002 was associated with larger declines (47–84%) in a total of nine taxa ($F > 6.35$; df = 1,> 8.9; $P < 0.015$). Reductions also were significant ($F > 10.1$; df = 1,> 8.9; $P < 0.003$) for all the broader taxonomic groups (Table 4). Given the relatively large changes in density for many taxa, the statistical power to discern a 50% change in density was high compared with those associated with the effects of *Bt* cotton alone. On average, 5.8 and 4.2 replicated blocks would have been necessary to discern 50% changes in density of the arthropod natural enemies examined in 2001 and 2002, respectively.

Multivariate Analyses of Individual Years. PRC analysis was used to further examine the time-dependent effect of *Bt* cotton and insecticide applications on the entire arthropod natural enemy community. PRCs for 1996–2003 supported the univariate analyses indicating no significant ($P > 0.121$) effect of *Bt* cotton on the community relative to non-*Bt* cotton (Fig. 2). The PRCs based on the first axes of the redundancy analysis explained 38–59% of the variation caused by treatment. The second axes were not significant ($P > 0.25$) in any year. There was no consistent pattern of decline or increase in the arthropod community over time in unsprayed *Bt* cotton in any year as depicted by the PRCs. There also was no pattern across years, suggesting the lack of any chronic effects through exposure of multiple generations of arthropod natural enemies to *Bt* toxins in a defined area (Fig. 2) as indicated in univariate analyses above. There were no consistent patterns in the species weights, which denote the



Species weights

	1996	1999	2000	2001	2002	2003
D. reticulata	0.34	-0.79	1.28	0.5	0.5	-0.75
M. celer	2.31	1.1	1.08	1.37	0.54	1.28
Salticidae	0.5	0.32	-0.24	0.01	0.11	0.27
Other Araneida	-0.35	0.88	-1.12	0.03	0	1.88
C. vittatus	0.25	0.19	2.12	-0.2	-0.35	-1.05
H. convergens	-0.95	0.24	1.45	1.14	0.21	-0.41
Other Coccinellidae	0.45	2.04	-0.01	0.1	0.24	0.23
Anthicidae	0.56	0.54	-0.54	1.15	0.07	-0.97
G. punctipes	1.45	0.41	0.64	1.33	3.48	2.5
G. pallens	-0.42	0.5	-0.57	0.18	2.3	-1.83
O. tristicolor	1.94	1.12	2.57	1.32	-0.13	0.73
N. alternatus	0.17	1.04	1.2	-0.05	1.81	1.72
Z. renardii	0.15	0.8	-0.53	0.14	0.13	1.37
Sinea spp.	0.2	-0.07	0.13			0.08
L. hesperus	1.29	1.79	0.48	1.24	2.26	0.56
P. seriatus	1.66	1.43	-0.15	0.93	1.41	-0.07
S. albofasciatus	-0.31	1.29	0.55	0.65	0.05	-0.49
R. forticornis	0.16	-0.23	0.08	0.05	-0.04	0.1
C. carnea	1	1.3	2.62	0.24	0.29	0.46
Drapetis sp.	1.02	0.91	0.93	3.92	0.57	0.41
Hymenoptera	0.85	3.18	1.37	1.36	0.17	-1.39

Fig. 2. PRCs showing the effects of *Bt* cotton, insecticide sprays, and glyphosate resistance on the arthropod natural enemy community over growing seasons between 1996 and 2003 in Maricopa, AZ. The PRCs show the effect of *Bt* cotton or sprayed cotton relative to a standard (unsprayed, non-*Bt*, non-RR [Roundup-Ready] cotton), which is represented by the y = 0 line. The *P* value denotes the significance of each treatment curve relative to the standard over all dates based on an *F*-type permutation test. The triangles near the bottom of graphs in 2001 and 2002 denote the dates of insecticide applications. The greater the species weight, the more the response for that species resembles the PRCs. Negative weights indicate an opposite pattern. The product of the species weight and the canonical coefficient for a given treatment and time estimates the natural log change in density of that species relative to the standard.

degree to which the PRC resemble the response of a given taxon, with the exception that the more abundant taxa tended to have higher positive species weights.

In contrast, the PRCs for 2001 and 2002, where insecticides were applied as needed in subplots of *Bt* and non-*Bt* cotton, showed distinctive patterns reflecting the negative effects of insecticides (Fig. 2). In sprayed plots in 2001, there was no response to the initial application of buprofezin for *B. tabaci* control on 12 July; however, there was a rapid decline in the arthropod community in 2001 after the applications of oxamyl and acephate for *L. hesperus* control beginning 20 July. Populations recovered somewhat in late August to mid-September. Similarly, in 2002 populations in the treated splits of the non-*Bt* main plot responded negatively to the first broad-spectrum spray for Lepidoptera on 12 July. An application of oxamyl on 25 July for *L. hesperus* control in *Bt* subplots led to similar declines in arthropod populations (Fig. 2), although there was a significant ($P < 0.05$) difference between sprayed *Bt* and non-*Bt* cotton because of the lepidopteran sprays. Additional applications of broad-spectrum materials in mid- to late-August did not allow populations in sprayed subplots to recover. The PRCs for these years based on the first axes of the redun-

Table 5. Overall change in mean densities of arthropods (per 50 sweeps) in *Bt* relative to non-*Bt* cotton (5 yr) and in sprayed relative to unsprayed cotton (2 yr), Maricopa, AZ, 1999–2003

Taxa	1999–2003			2001–2002		
	Non-*Bt* density[a]	Prop. Δ[b]	Power[c] (20% effect)	Unsprayed density[a]	Prop. Δ	Power (20% effect)
Dictyna reticulata	0.62 ± 0.05	0.121 (0.56)	0.76	0.62 ± 0.07	−0.443 (**0.02**)*	0.23
Misumenops celer	2.59 ± 0.28	−0.038 (0.42)	0.97	1.30 ± 0.13	−0.410 (<**0.01**)*	0.41
Salticidae	0.33 ± 0.07	−0.268 (0.07)	0.38	0.11 ± 0.02	−0.143 (0.71)	0.24
Other Araneida	0.63 ± 0.14	−0.233 (**0.02**)*	0.81	0.10 ± 0.04	0.154 (0.73)	0.18
Collops vittatus	1.65 ± 0.29	−0.062 (0.51)	0.86	0.67 ± 0.09	−0.349 (**0.02**)*	0.57
Hippodamia convergens	1.20 ± 0.13	−0.189 (**0.04**)*	0.87	0.48 ± 0.12	−0.613 (**0.03**)*	0.26
Anthicidae	1.48 ± 0.23	−0.095 (0.33)	0.43	0.32 ± 0.10	−0.439 (0.21)	0.19
Other Coccinellidae	0.59 ± 0.18	−0.132 (0.56)	0.48	0.20 ± 0.04	−0.538 (**0.05**)*	0.35
Geocoris punctipes	7.30 ± 1.69	−0.176 (**0.01**)*	0.92	6.22 ± 0.37	−0.781 (<**0.01**)*	0.99
Geocoris pallens	4.30 ± 0.79	0.058 (0.38)	0.99	2.08 ± 0.25	−0.677 (<**0.01**)*	0.89
Orius tristicolor	4.89 ± 0.67	0.054 (0.21)	0.86	5.39 ± 0.26	0.270 (**0.01**)*	0.69
Nabis alternatus	2.53 ± 0.25	−0.238 (<**0.01**)*	0.97	1.01 ± 0.17	−0.837 (<**0.01**)*	0.63
Zelus renardii	0.71 ± 0.20	−0.011 (0.77)	0.88	0.05 ± 0.02	−0.714 (**0.05**)*	0.40
Sinea spp.	0.01 ± 0.01	0.370 (0.74)	0.49	—	—	—
Lygus hesperus	18.8 ± 2.23	−0.073 (0.35)	0.83	34.5 ± 2.28	−0.557 (<**0.01**)*	0.71
Pseudatomoscelis seriatus	10.3 ± 2.33	0.044 (0.98)	0.94	1.80 ± 0.20	−0.398 (**0.02**)*	0.42
Spanogonicus albofasciatus	2.99 ± 0.53	0.052 (0.38)	0.80	0.73 ± 0.10	0.234 (0.65)	0.19
Rhinacloa forticornis	0.26 ± 0.07	−0.160 (0.31)	0.70	0.03 ± 0.02	0.000 (0.96)	0.33
Chrysoperla carnea s.l.	2.27 ± 0.21	−0.042 (0.56)	0.87	4.08 ± 0.24	−0.105 (0.26)	0.56
Drapetis nr. *divergens*	19.3 ± 4.31	−0.118 (**0.02**)*	0.99	17.8 ± 2.35	−0.387 (<**0.01**)*	0.46
Aphelinid parasitoids[d]	6.18 ± 1.26	−0.273 (0.21)	0.39	3.64 ± 0.63	−0.317 (0.08)	NA
Other Hymenoptera	1.72 ± 0.20	0.039 (0.59)	0.67	1.31 ± 0.15	−0.208 (0.13)	0.33
Araneida	4.31 ± 0.43	−0.064 (0.18)	0.98	2.56 ± 0.18	−0.424 (<**0.01**)*	0.51
Predaceous Coleoptera	4.92 ± 0.57	−0.112 (0.23)	0.84	1.68 ± 0.12	−0.465 (<**0.01**)*	0.38
Predaceous Heteroptera	19.7 ± 2.41	−0.070 (0.18)	0.99	14.8 ± 0.69	−0.386 (<**0.01**)*	0.98
Omnivorous Heteroptera	32.3 ± 2.12	−0.025 (0.62)	0.98	37.1 ± 2.37	−0.533 (<**0.01**)*	0.79
All taxa	84.5 ± 8.49	−0.064 (0.15)	0.99	78.9 ± 3.17	−0.345 (<**0.01**)*	0.93

[a] Overall means (±SE) based on seasonal means in four replicate main plots in each of 5 yr for *Bt* and non-*Bt* contrasts and 2 yr for unsprayed and sprayed contrasts.

[b] Prop. Δ 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. Δ are *P* values of randomized complete block ANOVA with years as a random factor on arthropod density using Proc Mixed (Littell et al. 1996); * $P < 0.05$.

[c] Power $(1 - \beta)$ to detect a 20% change in density (effect size) given the variance and replication associated with the experimental design ($\alpha = 0.05$).

[d] Immature parasitoids (no. per seventh node leaf) of the genera *Eretmocerus* and *Encarsia* attacking *Bemisia tabaci* hosts; samples from 2002 lost because of freezer malfunction.

dancy analysis explained 47–59% of the variation caused by treatment. The second axis was not significant ($P > 0.16$) in either year. In general, higher species weights were associated with taxa that showed significant population declines in sprayed subplots.

PRC results in 2003 where glyphosate-tolerant cottons were grown were similar to those observed in 1996, 1999, and 2000 (Fig. 2). The PRCs based on the first axis of the redundancy analysis explained 28% of the variation caused by treatment and was not significant ($P = 0.295$). Again, the second axis was not significant ($P = 0.14$).

Multi-Year Analyses and Power. Multi-year analyses were conducted by combining data from 1999 through 2003 where similar plot sizes were used. Contrasting unsprayed *Bt* to unsprayed non-*Bt* cotton in the multi-year analyses revealed significant declines in seasonal densities of five predator taxa in *Bt* cotton including other Araneida ($F = 6.60$; df = 1,27; $P = 0.016$), *H. convergens* ($F = 4.71$; df = 1,15; $P = 0.046$), *G. punctipes* ($F = 8.46$; df = 1,15; $P = 0.011$), *N. alternatus* ($F = 21.4$; df = 1,15; $P = 0.0003$), and *D.* nr. *divergens* ($F = 7.54$; df = 1,15; $P = 0.015$; Table 5). There were no significant ($P > 0.05$) changes in density of broader taxonomic groups. In general, the changes in density were smaller than those observed in individual years; however, the increased sample size of the analyses improved power and allowed smaller changes to be detected. For many individual taxa and for all broader taxonomic groups the power to detect a change of 20% was >80%. A smaller increase in sample size for the combined analyses of insecticide effects over 2 yr also improved power and allowed significant, mostly negative, effects to be discerned for 13 individual taxa ($F > 6.16$; df = 1,>12; $P < 0.045$) and all broader taxonomic groups ($F > 15.7$; df = 1,>21; $P < 0.0006$) (Table 5).

Power curves as a function of effect size for selected individual taxa and broader taxonomic groups show that the multi-year analyses had sufficient power to discern changes in density between *Bt* and non-*Bt* cotton of 10–20% with >80% power (Fig. 3). In general, analyses of taxa or groups that occurred at higher densities had higher power for a given effect size. The power of the multi-year analyses of insecticide effects was lower because of smaller sample sizes; however, the effects of insecticides were much larger than those of *Bt* cotton and so power to detect smaller differences was unnecessary. Nonetheless, the power to detect changes <20% was >80% for all the selected individual

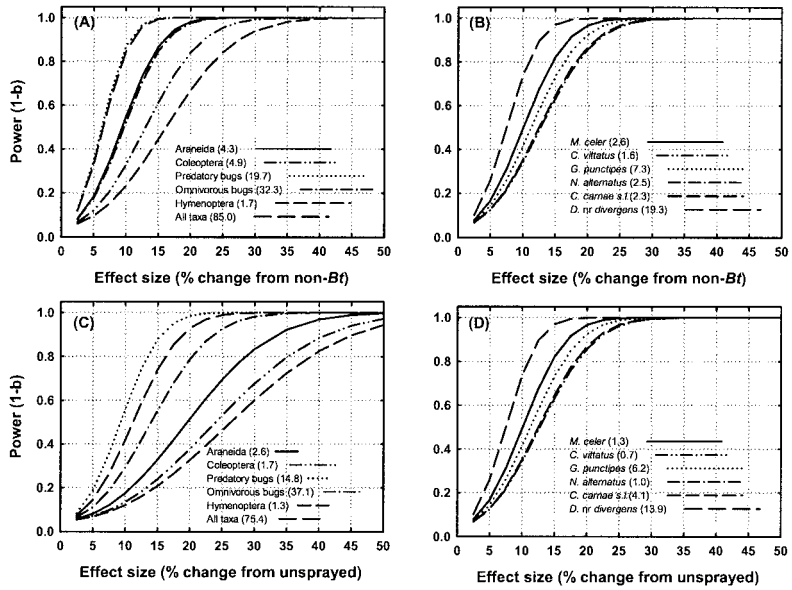


Fig. 3. Statistical power as a function of effect size (percent change in non-*Bt* or unsprayed cotton) for arthropod groups (A and C) and selected individual predator taxa (B and D). Results based on analyses of a combined 5-yr data set with block and year entered as random effects. The numbers in parentheses after the taxon designation represents the mean density (per 50 sweeps) over 5 yr.

taxa examined and many of the taxonomic groups (Fig. 3).

A final analysis examined the effect of years and replicate blocks per year on statistical power for three representative species. Again, patterns were similar for all the species examined and so only the results for *G. punctipes* will be shown (Fig. 4). Based on the underlying experimental design used here ($n = 4$), a moderate effect size (≈50%) was discernable with 80% power in any average single year of study. Detecting more subtle effects would require more years of study at this level of replication. Three years, on average, was suitable for detecting effects as small as 20% with 80% power for this species. Increasing replication to six or eight replicate blocks per year rapidly shifted the power curve to the left. However, even with eight replicates per year, a single average year was insufficient to detect a change of 20%. Power curves were shifted to the left for *D.* nr. *divergens* (high average density) and to the right for *C. vittatus* (low average density) reflecting lower relative variability for species occurring at higher densities. Regardless of the number of replicates per year, the advantage of additional years appeared to decline rapidly after 3 yr for the three species examined.

Summary of Effects on Natural Enemies. Averaged over all individual taxa in each year between 1999 and 2003, and irrespective of statistical significance, the proportional change in density in unsprayed *Bt* relative to non-*Bt* cotton varied from −18.3% in 1999 to +13.0% in 2000 (Table 6). Averaged over all taxa and years, the reduction in natural enemy density in *Bt* cotton was 5.8%. Very few taxa were significantly affected in *Bt* cotton in any year; however, for those that were, the effect ranged from −36.4 to −32.3%. Over all years and taxa, significant population reductions in unsprayed *Bt* compared with non-*Bt* cotton averaged 19.1%. Irrespective of statistical significance, insecticide effects averaged −33.0% in sprayed cotton compared with unsprayed cotton over 2 yr and all taxa. A total of 13 individual taxa were significantly reduced in sprayed cotton by an average of 47.7% over a 2-yr period (Table 6). Again, the lack of any consistent pattern in proportional reductions across years supports the absence of chronic effects caused by *Bt* toxins.

Key Pest Abundance

The primary target of *Bt* cotton in Arizona, *P. gossypiella*, was significantly reduced in *Bt* cotton in all years ($F > 7.90$; df = 1,>3; $P < 0.028$), with the exception of 2001, where the insect was not found in either *Bt* or non-*Bt* cotton (Table 7). Insecticide applications had no additional affect on this pest. Densities of other target lepidopteran pests, primarily *Spodoptera exigua* (Hübner)and *Trichoplusia ni* (Hübner), were significantly reduced in *Bt* cotton in 3 of 5 yr ($F > 14.1$; df = 1,>19.6; $P < 0.0013$) and significantly reduced by insecticide applications in 1 of 2 yr ($F = 15.4$; df = 1,29.8; $P < 0.0005$). *L. hesperus* was unaffected by *Bt* cotton in any year but was reduced by insecticide applications in both years that applications were made ($F > 26.3$; df = 1,>8.9; $P < 0.0003$). Finally, *B. tabaci* was unaffected by *Bt* cotton in all years, but insecticide applications in 2001 and 2002 significantly reduced densities of both nymphs

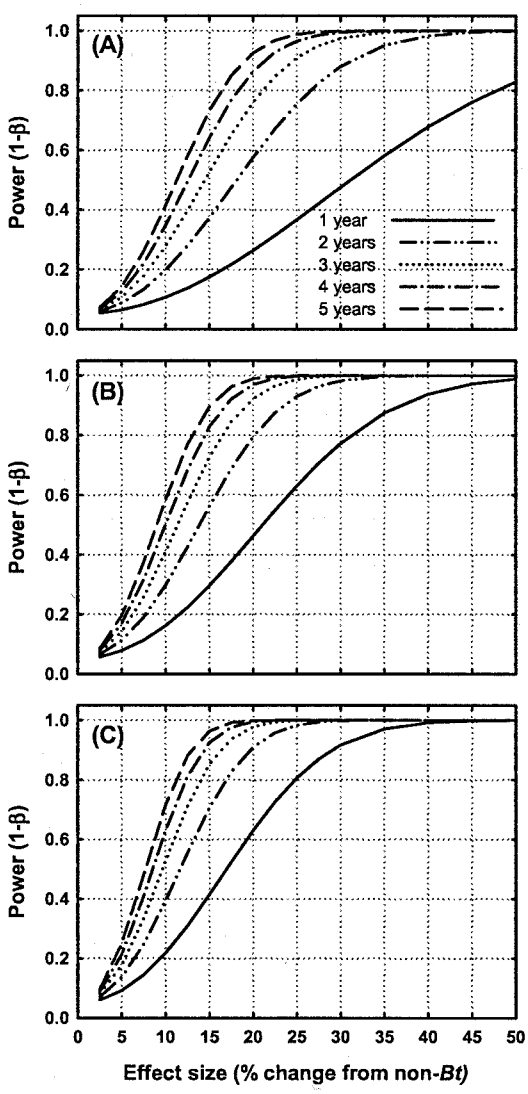


Fig. 4. Statistical power as a function of effect size (percent change in non-*Bt* cotton) for multi-year analyses of *G. punctipes*. (A) Four replicate blocks per year. (B) Six replicate blocks per year. (C) Eight replicate blocks per year. Results for 1 yr based on the average SD over all 5 individual yr; results for 2, 3, and 4 yr based on the average SD over all possible combinations of 2, 3, and 4 yr, respectively.

($F > 6.95$; df = 1,>9; $P < 0.027$) and adults ($F > 6.05$; df = 1,>11.8; $P < 0.012$).

Discussion

A long-term assessment of the impact of *Bt* cotton producing the Cry1Ac δ-endotoxin over multiple generations of 22 representative arthropod natural enemies taxa failed to show any evidence of chronic long-term effects in a defined cotton production area. Minor reductions in population densities of five natural enemy taxa in *Bt* cotton were detected in multi-year analyses, but a companion study measured similar levels of natural control by the natural enemy community in *Bt* and non-*Bt* cotton (Naranjo 2005), suggesting that these reductions may not be ecologically meaningful. In comparison, conventional alternatives to the use of *Bt* cotton, as represented by the positive controls in this study, were many times more damaging to the natural enemy community, causing much larger reductions in density and affecting a broader range of taxa. These latter findings are consistent with recent commercial-scale, nontarget studies contrasting *Bt* cotton to conventional non-*Bt* cotton production systems (Head et al. 2005, Torres and Ruberson 2005, Whitehouse et al. 2005). Such disruptions by broad-spectrum insecticides repeatedly have been shown to compromise the natural biological control of cotton pests (e.g., Leigh et al. 1966, Eveleens et al. 1973, Stoltz and Stern 1978).

Table 6. Summary of the effects of *Bt* cotton and insecticide sprays relative to non-*Bt* and unsprayed cotton, respectively, on seasonal abundance of arthropod natural enemies, Maricopa, AZ, 1999–2003

	Prop. Δ[a] from non-*Bt*		Prop. Δ from unsprayed	
	All taxa	Statistically significant taxa[b]	All taxa	Statistically significant taxa[b]
1999	−0.183	−0.364 (1)	—	—
2000	0.130	(0)	—	—
2001	0.068	(0)	−0.305	−0.578 (4)
2002	−0.152	−0.378 (2)	−0.393	−0.551 (9)
2003	−0.038	−0.323 (1)	—	—
All years	−0.058	−0.191 (5)	−0.330	−0.477 (13)

[a] Prop. Δ is the proportional change in density in *Bt* cotton relative to non-*Bt* cotton or sprayed cotton relative to unsprayed cotton.
[b] Taxa for which the repeated-measures ANOVA P value was < 0.05. Values in parentheses indicated the no. of taxa affected.

Univariate analyses of individual taxa indicated a total of six statistically significant differences between *Bt* and non-*Bt* cottons over 6 yr of study and 127 separate mean comparisons. This proportion (0.047) is nearly equal to the type I error rate of 0.05 used to define statistical significance of these contrasts, and so these differences may not be statistically meaningful. This interpretation is further supported by multivariate analyses of the entire arthropod natural enemy community in individual years using multivariate PRC. Although separate analyses each year generally indicated no negative effects, combined analyses across the 5 yr that used similar plot sizes revealed a significant average decline of ≈19% in five predator taxa representing four orders, including *H. convergens*, *G. punctipes*, *N. alternatus*, *D.* nr *divergens*, and a group of miscellaneous spiders. Combining the data sets simply allowed for a larger sample size and correspondingly greater statistical power to discern smaller changes in density. Over all taxa examined, there was a slight numerical trend toward lower population densities in *Bt* relative to non-*Bt* cotton averaging ≈6%. A declining trend also was found for natural enemy density (as a group) and family-level richness by Sisterson et al. (2004) in Arizona. Similarly, Men et al. (2003) showed

Table 7. Seasonal mean densities of selected pest insects in *Bt* and non-*Bt* and sprayed and unsprayed cotton, Maricopa, AZ, 1999–2003

Year	Comparison	*Pectinophora gossypiella* larvae (per 100 bolls)	Lepidoptera larvae (50 sweeps)	*Bemisia tabaci* nymphs (per leaf disk)	*Bemisia tabaci* adults (per leaf)
1999	Non-*Bt*	35.0 ± 13.9*	0.78 ± 0.15	34.1 ± 6.34	22.1 ± 4.84
	Bt	0.00	1.06 ± 0.15	33.2 ± 6.52	18.5 ± 4.49
2000	Non-*Bt*	50.2 ± 8.04**	0.45 ± 0.05**	6.26 ± 1.63	8.31 ± 2.58
	Bt	0.00	0.09 ± 0.03	4.93 ± 0.76	4.75 ± 0.63
2001	Non-*Bt*	0.00	1.04 ± 0.11**	6.38 ± 1.32	10.2 ± 2.31
	Bt	0.00	0.54 ± 0.15	5.79 ± 0.60	8.27 ± 1.51
	Unsprayed	0.00	1.41 ± 0.23	6.83 ± 1.20*	9.25 ± 1.26*
	Sprayed	0.04 ± 0.04	0.98 ± 0.26	3.31 ± 0.53	5.07 ± 1.05
2002	Non-*Bt*	1.86 ± 0.46**	0.93 ± 0.15**	31.9 ± 5.61	36.9 ± 3.16
	Bt	0.12 ± 0.12	0.17 ± 0.04	34.8 ± 10.6	38.01 ± 5.64
	Unsprayed	1.00 ± 0.40	0.82 ± 0.25**	33.3 ± 7.25**	37.4 ± 5.59**
	Sprayed	1.06 ± 0.60	0.22 ± 0.06	6.29 ± 0.56	6.22 ± 0.67
2003	Non-*Bt*	5.50 ± 1.60*	0.51 ± 0.13	6.29 ± 0.67	6.36 ± 0.82
	Bt	0.00	0.25 ± 0.06	6.31 ± 0.67	5.57 ± 0.48

Values are seasonal means (±SE) based on 1–16 sample dates depending on year and pest taxon with four replicate plots each year.

Significant differences based on repeated-measures ANOVA using Proc Mixed (Littell et al. 1996) between non-*Bt* and *Bt* and between unsprayed and sprayed cottons; * $P < 0.05$ and ** $P < 0.01$. In cases where densities were zero for *P. gossypiella* larvae, a *t*-test on the LS means was used to determine if the nonzero mean was significantly more than zero.

a slight numerical decline in species richness and diversity (significant in 1 of 3 yr) of the natural enemy community in unsprayed *Bt* compared with unsprayed non-*Bt* cotton in China.

The causes of declines in densities of predators in this study are uncertain but could be associated with sampling error, declines in target or nontarget prey abundance, or sublethal effects resulting from exposure to *Bt* toxins. Sampling error seems an unlikely explanation because populations were consistently lower in *Bt* cotton for the five taxa in the majority of years. There also was no obvious difference in the canopy structure between *Bt* and non-*Bt* cotton that could have affected sampling efficiency. A decline in target or nontarget prey is a likely mechanism. Although most larval stages of the target, *P. gossypiella*, are relatively invulnerable to natural enemies because they feed on seeds inside the cotton boll (Henneberry and Naranjo 1998), eggs are laid externally on vegetative structures up until early July, and eggs and neonates are vulnerable to predation and parasitism during this period. Eggs are hidden beneath the calyx of the cotton boll from July onward where they and eclosing neonates are somewhat protected; however, smaller predators such as *Orius tristicolor* (White) may attack eggs and neonates in these areas. The insect pupates in crevices in the soil surface, where they may be vulnerable to predation and parasitism. Little is known about natural enemy induced mortality of the nocturnal adult stage. Other potential lepidopteran prey occurred at low densities, but were less abundant on *Bt* cotton in most years (see Table 7). Thus, reductions in target prey could have influenced densities of affected predators. Both Daly and Buntin (2005) and Whitehouse et al. (2005) observed significant declines in populations of *Nabis* spp. in *Bt* corn and cotton, respectively, further suggesting that reductions in lepidopteran prey in *Bt* crops may play a role in the dynamics of this predator. In contrast, *B. tabaci* was the most abundant prey, but densities of immature and adult stages were similar in both *Bt* and non-*Bt* cotton. Direct feeding on the plant by *G. punctipes* and *N. alternatus* could expose these predators to *Bt* toxins. However, Armer et al. (2000) found no negative effects for *Geocoris* and *Nabis* spp. feeding directly on *Bt* potato foliage, and plant-feeding on *Bt* cotton leaves containing Cry1Ac had no effect on longevity of immature or adult stages of *G. punctipes* or *O. tristicolor* (unpublished data). Ponsard et al. (2002) observed modest declines (≈27%) in longevity of adult *G. punctipes* and *O. tristicolor* (but not *Nabis* spp. or *Zelus renardii* Kolenati) feeding strictly on *Bt*-intoxicated *S. exigua* in the laboratory. It is unlikely that these predators would feed exclusively on such caterpillars in the field, and there were no effects of *Bt* cotton on *O. tristicolor* here. In addition, follow-up studies with *G. punctipes* feeding strictly on *Bt*-intoxicated *S. exigua* or on intoxicated *S. exigua* plus *P. gossypiella* eggs suggest that *S. exigua* larvae are a poor quality prey (unpublished data) and may partially explain the results of Ponsard et al. (2002). Pollen and nectar 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. Studies with other coccinellids and *O. insidiosus* feeding on *Bt* corn pollen showed no negative effects (Pilcher et al. 1997, Al-Deeb et al. 2001, Lundgren and Wiedenmann 2002). Adults of *D.* nr. *divergens* largely specialize on adult *B. tabaci* (Hagler 2002), which are phloem feeders and unlikely to possess *Bt* toxins in their bodies. However, the larval stages of this fly are subterranean, and it is possible that subtle changes in the soil-dwelling fauna on which they prey affected adult abundance in *Bt* cotton. While reductions in target prey density may explain reductions in most of the five predators affected here, further controlled toxin exposure studies may be warranted to fully characterize potential sublethal effects.

The need for long-term field studies to examine potential ecological impacts of transgenic crops has been advocated repeatedly as a critical component of the postcommercialization testing process (National Research Council 2002, FIFRA Scientific Advisory Panel 2002, 2004). These reports further emphasize the general lack of guidelines for conducting such studies. Among the important issues identified were plot sizes, replication, sampling methods, sampling intensity, appropriate positive and negative controls, taxonomic coverage, and clearly specified endpoints. The results of this study may provide at least some initial guidance on several of these issues.

Plot size can be an important factor in evaluating population level toxicological effects with optimum plot size largely driven by the mobility, phenology, and ecological requirements of the species under consideration (Jepson and Thacker 1990, Sherratt and Jepson 1993, Prasifka et al. 2005). While a spatial scale representative of commercial production may be desirable for such evaluations, it may not always be feasible or economically viable. Studies in 1996 were conducted in plots ≈1.2–2.0 ha in size. While these would not be considered to be on a scale commensurate with commercial fields of cotton in Arizona, they are at least an order of magnitude greater than those used in 1999–2003 (0.12–0.17 ha), which were of a scale similar to that used in many of the nontarget studies cited herein. Despite the large difference in plot size between 1996 and 1999–2003, the magnitude of changes in density of the 22 arthropod taxa observed were similar between *Bt* and non-*Bt* cotton (see Tables 2 and 3). In addition, the large plots in 1996 were more variable as evidenced by the larger number of replicates that would have been required to discern a similar change in population density with equal power compared with the smaller plots used in 1999–2003. Larger plots are typically more heterogeneous and require a greater sampling effort for more precise estimation of abundance (Jepson et al. 1994). Additional evidence of the suitability of smaller plots was provided by the positive control treatments where large differences between arthropod densities were observed between sprayed and unsprayed plots over extended portions of the season. Similar resolution was shown in even smaller plots used to test alternative insecticide regimens for *B. tabaci* (Naranjo et al. 2004). Thus, even though there may be relatively rapid recolonization of plots smaller than 0.5 ha by some species (Prasifka et al. 2005), it may be possible to conduct meaningful studies of nontarget effects of at least some species in the relatively small plots used here.

Sampling method is another important consideration in field tests. Whole plant samples provide absolute density but are extremely costly in terms of both labor and time. Sweep nets are a common relative sampling tool in field crop and have the advantages of being easy to use, fast, and providing coverage of a large area of the crop canopy. Results here indicate that similar patterns of change between *Bt* and non-*Bt* cotton were measured with both methods but that whole plant samples were much more variable and detected fewer taxa, especially those that occurred at very low densities. While sweep net samples may provide only relative density estimates, they are suitable for comparative studies like those undertaken here.

The particular taxa to evaluate in nontarget studies is another important factor. Jepson et al. (1994) and Andow and Hilbeck (2004) suggested that the nontarget taxa examined should consistently occupy the crop studied and have the potential for exposure to *Bt* toxins, either directly or indirectly through trophic interactions. Many natural enemies common to crop habitats fulfill these requirements, and because of their interactions with target and nontarget herbivores in the system may be among the more sensitive indicators of potential risk (Cannon 2000). Their potential value in biological control also makes them desirable taxa for study. The pooling of individual species into broader taxonomic groups also should be carefully considered. Many nontarget studies have pooled species at the order or family level. Results here indicate that such pooling may obscure effects on individual species. For example, none of the broader taxonomic groups analyzed here (e.g., Araneida, Coleoptera, Heteroptera) reflected the significant changes observed for the five individual taxa that were found to have declined in *Bt* cotton (see Table 5).

A final issue involves the interrelated factors of sample size, statistical error, and the level of change that is desirable to detect in nontarget studies. This issue was partly addressed here through the estimation of statistical power for underlying experimental designs in yearly and multi-year analyses. In general, the statistical power of repeated-measures ANOVA with four replicates to detect even modest (50%) changes in arthropod density in *Bt* cotton here was relatively poor. On average ≈1.7 times more replicates would have been needed to detect this level of change in a single year for the "average" taxon. Clearly, an even greater sample size would be necessary to detect the smaller changes that were observed in all years. Using three representative species, it was further shown that the relationship between power and sample size can be highly variable over the individual years of a study and that within a repeated-measures design, increasing the number of sample dates does relatively little to improve power (see Fig. 1). These results may be useful in designing future short-term studies, but the power to detect smaller changes was improved greatly by combining annual data sets. A 5-yr combined analysis allowed small changes in population density (<20%) to be detected with >80% power for a set of representative species and broader taxonomic groups. Based on variability of the underlying experimental design, there was a trade-off in power between increased replication within a single year and repetition of the experiment over multiple years. Because den-

sities of natural enemy populations changed over years, average variation increased when additional years of study were pooled. This suggests that increased replication in any single year may be a more efficient way to increase power (Fig. 4). However, there also may be large variability in power for a given effect size from year to year (Fig. 1), and there will clearly be environmental and other factors that affect arthropod populations from year to year independent of experimental treatment effects. Thus, it would be prudent to use power analyses to suggest reasonable samples sizes each year and to gain not only additional power but also more robust assessment of community responses through repetition over years. Regardless of sample size per year, results here suggest that only marginal gains in power are afforded with a study duration >3 yr. Finally, it should be noted that this study involved repeated experiments at the same general field site but plots did not occupy the same ground each year, which may have contributed additional year to year variation. Yearly repetition using the same randomization on the same ground might reduce variation and thus sample size requirements. The colonization ability of nontarget taxa under consideration and the proximity of their source populations to experimental plots will ultimately determine how much additional variation, if any, is added by using new plots each year. Similarly, combining studies from multiple sites (and research groups) would likely increase sample size requirements but may nonetheless represent an efficient means of increasing power while at the same time improving the assessment of nontarget community responses.

Results here may help guide future studies, but they cannot address the question of how small or large an effect is needed to trigger concern. This will depend on the system under study, ecological factors, and societal decisions based on the level of change that is acceptable (Underwood 1997, Marvier 2002). Naranjo (2005) suggested that an average change of ≈20% in a small number of species may not be ecologically meaningful in terms of the biological control potential of the natural enemy community. Ultimately, the assessment of environmental impacts such as nontarget effects need to weight the importance of both type I and type II error rates as well as biologically relevant effect sizes to provide meaningful guidelines for experimental design (Di Stefano 2003).

In summary, the results of a multi-year year study indicate that the effects of transgenic *Bt* cotton on a select, but representative, natural enemy community seem to be relatively minor, especially in comparison with the alternative use of broader-spectrum insecticides, and are likely explained by changes in prey density in most cases. There were no indications of any long-term effects of exposure to *Bt* toxins in the population dynamics of the natural enemy community within a defined area. Overall, this selective pest control technology should broaden opportunities for natural enemy conservation in cotton.

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