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LADY BEETLE SPECIES SHIFT IN BT AND NON-BT COTTON FIELDS

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Abstract

Season-long, grower-scale field experiments are considered the ideals for evaluating the ecological impact of transgenic plants on natural enemies. It is important to consider the extended community of arthropods throughout the season in a sampling area representative of actual usage. Results from field experiments, however, are difficult to interpret due to often-confounding biotic and abiotic influences. Surveys of paired, adjacent Bt and non-Bt cotton fields near Tifton, GA, from 2002 to 2004 showed that most canopy-dwelling predators were similarly abundant in both Bt and non-Bt cotton. This was, however, not the case for the lady beetle *Hippodamia convergens* Guerin-Meneville. This beetle was more abundant in non-Bt cotton and became more numerous after pyrethroid applications to control heliothine infestations in non-Bt fields. In contrast, populations of the lady beetle *Harmonia axyridis* Pallas decreased in the same fields. In the laboratory, the susceptibility of these two species to the pyrethroid insecticide used in the fields (lambda-cyhalothrin) was tested. *H. convergens* was found to be highly tolerant to recommended dosages of the insecticide lambda-cyhalothrin, while *Ha. axyridis* was highly susceptible. The increase of *H. convergens* populations in sprayed non-Bt cotton appears to be related to pyrethroid tolerance and subsequent escape from competition with *Ha. axyridis*, which is highly susceptible to the pyrethroid. These results are the first to suggest that lady beetle interspecific competition in the field is affected by insecticide use. Therefore, differences in abundance of predator species in Bt and non-Bt cotton fields can be related to indirect effects related to management decisions rather than the technology *per se*, and such effects should be considered in interpreting data regarding risk assessment.

Introduction

Bt-transgenic cotton has been widely planted in the US for nearly a decade. Prior to and during this same period, effects of Bt-cotton on natural enemies have been extensively evaluated. A large body of literature has resulted from these studies, with essentially two conclusions: Bt toxins are unlikely to exert direct adverse effects on natural enemies, but indirect effects may result. Considering the potential risks imposed by indirect interactions in Bt crop systems on nontarget organisms (arthropod natural enemies, pollinators and other non-pests), emerging risk assessment guidelines strongly recommend long-term, large-scale field experiments to allow the studied organisms to be exposed to the toxins for multiple generations and under natural conditions.

Conservation of natural enemies in cotton has become more important and feasible following boll weevil eradication, adoption of Bt-cotton, and increased availability of selective insecticides. In this context, it is critical to understand any adverse effects of Bt-cotton on natural enemy populations and function. When the interactions of natural enemies and Bt-cotton have been studied in experimental fields, the taxon-specific results range from greater abundance to equal abundance to reduced abundance in Bt relative to non-Bt cotton (Fitt et al., 1994; Luttrell et al. 1995; Flint et al., 1995; Hagerty et al., 2000; Armstrong et al., 2000; Men et al., 2003; Sisterson et al., 2004). The clear trend is that always one or more species varies between cotton types, although usually not consistently, as most experiments are not sufficiently long or spatially-diffuse to account for spatial or environmental variability. Therefore, in this study we surveyed predatory arthropod in cotton fields throughout each of three successive seasons, covering multiple insect generations, in grower fields representative of production systems in the region. The results help to understand the importance of Bt-cotton in relation to insecticide use for conserving natural enemies, and underscores potential pitfalls in interpreting risk-assessment results from field studies.

Material and Methods

Site description

This study was conducted over 3 cotton seasons (2002-2004) in three pairs of commercial Bt and non-Bt cotton fields near Tifton, GA, using grower fields located between coordinates 31^{o} 45'N, 83^{o} 63'W - 31^{o} 51'N, 83^{o} 55'W, and 3 to 17 km distant from each other. The fields ranged in size from 10 to 32 acres and were variously in a peanut, tobacco, and watermelon two-year rotation. The sampled area in each location was a delineated portion of a total area ranging from 25 to 80 acres cultivated with cotton or with peanut, tobacco or watermelon, and with the fields separated from one another by water ditches or field roads. In the first season, the fields were planted with Bt-cotton variety DPL 458RR and non-Bt cotton DPL 491. In 2003 and 2004 all fields were cultivated with the Bt-cotton variety DPL 555RR and non-Bt cotton variety DPL 493. The Bt-cotton variety DPL 458RR was the second most planted Bt-cotton variety in 2002 and, DPL 555RR was the most planted variety in GA in 2003 and 2004 (NASS, 2004).

The fields were managed using standard grower practices. Insecticides were applied, based on scouting data and using economic thresholds for pest infestation in both Bt and non-Bt fields for Georgia (Georgia Pest Management Handbook, 2002-2004). All fields received preventive in-furrow treatments (of aldicarb; see Table 1) to manage thrips. In practice, the non-Bt fields received insecticide applications to manage heliothine larvae and both Bt and non-Bt fields were treated to manage stinkbugs and whitefly infestations.

Table 1. Timing of insecticide applications to manage pest infestations (Th, thrips; He, bollworms; Stb, stinkbugs; Wh, whiteflies) in the experimental fields near Tifton, GA, 2002-2004.

Dates (Fields)[1]	**Non-Bt cotton**	**Bt-cotton**	**Pest**[4]
2002			
Planting (C,M,T)	aldicarb 20G (560)[2]	aldicarb 20G (560)	Th
8-9 Jul (C,M,T)	spinosad (100)	-	He
10-12 Aug (C,M)	λ-cyhalothrin (34) + thiodicarb (680)	-	He
14 Aug (T)	-	dicrotophos (390)	Stb
5-7 Sep (C, M)[3]	pyriproxifen (60)	pyriproxifen (60)	Wh
2003			
Planting (C,M,T)	aldicarb 20G (560)	aldicarb 20G (560)	Th
8 Jul (O)	spinosad (100)	-	He
13 Jul (M)	spinosad (100)	-	He
14 Jul (C)	λ-cyhalothrin (30)	-	He
21 Jul (C)	λ-cyhalothrin (45)	-	He
3-5 Aug (M,O)	λ-cyhalothrin (45)	-	He+Stb
30 Aug (O)[3]	bifenthrin (70)	bifenthrin (70)	Stb
2004			
Planting (C,M,F)	aldicarb 20G (560)	aldicarb 20G (560)	Th
2-7 Jul (C,M,F)	λ-cyhalothrin (45)	-	He
15 Jul (C, M)	spinosad (100)	-	
29 Jul (C)	-	dicrotophos (420)	Stb
5 Aug (C)	ζ-cypermethrin (160)	-	He+Stb
17 Aug (M, F)	ζ-cypermethrin (210)	ζ-cypermethrin (210)	He+Stb
31 Aug (M)[3]	-	acephate (810)	Stb

[1]C = Chula; M = Marchant; T = Ty Ty; O = Old House; and F = Frazier fields. [2]Rate in grams of active ingredient per hectare. [3]Insecticide application after terminating experimental sampling. [4]Th (*Frankliniella occidentalis*, *Frankliniella fusca* and *Thrips tabaci*), He (*Helicoverpa zea* and *Heliothis virescens*), Stb (*Nezara viridula* and *Euschistus servus*). Economic thresholds: He = 8-10% of plants with small larva in terminals, squares, or fruit; and Stb = 18-20% bolls of ~2.5 cm diameter with internal damage; whiteflies = plants infested and honeydew on plants (Georgia Pest Management Handbook, 2004).

Insect sampling

Predatory insects and spiders were sampled with a standard 100-cm long white cloth. The cloth was spread on the ground between two rows of cotton. The plants on both rows adjacent to the cloth were vigorously shaken over the cloth. Predators dropping on the cloth were immediately identified, sorted by stages (i.e., immatures and adults), and counted. This sorting procedure was based on previous scouting records for Georgia cotton and on practical identification provided in Knutson and Ruberson (1996). A total of 40 samples was taken along a transect from border to border in each of the Bt and non-Bt cotton fields on each sampling date (12 weeks per season from mid June to late August/early September).

Toxicity test for lady beetles

After detecting changes in the relative abundance of the lady beetles *Hippodamia convergens* and *Harmonia axyridis* (increase and decrease in non-Bt fields, respectively), adults of both species were collected from studied fields and maintained in the laboratory on *Sitotroga cerealella* eggs. After one week in the laboratory, adult susceptibility to lambda-cyhalothrin was assayed using dry insecticide residue on cotton leaves. Cotton leaves from plants growth in greenhouse were treated with lambda-cyhalothrin (Karate-Z 2.08, Syngenta) at four concentrations [0, 0.0125, 0.025 and 0.0406 lbs (AI) /acre] using a Potter spray tower (20 psi, 0.5 ml material applied per leaf, mixed in a concentration equivalent to 8 gallons per acre). One hour later, 30 adults of each species per concentration were confined on treated leaves at rate of 3 individuals per ventilated cage (10 replicates for each treatment) and maintained in the ventilation tower described in Ruberson & Fairbanks (2003). Mortality was evaluated at 24h intervals until 72h post-introduction.

Statistical analysis

The predators collected in drop cloth sampling and sorted by species, genus or family were summed up into 40 samples per field on each sampling date. All count data were square root (x+0.5) transformed prior to univariate analyses, but untransformed data are presented. The results were submitted to one-way or two-factor repeated measures analysis of variance (ANOVA) with repeated measures on sampling dates within season and sampling date and years for two factors, respectively, with field as a blocking factor since the predator samplings were carried out on the same fields over the season (SAS Institute 2001). In addition, orthogonal contrasts were run to test the null hypothesis that on each sampling date over the season the mean abundance for each taxon did not differ significantly between Bt and non-Bt cottons.

The changes in predatory community dynamics in Bt-cotton compared to non-Bt cotton as the control system throughout the season were investigated using principal response curve (PRC) analysis. Briefly, the PRC models the treatment community response pattern ($T_{dtk} = b_k\, c_{dt}$) for each species as a multiple of abundances weight (b_k) and the canonical coefficients (c_{dt}) of the partial redundancy analyses (RDA). The species weight (b_k) represents the regression coefficients for each species over sampling time and the canonical coefficient (c_{dt}) the regression coefficient for whole community over sampling time. These parameters were generated using the software CANOCO 4.5 for Windows (Lepš & Šmilauer 2003). For each set of analyses, the null hypothesis that the PRC does not explain significant treatment variance was tested using an F-type test obtained by permutating whole time series in the partial RDA from which that PRC was obtained. Random permutation using the Monte-Carlo method (999 permutations) was also performed for significant PRC to test the null hypothesis that on each sampling date, the principal response c_{dt} did not differ significantly between cotton types.

Results and Discussion

Abundance of predators on Bt and non-Bt cotton

A total of 35,342 and 35,128 predators (insect and spiders; excluding fire ants) were collected in Bt and non-Bt cotton fields over three seasons 2002-2004, sampled during 12 weeks within each season. Fire ants alone accounted for numbers comparable to that of all other predatory insects and spiders pooled (Bt: 34,253 ants; Non-Bt: 30,682 ants). The species composition was typical for predator communities in cotton ecosystem in the region (Knutson & Ruberson, 1996), with only two additional taxa. The predatory big-eyed bug, *Geocoris floridanus* Blatchley and predatory stink bug *Stiretrus anchorago* (Fab.) were collected in 2003 and 2004. Repeated measures ANOVA within each season revealed differences in abundance of 2 out of 19, 1 out 20, and 3 out of 21 predatory taxa in the 2002, 2003, and 2004 seasons, respectively. Orthogonal contrasts between Bt and non-Bt cotton for significant taxa demonstrated that *Hippodamia convergens* and *Scymnus* spp. were more abundant in non-Bt and Bt-cotton, respectively, for 2 weeks in 2002. In 2003, *Coleomegilla maculata* was more abundant for a 3-week period in Bt-cotton; and in 2004 *C. maculata* and *Harmonia axyridis* were more abundant in Bt-cotton for 2 weeks, while *H. convergens* was more abundant in non-Bt cotton for 4 successive weeks. Among all 21 taxa recorded throughout the study, few taxa were significantly more or less abundant in Bt or non-Bt cotton within seasons, and when data for all three seasons for the 18 taxa that occurred all seasons were considered, only *H. convergens* was consistently more abundant in non-Bt than in Bt-cotton during three successive weeks (Table 2, Fig. 1).

Table 2. Seasonal mean of predators collected in 40-drop cloth samples per sample date in cotton fields from 2002 to 2004 near Tifton, GA (pooled for all 3 years).

Predators	**Bt-cotton**	**Non-Bt cotton**	**F**[a]
Chrysoperla rufilabris larvae	3.9 ± 0.48	4.3 ± 0.55	0.33
Coccinella septempunctata	10.1 ± 1.88	14.6 ± 2.98	1.13
Coleomegilla maculata	4.1 ± 0.62	2.3 ± 0.36	3.62
Diomus spp.	6.5 ± 1.05	7.9 ± 1.18	2.27
Geocoris punctipes	36.4 ± 3.86	44.1 ± 4.41	0.79
Geocoris uliginosus	2.5 ± 0.38	3.1 ± 0.49	0.68
Harmonia axyridis	26.1 ± 3.63	22.6 ± 3.38	1.25
Hippodamia convergens	4.5 ± 0.56	14.7 ± 2.31	6.33**
Micromus sp. larvae	8.3 ± 0.99	10.5 ± 2.17	0.03
Nabis spp.	12.4 ± 1.44	9.7 ± 1.13	2.08
Notoxus monodon	8.1 ± 1.18	6.8 ± 1.06	0.27
Orius insidiosus	34.7 ± 4.27	38.7 ± 5.02	0.18
Podisus maculiventris	1.9 ± 0.31	2.7 ± 0.38	1.55
Scymnus spp.	57.7 ± 5.91	50.3 ± 4.70	0.38
Solenopsis invicta	317.1 ± 22.65	284.1 ± 21.12	0.33
Sinea spp. + *Zelus* spp.	2.2 ± 0.29	2.0 ± 0.24	0.01
Spiders[c]	103.7 ± 8.37	87.1 ± 6.57	4.86
Syrphid fly larva	2.4 ± 0.52	2.5 ± 0.57	0.00

[a]ANOVA results (F-test) from repeated-measures procedure of SAS. Asterisks indicate significant difference between *Bt* and non-*Bt* cotton (p<0.01).

Principal response curve (PRC) diagrams show consistent variability in canopy predator communities throughout seasons, except for 2002 (F=3.42, p=0.604) (Fig. 2). Of the total variance in the species abundance in the seasons 2003 and 2004, 63.7 and 63.9% are explained by sampling date, and 9.2 and 10.1% are explained by the treatment (Bt-cotton), respectively. The PRC results were highly significant in 2003 (F=7.29, p=0.001) and in 2004 (F=8.64, p=0.002), and indicated that 52.1% and 56.8% of the variation in the community construct was due to sampling date and Bt-cotton interactions (Fig. 2). The Monte Carlo permutation test detected significantly higher species abundance in Bt-cotton in the last week of July, and in the first and last week of August of 2003, and lower abundance in the two last weeks of July and in the first week of August in 2004 (Figs. 1 and 2).

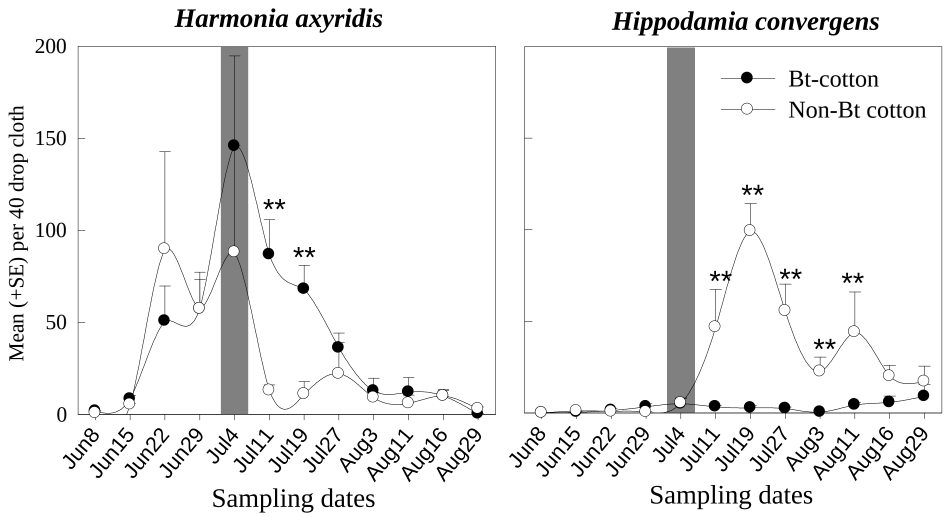

Fig. 1. Population dynamics of larvae+adults of two lady beetle species during 2004 in Bt and non-Bt cotton fields, near Tifton, GA. Gray bars indicate time-spray of lambda-cyhalothrin on non-Bt cotton fields (table 1).

Species with high weight values are most likely to follow the deviations from abundance in the non-Bt cotton, as shown in the PRC diagram, while low values (>-.05 or <0.5) do not contribute strongly to the overall community response, and indicate a weak association or a response pattern that differs from that displayed in the diagram (Van den Brink & Ter Braak, 1999). Thus, only taxa that made significant relative contributions to the outcome are shown on the right side of the diagram. In 2003, the species weights based on the PRC diagram suggest that higher densities of *Solenopsis invicta*, *C. maculata*, *Nabis* spp., *H. axyridis*, *Micromus* sp., *Coccinella septempunctata* and spiders occurred in Bt-cotton (Fig. 2), whereas lower species abundance in 2004 Bt-cotton is strongly associated with *H. convergens*, *Geocoris uliginosus*, *Scymnus* sp., *Diomus* sp., *S. invicta*, *Chrysoperla rufilabris* and *Orius insidiosus* densities, which is consistent with univariate ANOVA results for those more abundant species (Table 2).

Further, consideration of species abundance on Bt-cotton relative to non-Bt cotton [exp($b_k c_{dt}$)] indicates that the density of *H. convergens* in Bt-cotton is estimated to be 0.0416 [exp(-0.9182*3.462)] times the field average in non-Bt-cotton (99.3 ± 35.8), while the density of *H. axyridis* is 3.1598 [exp(-0.9182*-1.253)=3.1598] times the field average in non-Bt cotton (11.3 ± 6.8) on 20 July 2004. Interpreting these results strictly from the statistical results (univariate ANOVA and multivariate PRC), we would reach the erroneous conclusion that populations of *H. convergens* are significantly adversely affected by Bt-cotton compared to non-Bt cotton.

<u>Toxicity of lambda-cyhalothrin to lady beetles</u>

The significant changes in lady beetle species abundance in the 2003 and 2004 relative to cotton type seems to be related to insecticide use (Table 1 and Fig. 2). In both years, differences were detected by PRC analyses on sampling dates following lambda-cyhalothrin applications. In 2002 and generally in 2003, early-season insecticide application to manage bollworm populations was done with a selective insecticide (spinosad; Tillman & Mulrooney, 2000). Exceptions occurred in 2003 following two applications of lambda-cyhalothrin applied to the non-Bt cotton at the Chula farm (C). In this field, insecticide applications suppressed populations of both pests and predators to a very low level. As the average used in our study is produced from counts of all three-field pairs, the Chula results skewed the mean toward higher species abundance in Bt-cotton fields right after insecticide application, on sampling date 20 July 2003 (Fig. 2). The opposite pattern occurred in 2004 when lambda-cyhalothrin was applied early and in all non-Bt fields, although the same pattern for higher species abundance in Bt-cotton would be expected since lambda-cyhalothrin was sprayed in all three non-Bt cotton fields in 2004 at approximately the same time.

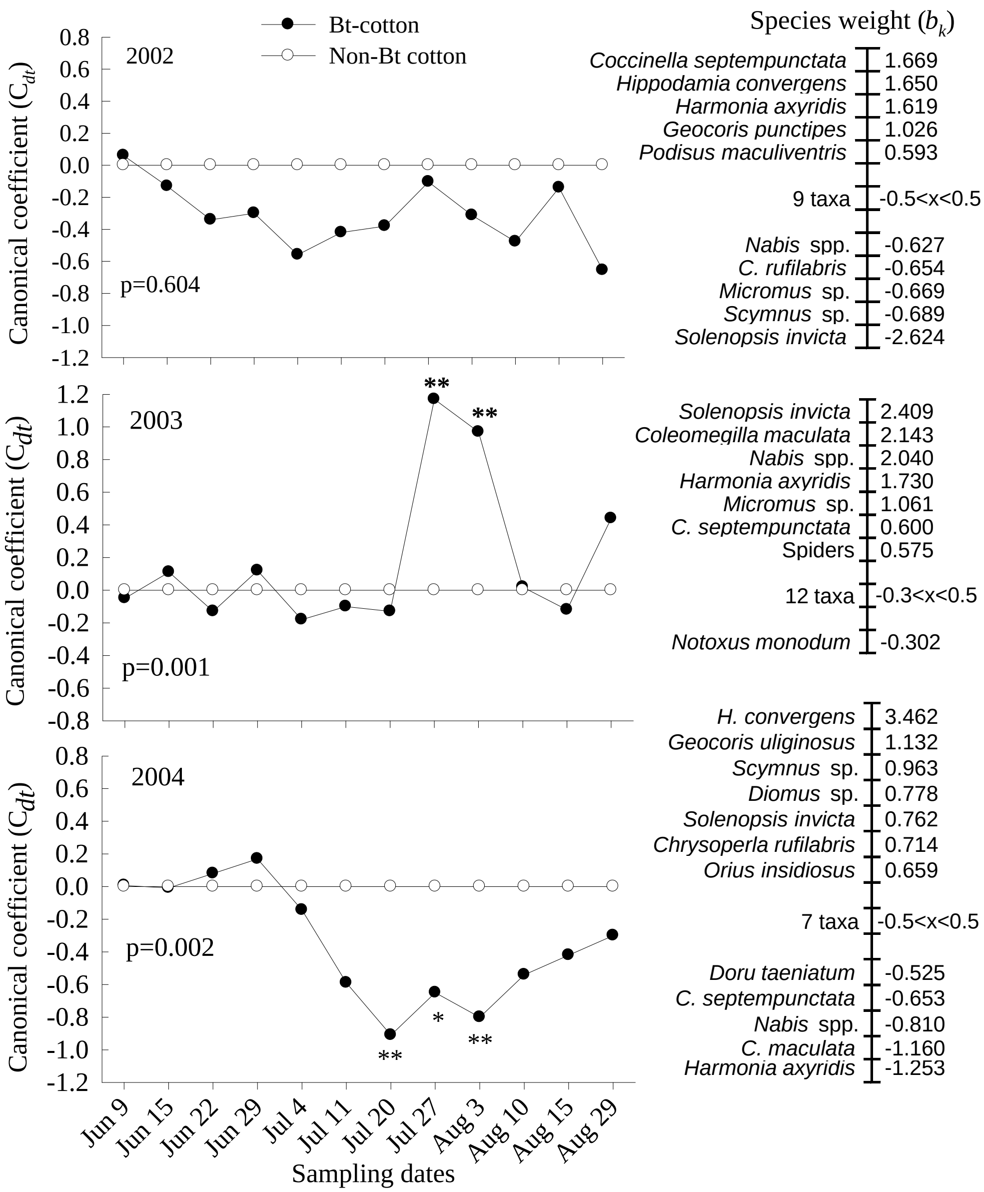

Fig. 2. Principal response curves (PRC) and species weights for predators collected in drop cloth sampling from 2002 to 2004, Tifton, GA. The PRC curves show the main effect of B*t*-cotton on predator community relative to non-Bt cotton (y = 0 line). The p-value indicates significance of the PRC diagram across all sampling dates, and the asterisks (**p<0.01 and *p<0.05) indicate the level of significance between cotton types for specified sampling date.

The greatest contributors to the pattern of the PRC diagram in 2004 were *H. convergens* and *H. axyridis.* Populations of these two lady beetle species shifted between Bt and non-Bt cotton [ca. the highest and lowest species weight (Figs. 1 and 2)], and the differences in this study are hypothesized to be a result of differential tolerance to lambda-cyhalothrin by these species and, hence, release from interspecific competition. *H. axyridis,* although capable of outcompeting *H. convergens,* is highly susceptible to lambda-cyhalothrin. In contrast, *H. convergens* is highly tolerant. *H. convergens* caged on treated and untreated leaves for 24h survived >93% and 100%, respectively, while only 10, 3.3, and 0% *H. axyridis* survived 24h on treated leaves, compared to 96.6% of adults surviving on untreated leaves (Table 3). Similar results were observed 48 and 72h later, with >86% of *H. convergens* and 0% of *H. axyridis* surviving when caged on treated leaves.

Table 3. Survival of two lady beetles caged on cotton leaves untreated and treated with lambda-cyhalothrin in three concentrations.

Treatments	**Survival (%) per time exposure (h)**		
(lbs of AI/Acre)	**24h**	**48h**	**72h**
Harmonia axyridis			
0	96.6	90.0	90.0
0.0125	10	3.3	0
0.0250	3.3	0	0
0.0406	0	0	0
Hippodamia convergens			
0	100.0	96.7	96.7
0.0125	93.3	93.3	93.3
0.0250	93.3	86.7	86.7
0.0406	96.7	96.7	96.7

The tolerance of *H. convergens* to the pyrethroid lambda-cyhalothrin could be a local population trait. Tillman & Mulrooney (2000) treated cotton fields near Tifton, GA, with lambda-cyhalothrin [0.028 kg (AI)/ha] in 1996, and found no difference in mortality for populations of this predator in treated and untreated fields. Survival was >82% for bugs caged on leaves collected from treated fields 0, 24 and 48 h after. Riddick et al. (2000) reported that, in 1994, higher densities of *H. convergens* were found in non-Bt potato fields near Beltsville, MD, treated with esfenvalerate with lower densities of *H. axyridis*; while in 1995, *H. axyridis* was more abundant relative to *H. convergens* and no major difference in predator abundance was observed between Bt and non-Bt potatoes. On the other hand, using *H. convergens* from a commercial source and exposing them to the pyrethroid cyfluthrin, Elzen et al. (1998) found 93.3, 86.7 and 76.7% mortality of adults caged on treated cotton leaves after 30 min, 24 h and 72 h. Lack of other published results with lambda-cyhalothrin toxicity to *H. convergens* limits broadening of our conclusions. Nevertheless, it is apparent that in our study, the tolerance of *H. convergens* for pyrethroids (and lambda-cyhalothrin in particular) relative to *H. axyridis* significantly affected the results of the risk assessment studies.

Summary

The 3 yr predator survey in paired Bt and non-Bt cotton fields under standard grower practices provides no evidence for adverse effects of Bt-cotton on the population dynamics of predators found in our cotton ecosystem. The major difference between Bt and non-Bt cotton that persisted across seasons was relative to the interaction between the lady beetles *H. convergens* and *H. axyridis*. Our findings suggest that local populations of *H. convergens* exhibited resistance to the insecticide used in non-Bt cotton fields, thereby escaping from competition with *H. axyridis*, which is highly susceptible to the lambda cyhalothrin used. These results suggest the need for care when interpreting field results on risk assessment of transgenic plants relative to natural enemy communities under standard grower practices that rely on insecticide to manage pest populations not targeted by the modified resistant plant.

Also, our summarized results showed that variation on sampling dates and on a seasonal basis could be common for some species but become neutral across long-term analyses (Table 2). These findings underscore the suggestions of Candolfi et al. (2004) and O'Callaghan et al. (2005) that population level effects, rather than individuals, and large-scale studies over sufficiently long periods to account for environmental variability, should be the ultimate endpoints in field assessments, despite limitations and challenges common to field work.

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