A Multiyear, Large-Scale Comparison of Arthropod Populations on Commercially Managed *Bt* and Non-*Bt* Cotton Fields

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ABSTRACT Field studies were conducted in 2000–2002 to compare foliage-dwelling arthropod populations on *Bacillus thuringiensis* Berliner (*Bt*) (Bollgard) cotton and non-*Bt* (conventional) cotton season-long in South Carolina, Georgia, northern Alabama, and southern Alabama. For each of these four regions, three or four paired fields were sampled weekly in each of the 3 yr. Each pair of fields consisted of a *Bt* and a non-*Bt* cotton field, both at least 5 ha in size. The dominant arthropod taxa collected included target pests (heliothine moths and *Spodoptera* spp.), nontarget pests (stink bugs and plant bugs), and generalist natural enemies [*Geocoris* spp., *Orius* spp., *Solenopsis invicta* (Buren), ladybeetles, and spiders]. Where target pests were present, particularly *Helicoverpa zea* (Boddie), their numbers were consistently significantly lower in the *Bt* cotton fields. Natural enemy populations generally were not significantly different between the *Bt* and the non-*Bt* cotton fields (50% of all comparisons) and, where significant differences were present, natural enemy abundance usually was higher in the *Bt* than the non-*Bt* cotton fields. These differences were correlated with lower insecticide use on the *Bt* than the non-*Bt* cotton fields, particularly in South Carolina, where target pest pressure was heaviest. When presented with insect eggs or larvae as prey items, the larger natural enemy populations in *Bt* cotton fields exhibited significantly higher predation rates. These results show that *Bt* cotton has no significant adverse impacts on the nontarget arthropod populations studied and, compared with insecticide-treated non-*Bt* cotton, *Bt* cotton supports higher natural enemy populations with significant positive impacts on biological control.

KEY WORDS *Bacillus thuringiensis*, Bollgard, nontarget effects, biological control, transgenic plants

THE USE OF TRANSGENIC crops that express insecticidal proteins derived from *Bacillus thuringiensis* Berliner (*Bt*) is revolutionizing production agriculture on a global scale. The most widely used products are *Bt* cotton expressing a Cry1Ac protein and *Bt* corn expressing a Cry1Ab protein. For example, *Bt* cotton expressing Cry1Ac, sold as Bollgard or Bollgard II, is grown commercially in the United States, Mexico, Colombia, Argentina, China, Australia, and South Africa on >7,000,000 ha worldwide (James 2004). Reasons for the widespread adoption of *Bt* cotton by growers include increased productivity, reduced use of synthetic pesticides, and less risk associated with decision making (Edge et al. 2001, James 2002, Huang et al. 2003). In addition, *Bt* cotton has been shown to have significant benefits for human health by reducing the exposure of growers to toxic insecticides in China (Hossain et al. 2004).

Bt cotton also has the potential to significantly benefit the environment. By significantly reducing broad-spectrum insecticide use for lepidopteran pests such as *Heliothis virescens* (F.), *Helicoverpa* spp., and *Pectinophora gossypiella* (Saunders), *Bt* cotton can reduce the toxic effects of these insecticides (Edge et al. 2001, EPA 2001, James 2002). Many laboratory and small-scale (i.e., plot sizes $\ll$ 1 ha) field studies conducted with Cry1Ac protein and *Bt* cotton indicate that *Bt* cotton only has direct toxic effects on certain species of Lepidoptera (Mendelsohn et al. 2003), in contrast with the chemistries that *Bt* cotton replaces such as pyrethroids and carbamates that have been documented to adversely impact many orders of nontarget arthropods and other invertebrates (Turnipseed et al. 2001, Wu and Guo 2004).

However, no large-scale, multilocation studies have been published on the nontarget impacts of *Bt* cotton nor have published studies evaluated how the adoption of *Bt* cotton affects nontarget arthropods under commercial production conditions. Therefore, this paper reports results from a 3-yr study conducted in four

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Table 1. Summary of arthropod sampling carried out in the four regions and 3 yr of the study

Location		Sampling regimen			
State	Year	No. farms	No. stations and samples[a]	No. sample times	Sampling period
South Carolina	2000	4	1 (5)	6	6/20–7/25
	2001	4	10 (1)	6	7/9–8/13
	2002	3	10 (1)	6	6/14–7/31
Georgia	2000	3	4 (1)	11	6/9–9/27
	2001	3	10 (1)	11	6/6–9/6
	2002	3	10 (1)	12	6/11–8/30
North Alabama	2000	4	10 (1)	8	6/16–9/2
	2001	4	10 (1)	7	6/26–8/9
	2002	4	10 (1)	8	6/19–8/7
South Alabama	2000	4	4 (10)	14	6/1–8/26
	2001	3	4 (10)	15	6/1–8/30
	2002	3	4 (10)	16	5/25–8/31

[a] Values are the no. sampling stations established in each field, with the no. samples taken per station on each sampling date in parentheses.

regions of three U.S. states (north and south Alabama, Georgia, and South Carolina) comparing arthropod populations on commercially managed *Bt* (Bollgard) and non-*Bt* (conventional) cotton (see also the papers in this volume by Naranjo 2005a, b, Torres and Ruberson 2005, Whitehouse et al. 2005). Sampling focused on foliage-dwelling arthropods and particularly generalist predators because of the ecological and economic importance of these groups; previous studies indicated that predators are the dominant natural enemies in cotton fields in these regions (McGriff and Ruberson 1999).

Materials and Methods

Locations. From 2000 to 2002, three or four pairs of *Bt* (Bollgard; Monsanto Company, St. Louis, MO) and conventional cotton fields were monitored in each of South Carolina, Georgia, and north and south Alabama (Table 1). Fields were all at least 5 ha in size and were as large as 30 ha. Pairs of fields were located on the same farm and were chosen to be as similar as possible in location, variety, tillage practices, and border vegetation. All fields were commercially managed according to standard practices for that region, including applications of appropriate insecticides when economic thresholds were exceeded. Insecticide treatments generally were targeted against either lepidopteran pests, such as *H. virescens, Helicoverpa zea* (Boddie), and *Spodoptera* spp., or sucking bugs, such as plant bugs and stink bugs. Records were kept of all insecticide applications.

Sampling Methods. At each of the locations, arthropod populations were sampled at least weekly throughout the course of the season, except in the case of South Carolina where the sampling duration was shorter (Table 1). Sampling was initiated 2–3 wk after plant emergence and continued until plant defoliation occurred. This resulted in at least 6 and as many as 16 sampling dates for each of the locations in each of the years. Sampling typically began in early June and ended in late August or September (Table 1).

The number of samples taken on each sampling date varied among locations and years (Table 1). At the South Carolina sites in 2000, five subsamples were taken from around a single centrally located station in each field and these were combined into a single sample. In 2001 and 2002, a diagonal transect of ten sampling stations was established in each field, and a single sample was taken at each station on each sampling date. At the Georgia sites in 2000, fields were divided into four quadrants and one sample was taken from around a single centrally located station in each quadrant on each sampling date. In 2001 and 2002, a transect of 10 sampling stations was established in each field as described for South Carolina, and a single sample was taken at each station on each sampling date. At the north Alabama sites, a transect of 10 sampling stations was established in each field and a single sample was taken at each station on each sampling date in all 3 yr. At the south Alabama sites, fields were divided into four quadrants, and 10 samples were taken from random locations within each quadrant on each sampling date in all 3 yr.

Each arthropod sample consisted of examining 5–10 whole plants for pest Lepidoptera and using combinations of beat buckets and drop cloths to sample for nontarget pest and natural enemy species. Beat buckets were used until plants were 35 cm tall, and 1-m^2 beat sheets were used as described by Shepard et al. (1974). For both beat buckets and beat sheets, each sample consisted of collecting arthropods from a minimum of 2 m of row. The collected arthropods were identified at least to the family level and to the species level in most cases. The individuals of each taxon were counted and the totals for each taxon in each sample were converted to abundances per meter of row.

The primary target pests sampled were *H. zea, H. virescens,* the armyworms *Spodoptera exigua* (Hübner) and *S. frugiperda* (J.E. Smith), and *Pseudoplusia includens* Walker. The most significant foliage-dwelling nontarget pests sampled were nymphal and adult plant bugs [primarily *Lygus lineolaris* (Palisot de Beauvois)] and stink bugs (*Euschistus* spp.). The dominant foliage-dwelling nontarget natural enemy species sampled were *Geocoris spp.* nymphs and adults [primarily consisting of *G. punctipes* (Say)], *Orius spp.* nymphs and adults [primarily *Orius insidiosus* (Say)], *Nabis spp.* nymphs and adults, ladybeetle larvae and adults (Coccinellidae), lacewing larvae and adults (Chrysopidae and Hemerobiidae), spiders (primarily Araneidae, Lycosidae, Oxyopidae and Thomisidae), and ants (primarily the fire ant, *Solenopsis invicta* Buren).

For each of the four regions, representative voucher specimens were deposited in the respective university collections (South Carolina, Clemson University; Georgia, University of Georgia; and Alabama, Auburn University).

Sentinel Prey Studies. In three regions (South Carolina, Georgia, and south Alabama) in 2002, sets of *H. zea* eggs were placed in the paired fields and recovered after 24 h to estimate rates of egg predation by natural enemies. The eggs used were <24 h old and were individually attached to the leaves of cotton plants with a fine paintbrush using bovine serum albumin as an adhesive. Eggs were evenly distributed on randomly chosen upper, middle, and lower leaves of each designated cotton plant, mimicking natural heliothine oviposition patterns (Parker and Luttrell 1998). All studies were performed in the last week of July or the first week of August using at least 10 sets of 15 eggs in both the *Bt* cotton and non-*Bt* cotton fields, with each set of eggs placed on a different cotton plant. This time period was chosen because the abundance of arthropod natural enemies in cotton fields in these regions peaks at about this time and because natural enemy control of damage to cotton fruiting structures would be particularly valuable during this period.

In addition, 10 sets of 10 1–2 d old first-instar beet armyworm were placed out in both the *Bt* cotton and non-*Bt* cotton fields in South Carolina, with each set placed on a different cotton plant, and recovered after 24 h to estimate rates of larval predation by natural enemies. Each set of larvae were placed as a cluster on an individual plant, mimicking a recently hatched armyworm egg mass. The focal plant and surrounding cotton plants were examined for the presence of larvae and any evidence of larval feeding.

Statistical Analysis. For each region in each of the 3 yr, individual statistical analyses were run on the following taxa because of their abundance and importance: bollworms (consisting of larval *H. zea* and *H. virescens*); armyworms (larval *S. exigua* and *S. frugiperda;* plant bugs nymphs and adults; stink bugs nymphs and adults; big-eyed bug nymphs and adults (*Geocoris* spp.); minute pirate bug nymphs and adults combined (*Orius* spp.); lacewing larvae and adults (Chrysopidae and Hemerobiidae); ladybeetle larvae and adults (Coccinellidae); fire ants (*S. invicta*); and spiders. Analyses focused particularly on larval and nymphal stages where possible, because these stages are less mobile than the adults and more sensitive to local conditions. Therefore these stages are more likely to be affected by insecticides or *Bt* proteins. For each of these numerically abundant taxa, repeated-measures analyses of variance (ANOVA) was used to compare population densities in the *Bt* cotton and non-*Bt* cotton fields. The effects of farm (blocking factor), cotton type (*Bt* or non-*Bt* cotton), date, and the interaction of date and cotton type on the abundance of natural enemies and herbivores were analyzed with mixed model, repeated-measures ANOVA using the PROC MIXED procedure of SAS (SAS Institute 2001). A compound symmetry structure was used to model the covariance among sampling dates. This structure was chosen because it requires relatively few assumptions about how the data are autocorrelated. All data were $\log(n + 1)$-transformed before analysis. Nontransformed means and SEs are reported in the tables and figures.

The number of insecticide applications on *Bt* cotton and non-*Bt* cotton fields within a region in each of the 3 yr, and the level of egg and larval predation in *Bt* cotton and non-*Bt* cotton fields within the regions, were compared using *t*-tests adjusted for unequal variances (SAS Institute 1995). The difference in the number of insecticide applications on non-*Bt* cotton and *Bt* cotton fields was regressed against the relative size of fire ant populations (the most abundant predator in these systems) in each region and year (SAS Institute 1995).

Results

The most abundant foliage-dwelling cotton pests in all regions were various lepidopteran and heteropteran species. The lepidopteran species are target pests for *Bt* cotton because they are all controlled to varying degrees by the Cry1Ac protein; *H. virescens* is completely controlled by Bollgard, *H. zea* is partially controlled by Bollgard, and *Spodoptera* spp. are essentially unaffected by Bollgard (Stewart et al. 2001). Of these species, the most common target pest in all of the regions studied was the corn earworm, *H. zea*, and it was still relatively rare in all of the years of study (Table 2). Other lepidopteran pests of cotton such as armyworms (*Spodoptera* spp.) were very sporadic, only occasionally occurring in any given region in any given year. Nontarget sucking pests, particularly plant bugs (*Lygus spp.*) and to a lesser extent stink bugs (primarily *Nezara viridula* L., but also *Acrosternum hilare* (Say) and *Euschistus* spp.), were consistently present in all regions and years.

Generalist predators dominated the foliage-dwelling arthropod community in all regions and years (Table 3). Fire ants were the most common species, averaging >10 individuals per meter of row in Georgia. However, big-eyed bugs (*Geocoris* spp.), minute pirate bugs (*Orius* spp.), ladybeetles (Coccinellidae), and spiders (Araneae) were all present at seasonal average densities ranging from one to five individuals per meter of row in most regions.

Comparisons of Arthropod Populations in *Bt* Versus Non-*Bt* Cotton. The average number (±SE) of insecticide applications on *Bt* and non-*Bt* cotton fields in the four regions in each of the 3 yr is shown in Table 4. The insecticides commonly used included the pyrethroids λ-cyhalothrin (Karate, Syngenta Crop Protection, Greensboro, NC) and ζ-cypermethrin (Fury, FMC Corp., Philadelphia, PA), the organo-

Table 2. Mean no. (±SE) selected plant-dwelling arthropod pests per meter of row over the course of the season in sets of commercially managed conventional (non-*Bt*) cotton fields in four regions of the U.S. Cotton Belt over 3 yr

Location		Target pests[a]		Nontarget pests[b]			
State	Year	Heliothine	Armyworm	PB nymph	PB adult	SB nymph	SB adult
South Carolina	2000	0.18 ± 0.05	0	0.18 ± 0.04	0.01 ± 0.01	0	0.01 ± 0.01
	2001	0.13 ± 0.03	0	0.26 ± 0.03	0.02 ± 0.01	0.01 ± 0.01	0.03 ± 0.01
	2002	0.50 ± 0.07	3.32 ± 0.61	0.66 ± 0.10	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
Georgia	2000	0.02 ± 0.01	0	0.01 ± 0.01	0.04 ± 0.02	0.02 ± 0.01[c]	
	2001	0.19 ± 0.04	0	0.77 ± 0.11	0.07 ± 0.02	0.03 ± 0.01	
	2002	0.51 ± 0.06	0.18 ± 0.03	0.84 ± 0.08	0.28 ± 0.04	0.14 ± 0.03	
North Alabama	2000	<0.01	0	0.47 ± 0.05	0.04 ± 0.01	0.02 ± 0.01	0.01 ± 0.00
	2001	0.03 ± 0.01	0	1.16 ± 0.13	0.16 ± 0.03	0.04 ± 0.01	0.02 ± 0.01
	2002	0.08 ± 0.03	0.02 ± 0.01	1.56 ± 0.21	0.09 ± 0.02	0.03 ± 0.01	0.05 ± 0.01
South Alabama	2000	0.02 ± 0.00	<0.01	<0.01	<0.01	<0.01	0.01 ± 0.00
	2001	0.02 ± 0.00	<0.01	<0.01	<0.01	<0.01	<0.01
	2002	0.06 ± 0.00	0.01 ± 0.00	<0.01	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00

[a] Heliothine, larvae of *Helicoverpa zea* and *Heliothis virescens;* Armyworm, larvae of *Spodoptera frugiperda* and *S. exigua.*
[b] PB, plant bugs (*Lygus spp.*); SB, stink bugs.
[c] Stink bug nymphs and adults were combined for the Georgia location.

phosphate dicrotophos (Bidrin, AMVAC Chemical Corp., Los Angeles, CA), and spinosad (Tracer; Dow AgroSciences LLC, Indianapolis, IN). The insecticide applications typically occurred between mid-July and mid-August, particularly in the case of the pyrethroids. Of the regions, lepidopteran pest pressure was highest in South Carolina (see Table 2), resulting in relatively more insecticide applications (particularly of pyrethroids for *H. zea* control) on the non-*Bt* cotton fields in this region than in other regions and significantly more insecticide applications on the non-*Bt* than the *Bt* cotton fields in this region in all 3 yr (Table 4; *t*-tests, $P < 0.05$). In the other three regions, more insecticide applications tended to be applied to the non-*Bt* cotton fields than the *Bt* cotton fields but it was only significant in two cases: Georgia and south Alabama, both in 2002. In these regions, lepidopteran pest pressure was consistently relatively low (Table 2) and most of the applications were made for pest Heteroptera, with dicrotophos being the most commonly used insecticide. However, pest populations were higher in 2002 than in 2000 and 2001 (Table 2), resulting in the observed patterns of insecticide use in Georgia and south Alabama in that year.

The impacts of *Bt* cotton on populations of each of the important target and nontarget taxa are presented in Tables 5 and 6. Results are presented as the percent difference between the abundance of a given taxon in *Bt* cotton fields and the abundance in non-*Bt* cotton fields in a given region and year. A positive value represents a significantly greater abundance of that taxon in the *Bt* cotton fields relative to the non-*Bt* fields, whereas a negative value represents a significantly lower abundance in the *Bt* cotton fields.

Target Pest Lepidoptera. As expected, the target lepidopteran pests of *Bt* cotton, particularly the heliothines, generally were present at significantly lower densities on *Bt* cotton than on non-*Bt* cotton (Table 5), despite the use of insecticidal sprays on the non-*Bt* cotton fields to control these pests. In north Alabama and Georgia, population densities of these pest species were too low in some years (particularly 2000) of the study to detect differences. Similarly, armyworms tended to be too rare and sporadic in all regions for significant differences to be detected, except in South Carolina in 2002.

Nontarget Pest Heteroptera. In South Carolina, where significantly more insecticide applications were made on the non-*Bt* cotton fields than the *Bt* cotton fields in all 3 yr of study (Table 4), nymphal and adult populations of plant bugs were significantly lower on the non-*Bt* cotton fields in all 3 yr (Table 5), presumably as a direct result of these insecticide sprays. A similar pattern was observed with nymphal and adult stink bugs in 2002. In other years, stink bugs seldom were present in sufficient numbers to detect differences (Table 2). In the other regions where the number of insecticide applications was comparable on the non-*Bt* cotton fields and the *Bt* cotton fields in all 3 yr of study, the abundance of nymphal and adult plant bugs and stink bugs generally was not significantly different between *Bt* and non-*Bt* cotton fields (Table 5). Of the 36 comparisons, 26 (72%) were not significantly different, 5 had significantly larger pest populations in the *Bt* cotton fields, and 5 had significantly lower pest populations in the *Bt* cotton fields.

Nontarget Generalist Predators. In South Carolina, populations of nymphal and adult big-eyed bugs, minute pirate bugs, damsel bugs, fire ants, and spiders were consistently significantly greater in the *Bt* cotton fields than in the non-*Bt* cotton fields in each of the 3 yr (Table 6), presumably reflecting the lower number of broad-spectrum insecticide applications on the *Bt* cotton fields compared with non-*Bt* cotton fields. In many of these cases, the natural enemy populations were two to four times greater in the *Bt* cotton fields. Similarly, in this region lacewing (Chrysopidae and Hemerobiidae) populations were significantly greater in the *Bt* cotton fields in 1 of the 3 yr.

Among the arthropod predators, ladybeetles were the one exception to the trend toward higher populations in the *Bt* cotton fields. Larval ladybeetle populations were significantly lower in South Carolina *Bt* cotton fields than in the non-*Bt* cotton fields in 2 of the

Table 3. Mean no. (±SE) selected plant-dwelling arthropod predators per meter of row over the course of the season in sets of commercially managed conventional (non-*Bt*) cotton fields in four regions of the U.S. Cotton Belt over 3 yr

Location		Predatory bugs[a]				Other predatory arthropods[b]					
State	Year	BE nymph	BE adult	Pirate	Damsel	LW larva	LW adult	LB larva	LB adult	Fire ant	Spiders
South Carolina	2000	0.35 ± 0.17	0.55 ± 0.10	0.32 ± 0.11	0.05 ± 0.03	0.24 ± 0.09	0.03 ± 0.01	2.86 ± 0.66	0.75 ± 0.13	4.55 ± 1.07	1.42 ± 0.25
	2001	0.14 ± 0.02	0.24 ± 0.03	0.51 ± 0.07	0.12 ± 0.13	0.25 ± 0.04	0.08 ± 0.18	2.50 ± 0.36	4.99 ± 0.73	4.04 ± 0.50	0.98 ± 0.11
	2002	0.36 ± 0.05	0.38 ± 0.05	0.97 ± 0.10	0.05 ± 0.01	0.11 ± 0.02	0.02 ± 0.01	6.93 ± 0.77	1.39 ± 0.17	2.53 ± 0.35	1.39 ± 0.10
Georgia	2000	0.99 ± 0.20	0.86 ± 0.09	0.31 ± 0.08	0.48 ± 0.10	0.05 ± 0.02	—[c]	1.59 ± 0.23	2.79 ± 0.27	11.8 ± 0.86	1.76 ± 0.21
	2001	1.15 ± 0.15	0.51 ± 0.06	0.95 ± 0.12	1.12 ± 0.18	0.64 ± 0.08	—	6.17 ± 0.61	1.93 ± 0.16	13.1 ± 0.95	5.35 ± 0.35
	2002	2.68 ± 0.22	1.90 ± 0.13	2.15 ± 0.20	0.68 ± 0.07	0.51 ± 0.06	—	6.00 ± 0.47	4.61 ± 0.29	24.4 ± 1.30	5.99 ± 0.27
North Alabama	2000	0.89 ± 0.07	0.19 ± 0.02	1.49 ± 0.13	0.09 ± 0.01	0.14 ± 0.02	0.04 ± 0.01	0.44 ± 0.08	0.41 ± 0.04	1.30 ± 0.12	1.01 ± 0.07
	2001	0.29 ± 0.03	0.27 ± 0.01	1.27 ± 0.13	0.06 ± 0.01	0.57 ± 0.05	0.09 ± 0.02	0.68 ± 0.11	0.16 ± 0.02	0.93 ± 0.12	1.09 ± 0.07
	2002	1.84 ± 0.18	0.35 ± 0.04	0.79 ± 0.10	0.42 ± 0.04	0.24 ± 0.04	0.08 ± 0.03	1.01 ± 0.13	0.36 ± 0.05	3.96 ± 0.39	2.45 ± 0.17
South Alabama	2000	<0.01	0.01 ± 0.00	<0.01	<0.01	0	<0.01	0.02 ± 0.00	0.05 ± 0.01	1.43 ± 0.05	0.08 ± 0.01
	2001	0	0.01 ± 0.00	0.02 ± 0.01	<0.01	0	<0.01	0.03 ± 0.00	0.03 ± 0.00	1.22 ± 0.04	0.06 ± 0.00
	2002	<0.01	0.01 ± 0.00	0	<0.01	<0.01	<0.01	0.06 ± 0.00	0.08 ± 0.00	2.34 ± 0.05	0.10 ± 0.00

[a] BE, big-eyed bugs (*Geocoris* spp.); Pirate, *Orius* spp. nymphs and adults; Damsel, *Nabis* spp.
[b] LW, lacewings (Chrysopidae and Hemerobiidae); LB, ladybeetles (Coccinellidae).
[c] Adult lacewings were not recorded for the Georgia location.

Table 4. Mean no. (±SE) insecticide sprays on sets of commercially managed non-*Bt* (conventional) cotton fields and *Bt* (Bollgard) cotton fields in four regions of the U.S. Cotton Belt over 3 yr

Locations		Insecticide sprays	
State	Year	*Bt*	Non-*Bt*
South Carolina	2000	1.5 ± 0.3a	4.0 ± 0.0b
	2001	1.0 ± 0.0a	2.5 ± 0.3b
	2002	0a	3.0 ± 1.0b
Georgia	2000	0	0.3 ± 0.3
	2001	0.7 ± 0.3	1.3 ± 0.3
	2002	0.3 ± 0.3a	1.7 ± 0.3b
North Alabama	2000	1.5 ± 0.3	1.8 ± 0.5
	2001	2.8 ± 0.5	3.3 ± 0.5
	2002	2.5 ± 0.6	2.5 ± 0.5
South Alabama	2000	1.0 ± 0.6	1.5 ± 0.9
	2001	1.3 ± 0.7	2.0 ± 0.6
	2002	2.3 ± 0.3a	4.3 ± 0.7b

Means within a row followed by different letters are significantly different ($P < 0.05$).

3 yr, and adult ladybeetle populations were not significantly different between the *Bt* and non-*Bt* cotton fields in any of the 3 yr (Table 6).

In the other three regions where insecticide use was comparable on *Bt* cotton and non-*Bt* cotton fields, natural enemy populations generally were not significantly different between *Bt* and non-*Bt* cotton fields. Of the 90 comparisons, 66 (73%) of the comparisons were not significant, 15 (17%) had greater populations in the *Bt* cotton fields, and 9 (10%) had lower populations in the *Bt* cotton fields (Table 6). Where a natural enemy population differed significantly between the *Bt* and non-*Bt* cotton fields in a particular region and year, the size of the difference tended to be smaller than in those observed for South Carolina (generally <2-fold).

To examine the quantitative relationship between insecticide use and predator populations, the difference in the number of insecticide applications on non-*Bt* cotton and *Bt* cotton fields was regressed against the relative size of fire ant populations (the most abundant predator in these systems) in each region and year (Fig. 1). The relative size of the fire ant population in the *Bt* cotton fields relative to the non-*Bt* fields was significantly positively correlated with the spray reduction on the *Bt* cotton fields (R^2 = 0.51, F = 10.3, df = 1,10, P = 0.0094).

Predation Rate in *Bt* and Non-*Bt* Cotton Fields. The percentage of sentinel prey items consumed over a 24-h period in *Bt* cotton fields was consistently greater than the percentage consumed in non-*Bt* cotton fields. Figure 2 shows the results of experiments run with sets of *H. zea* eggs in three of the regions in 2002. At all three locations, these experiments were preceded by at least one pyrethroid application for lepidopteran pests on the non-*Bt* cotton fields, and two λ-cyhalothrin applications occurred in the preceding 2 wk at the South Carolina site. The percentage of eggs consumed in the *Bt* cotton fields was significantly greater in South Carolina (t = 4.63, df = 17.9, P = 0.0002) and south Alabama (t = 3.19, df = 35.6, P = 0.003) but not in Georgia (t = 1.21, df = 7.56, P = 0.13), although the

Table 5. Percent difference in the abundance of selected plant-dwelling arthropod pests over the course of the season in sets of commercially managed *Bt* (Bollgard) cotton fields compared with paired non-*Bt* (conventional) cotton fields in four regions of the U.S. Cotton Belt over 3 yr

Location		df	Target pests[a]		Nontarget pests[b]			
State	Year		Heliothine	Armyworm	PB nymph	PB adult	SB nymph	SB adult
South Carolina	2000	1,36	−74	NS	75	700	NS	**200**
	2001	1,72	−77	NS	130	125	NS	NS
	2002	1,54	NS	−78	145	1,580	1,230	**679**
Georgia	2000	1,19	NS	NS	NS	NS	NS[c]	
	2001	1,55	−77	NS	NS	−56	NS	
	2002	1,54	−85	NS	NS	−49	NS	
North Alabama	2000	1,153	NS	NS	NS	NS	NS	NS
	2001	1,93	NS	NS	−44	−82	NS	NS
	2002	1,73	−90	NS	NS	−35	230	NS
South Alabama	2000	1,24	−87	NS	600	145	519	NS
	2001	1,18	−100	NS	NS	801	NS	NS
	2002	1,18	−85	NS	NS	NS	NS	NS

Positive numbers represent significantly greater numbers in the *Bt* cotton fields, negative numbers represent significantly lower numbers in the *Bt* cotton fields, and NS represents no significant difference between *Bt* and non-*Bt* cotton fields, based on repeated-measures ANOVA ($P < 0.05$).

[a] Heliothine, larvae of *Helicoverpa zea* and *Heliothis virescens;* Armyworm, larvae of *Spodoptera frugiperda* and *S. exigua.*

[b] PB, plant bugs (*Lygus spp.*); SB, stink bugs.

[c] Stink bug nymphs and adults were combined for the Georgia location.

trend was comparable. These patterns match those observed for the relative size of natural enemy populations (Table 6); generalist predator populations were consistently significantly larger in *Bt* cotton fields than in non-*Bt* cotton fields in South Carolina and south Alabama, and similar but smaller differences were present between *Bt* and non-*Bt* cotton fields in Georgia.

Comparable studies using larvae of *S. exigua* in South Carolina in 2002 produced similar results. Of 100 larvae placed out for 24 h, significantly fewer larvae were recovered in the *Bt* cotton fields than in the non-*Bt* cotton fields (1% compared with 51%; $t = 4.92$, df = 9, $P = 0.0008$). Some of the missing larvae may have moved onto neighboring plants but searches of these plants did not reveal any larvae and interplant movement of first-instar armyworms typically is limited.

Spatial and Temporal Variation in Arthropod Populations in Cotton. Factors other than insecticide applications clearly contributed to significant spatial and temporal variation in arthropod populations within and among all the regions.

Spatial Variation in Arthropod Populations. As noted earlier, lepidopteran pest populations tended to be higher in South Carolina than in the other regions (Table 2). These pests were almost nonexistent in north and south Alabama during the 3 yr of this study. However, of all the regions, plant bugs were most common in north Alabama, resulting in relatively high number of spray applications on both *Bt* and non-*Bt* cotton fields in this region (Table 4).

Table 6. Percent difference in the abundance of selected plant-dwelling arthropod predators over the course of the season in sets of commercially managed *Bt* (Bollgard) cotton fields compared with paired non-*Bt* (conventional) cotton fields in four regions of the U.S. Cotton Belt over 3 yrs

Location		df	Predatory bugs[a]				Other predatory arthropods[b]					
State	Year		BE nymph	BE adult	Pirate	Damsel	LW larva	LW adult	LB larva	LB adult	Fire ant	Spiders
South Carolina	2000	1,36	79	120	300	250	NS	NS	45	NS	47	69
	2001	1,72	177	69	66	124	NS	NS	NS	NS	NS	52
	2002	1,54	66	NS	380	330	79	NS	−39	NS	256	222
Georgia	2000	1,19	NS	NS	−41	NS	NS	—[c]	NS	NS	42	NS
	2001	1,55	NS	NS	NS	NS	NS	—	NS	NS	NS	NS
	2002	1,54	−36	−39	29	NS	47	—	NS	NS	84	NS
North Alabama	2000	1,153	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	2001	1,93	NS	NS	−33	62	NS	NS	NS	NS	−49	NS
	2002	1,73	−25	−39	NS	−32	NS	NS	NS	NS	−26	NS
South Alabama	2000	1,24	117	61	147	NS	NS	NS	57	127	13	NS
	2001	1,18	NS	NS	NS	NS	NS	NS	NS	NS	13	NS
	2002	1,18	NS	NS	NS	NS	NS	NS	20	NS	12	21

Positive numbers represent significantly greater numbers in the *Bt* cotton fields, negative numbers represent significantly lower numbers in the *Bt* cotton fields, and NS represents no significant difference between *Bt* and non-*Bt* cotton fields, based on repeated-measures ANOVA ($P < 0.05$).

[a] BE, big-eyed bugs (*Geocoris* spp.); Pirate, *Orius* spp. nymphs and adults; Damsel, *Nabis* spp.

[b] LW, lacewings (Chrysopidae and Hemerobiidae); LB, ladybeetles (Coccinellidae).

[c] Adult lacewings were not recorded for the Georgia location.

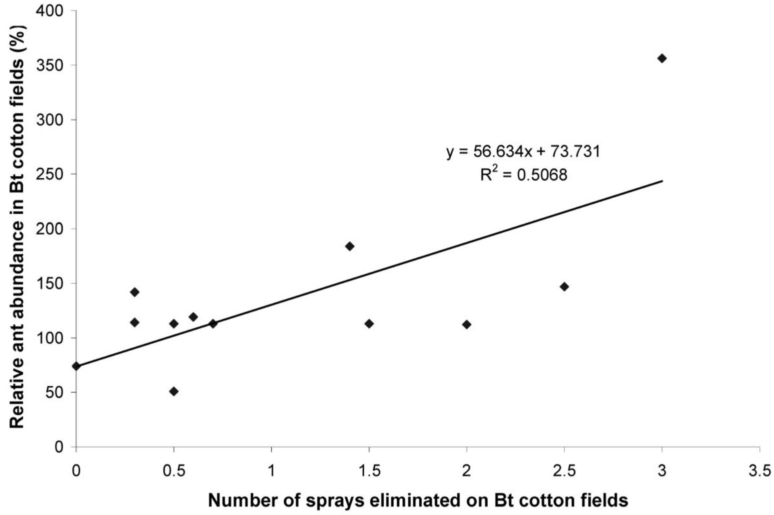

Fig. 1. Relative size of fire ant populations in *Bt* versus non-*Bt* cotton fields regressed against the difference in the number of insecticide applications on non-*Bt* versus *Bt* cotton fields. Values are averages for a region in each of the years 2000–2002.

Natural enemy populations also varied greatly among the regions (Table 3). Populations of most of the common generalist predators tended to be highest in South Carolina and Georgia and were consistently very low in south Alabama. However, different taxa did display different patterns across the regions. For example, fire ants and spiders were particularly common in Georgia, possibly because of the high levels of conservation tillage adoption.

Within regions, variability in arthropod populations was even greater. Table 7 shows the percent difference (where that difference was statistically significant) between the locations within a region with the highest and lowest abundances of the common pest and natural enemy taxa. These differences within a region generally were statistically significant (71 of 110 cases evaluated = 65%), and the size of the differences ranged up to 100-fold. The taxa that exhibited the greatest spatial variability were the predatory bugs (anthocorids and geocorids) and fire ants.

Temporal Variation in Arthropod Populations. As discussed earlier, pest populations were consistently higher in 2002 than in 2000 and 2001 in all of the regions, particularly in the case of the target lepidop-

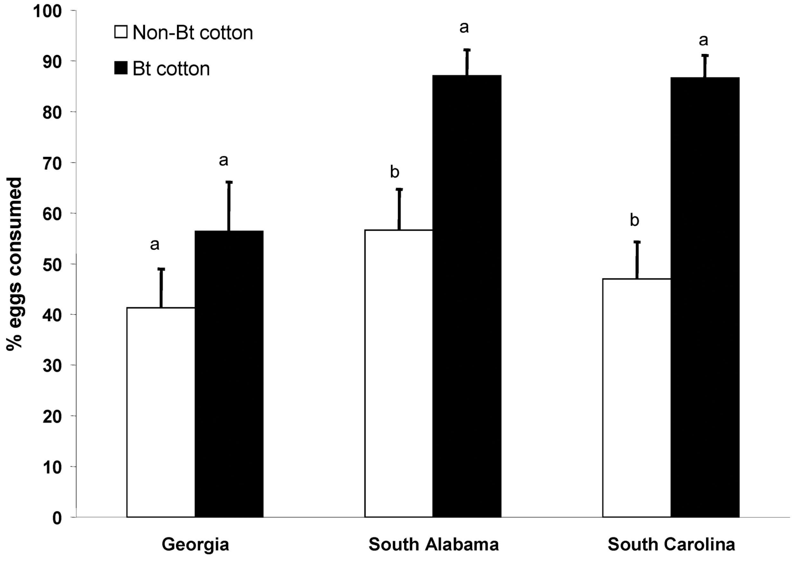

Fig. 2. Percentage (mean ± SE) of sentinel eggs consumed within 24 h in non-*Bt* and *Bt* cotton fields in each of three regions (Georgia, south Alabama, and South Carolina) in 2002. Bars within a region with the same letter above them are not significantly different ($P < 0.05$).

Table 7. Percent difference in the abundance of selected plant-dwelling arthropod pests and predators in sets of commercially-managed cotton fields in four regions of the U.S. Cotton Belt over 3 yr

Location		df	Target pests[a]		Nontarget pests[b]		Predatory bugs[c]		Other predatory arthropods[d]			
State	Year		Heliothine	Armyworm	PB	SB	Pirate	BE	LW larva	LB larva	Fire ant	Spiders
South Carolina	2000[e]	—	—	—	—	—	—	—	—	—	—	—
	2001	3,72	2,300	NS	576	NS	500	1,920	560	226	386	139
	2002	2,54	205	509	1,900	NS	100	351	NS	150	223	54
Georgia	2000	2,19	NS	NS	NS	NS	638	726	NS	163	97	112
	2001	2,55	NS	NS	NS	872	NS	NS	NS	NS	125	NS
	2002	2,54	189	573	165	238	509	129	148	277	160	54
North Alabama	2000	3,153	NS	NS	257	NS	378	122	NS	465	1,025	103
	2001	3,93	NS	NS	401	266	921	3260	88	667	3,250	176
	2002	3,73	NS	NS	182	NS	308	687	133	379	4,150	105
South Alabama	2000	3,24	754	10,000	NS	519	10,000	2930	NS	1720	153	805
	2001	2,18	NS	NS	NS	376	10,000	NS	NS	564	54	NS
	2002	2,18	43	NS	240	71	NS	NS	NS	NS	14	20

Values represent cases where sites within a region significantly varied in abundance based on repeated-measures ANOVA ($P < 0.05$) and are the percent difference between the sites with the greatest and lowest abundances; NS represents no significant difference among farms within a region.

[a] Heliothine, larvae of *Helicoverpa zea* and *Heliothis virescens;* Armyworm, larvae of *Spodoptera frugiperda* and *S. exigua.*

[b] PB, plant bug nymphs (*Lygus spp.*); SB, stink bug nymphs.

[c] Pirate, *Orius* spp. nymphs and adults; BE, big-eyed bug nymphs (*Geocoris* spp.).

[d] LW, lacewings (Chrysopidae and Hemerobiidae); LB, ladybeetles (Coccinellidae).

[e] No analysis of differences among sites within a region was performed for South Carolina in 2000 because of limited replication within sites.

teran species (Table 2). Predator populations also tended to be higher in 2002 in all of the regions, possibly because they were responding to the same factors as the pest species or because they were responding to the greater abundance of the prey species themselves (Table 3).

Discussion

Impact of *Bt* Cotton on Generalist Predator Populations. Across the four regions and 3 yr of this study, no consistent adverse impacts on foliage-dwelling generalist predators were observed in the large, commercially managed *Bt* cotton fields relative to the non-*Bt* cotton fields in each region (Table 6). In any given year, this amounted to weekly sampling throughout the course of the cotton growing season on 14 or 15 pairs of *Bt* and non-*Bt* cotton fields, with any given field being sampled repeatedly on each sample date. This supports the original conclusions of the risk assessment performed for *Bt* cotton (Mendelsohn et al. 2003) and is generally consistent with other studies carried out in various countries that have found few consistent differences between unsprayed *Bt* cotton and non-*Bt* cotton fields (Men et al. 2003, Sisterson et al. 2004, Naranjo 2005a, b, Torres and Ruberson 2005, Whitehouse et al. 2005). Where differences have been seen in these studies, they have tended to be small in magnitude, and their biological significance was unclear (Naranjo 2005a, b, Whitehouse et al. 2005).

Where significantly greater insecticide use occurred on the non-*Bt* cotton fields than the *Bt* cotton fields in this study, such as in South Carolina in all years and in Georgia and south Alabama in 2002, generalist predator populations were consistently significantly larger in the *Bt* cotton fields than in the non-*Bt* cotton fields (Table 6). In particular, populations of predatory Heteroptera, fire ants, and spiders were significantly larger in *Bt* than non-*Bt* cotton fields where treatments occurred for lepidopteran pests like *H. zea.* The size of this effect was significantly correlated with the difference in the number of insecticide applications on the non-*Bt* and *Bt* cotton fields (Fig. 1). This clearly shows that the smaller number of insecticide applications required for lepidopteran pests on *Bt* cotton results in substantial benefits to nontarget arthropod populations when cotton is grown under commercial conditions. Similar environmental impacts should be observed anywhere that the use of *Bt* cotton results in fewer insecticide applications than are required for non-*Bt* cotton in the same region. Smaller-scale field studies in various countries support this conclusion (Xia et al. 1999, Hagerty et al. 2001, 2005, Naranjo 2005a, b).

Consequences of Differences in Generalist Predator Populations. The differences in generalist predator populations between *Bt* and non-*Bt* cotton fields that had different numbers of insecticide applications had functional impacts on levels of biological control within these fields. Rates of egg and larval predation were significantly higher in the *Bt* cotton than the non-*Bt* cotton fields in South Carolina and south Alabama in 2002, and a similar nonsignificant trend was observed for Georgia cotton fields. This enhanced level of biological control probably was responsible for the significantly lower populations of fall armyworms observed in *Bt* cotton fields in South Carolina in 2002 (Table 6). In that year, armyworm populations were high in the non-*Bt* cotton fields and almost nonexistent in the *Bt* cotton fields, yet *Bt* cotton provides only minimal direct control of armyworms (Stewart et al. 2001). This sort of impact on rates of biological control has been reported for other nontarget pests such as aphids in both *Bt* potato fields and *Bt* cotton fields (Reed et al. 2001, Wu and Guo 2004). Aphid populations have been observed to be significantly lower in *Bt* crop fields than in corresponding non-*Bt* crop fields managed with broad-spectrum insecticides.

The enhanced level of biological control in *Bt* cotton fields with relatively fewer insecticide applications also may explain why ladybeetle larvae were less abundant in *Bt* cotton fields than in non-*Bt* cotton fields in South Carolina in 2000 and 2002 (Table 6). The generalist predators in *Bt* cotton fields would have decreased the abundance of ladybeetle prey such as lepidopteran eggs and aphids. Ladybeetles in cropping systems tend to be highly responsive to prey populations such as aphids, potentially explaining the larger numbers of ladybeetles found in the South Carolina non-*Bt* cotton fields. In addition, ladybeetles in the *Bt* cotton fields may have been exposed to more intraguild predation than those in the non-*Bt* cotton fields because of the higher abundance of ants and spiders in the *Bt* cotton fields.

Other Biotic and Abiotic Factors Affecting Arthropod Populations in Cotton. The significant spatial and temporal variation in arthropod populations observed in this study indicates that many factors beyond insecticide use significantly affect these populations. The variation within and among regions probably reflected differences in other management practices such as tillage and irrigation; differences in the surrounding land use patterns and particularly in the presence of source areas for the generalist predators; and differences in how the focal fields themselves were used in previous years. The variation across the years of the study probably reflected direct and indirect effects of abiotic factors such as temperature and precipitation. Cumulatively, these biotic and abiotic factors tended to have much larger effects on arthropod populations than the insecticide applications (e.g., compare Tables 5 and 6 with Table 7), and may wholly or partially obscure the impacts of *Bt* cotton use in studies that use a much more limited spatial or temporal scale than this study.

Potential Large-Scale Effects of *Bt* Cotton Adoption. The results of this study show that the adoption of *Bt* cotton over large areas can have significant benefits in terms of enhanced biological control in cotton systems. As *Bt* cotton adoption continues to increase in future years, these environmental benefits can be expected to accumulate, particularly in regions with historically heavy lepidopteran pest pressure and heavy insecticide use against these pests. In countries such as China, India, and Australia, where the number of insecticide applications for lepidopteran pests in cotton can exceed 10 in any given season (James 2002), these environmental benefits should be especially significant. Furthermore, in regions where cotton is grown among many other crops, such as India and much of China, *Bt* cotton adoption also may result in environmental benefits for these other crops; the impact on generalist predators moving among crops will be minimized by significantly reducing the number of insecticide applications that occur within cotton.

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References Cited

Edge, J., J. Benedict, J. Carroll, and H. Reding. 2001. Bollgard cotton: an assessment of global economic, environmental and social benefits. J. Cotton Sci. 5: 121–136.

Environmental Protection Agency [EPA]. 2001. *Bt* plant-incorporated protectants biopesticides registration action document. Benefits assessment. http://www.epa.gov/pesticides/biopesticides/pips/bt_brad2/5-benefits.pdf.

Hagerty, A. M., S. G. Turnipseed, and M. J. Sullivan. 2001. Impact of predaceous arthropods in cotton IPM. Proceedings of the 2001 Beltwide Cotton Conference, National Cotton Council, 9–13 Jan., Memphis, TN.

Hagerty, A. M., A. L. Kilpatrick, S. G. Turnipseed, M. J. Sullivan, and W. C. Bridges. 2005. Predaceous arthropods and lepidopteran pests on conventional, Bollgard, and Bollgard II cotton under untreated and disrupted conditions. Environ. Entomol. 34: 105–114.

Hossain, F., C. E. Pray, Y. Lu, J. Huang, C. Fan, and R. Hu. 2004. Genetically modified cotton and farmers' health in China. Int. J. Occup. Environ. Health. 10: 296–303.

Huang, J., R. Hu, C. Pray, F. Qiao, and S. Rozelle. 2003. Biotechnology as an alternative to chemical pesticides: a case study of *Bt* cotton in China. Agric. Econ. 29: 55–67.

James, C. 2002. Global review of commercialized transgenic crops: 2001. Feature: *Bt* cotton. ISAAA, Ithaca, NY.

James, C. 2004. Preview: global status of commercialized biotech/GM crops: 2004. ISAAA, Ithaca, NY.

McGriff, E., and J. R. Ruberson. 1999. A beltwide rating of beneficial insects. Proceedings of the 1999 Beltwide Cotton Conference, National Cotton Council, 3–7 Jan., Memphis, TN.

Men, X., F. Ge, X. Liu, and E. N. Yardim. 2003. Diversity of arthropod communities in transgenic *Bt* cotton and non-transgenic cotton agroecosystems. Environ. Entomol. 32: 270–275.

Mendelsohn, M., J. Kough, Z. Vaituzis, and K. Matthews. 2003. Are *Bt* crops safe? Nature Biotechnol. 21: 1003–1009.

Naranjo, S. E. 2005a. Non-target effects of *Bt* transgenic cotton: natural enemy abundance. Environ. Entomol. 34: 1193–1210.

Naranjo, S. E. 2005b. Nontarget effects of *Bt* transgenic cotton: function of the natural enemy community. Environ. Entomol. 34: 1211–1223.

Parker, C. D., and R. G. Luttrell. 1998. Oviposition of tobacco budworm (Lepidoptera: Noctuidae) in mixed plantings of non-transgenic and transgenic cottons expressing δ-endotoxin protein of *Bacillus thurigiensis* (Berliner). Southwest. Entomol. 23: 247–257.

Reed, G. L., A. S. Jensen, J. Riebe, G. P. Head, and J. J. Duan. 2001. Transgenic *Bt* potato and conventional insecticides for Colorado potato beetle management: comparative efficacy and non-target impacts. Entomol. Exp. Appl. 100: 89–100.

SAS Institute. 1995. JMP statistics and graphics guide, version 3. SAS Institute, Cary, NC.

SAS Institute. 2001. SAS, version 8.2. SAS Institute, Cary, NC.

Shepard, M., G. R. Carner, and S. G. Turnipseed. 1974. A comparison of three sampling methods of arthropods in soybean. Environ. Entomol. 3: 227–232.

Sisterson, M. S., R. W. Biggs, C. Olson, Y. Carriere, T. J. Dennehy, and B. E. Tabashnik. 2004. Arthropod abundance and diversity in *Bt* and non-*Bt* cotton fields. Environ. Entomol. 33: 921–929.

Stewart, S. D., J. J. Adamczyk, K. S. Knighten, and F. M. Davis. 2001. Impact of *Bt* cotton expressing one or two insecticidal proteins of *Bacillus thuringiensis* Berliner on growth and survival of noctuid (Lepidoptera) larvae. J. Econ. Entomol. 94: 752–760.

Torres, J. B., and J. R. Ruberson. 2005. Canopy- and ground-dwelling predatory arthropods in *Bt* and non-*Bt* cotton fields: patterns and mechanisms. Environ. Entomol. 34: 1242–1256.

Turnipseed, S. G., M. J. Sullivan, A. Hagerty and R. Ridge. 2001. Cotton as a model IPM system in the southeast: a dream or potential reality. Proc. 2001 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Whitehouse, M.E.A., L. J. Lewis, and G. P. Fitt. 2005. A comparison of arthropod communities in transgenic *Bt* and conventional cotton in Australia. Environ. Entomol. 34: 1224–1241.

Wu, K. M., and Y. Y. Guo. 2004. The evolution of cotton pest management practices in China. Annu. Rev. Entomol. 50: 31–52.

Xia, J. Y., J. J. Cui, L. H. Ma, S. X. Dong, and X. F. Cu. 1999. The role of transgenic *Bt* cotton in integrated insect pest management. Acta Gossypii Sinica. 11: 57–64.

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