Evaluation of Transgenic Soybean Exhibiting High Expression of a Synthetic *Bacillus thuringiensis cry1A* Transgene for Suppressing Lepidopteran Population Densities and Crop Injury

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J. Econ. Entomol. 102(4): 1640–1648 (2009)

ABSTRACT Several transgenic lines of soybean, *Glycine max* (L.) Merr., expressing a synthetic *cry1A* gene from *Bacillus thuringiensis* (Bt), were examined in replicated field trials in 2003–2007 for suppression of naturally occurring population densities of lepidopteran pests and the resultant crop injury that they caused. Bt soybean and negative controls (isogenic segregants and parental lines) were evaluated against velvetbean caterpillar, *Anticarsia gemmatalis* (Hübner); soybean looper, *Pseudoplusia includens* (Walker); and green cloverworm, *Hypena scabra* (F.). Population densities of these lepidopteran species were essentially absent in each of the Bt soybean entries evaluated throughout the growing season in every year of the study compared with moderate (5–10 larvae per row-m) to large (20–30 larvae per row-m) peak population densities in the negative control soybean entries. These lepidopteran populations caused significant plant injury in the non-Bt soybean plots, ranging from 53% defoliation in 2003 to 17.5% in 2007, compared with <1.5% defoliation (mostly 0.0% defoliation) in the Bt soybean plots. When two or three foliar insecticides were applied in August or September, as lepidopteran populations approached or exceeded economic threshold levels, pest populations were suppressed and defoliation was minimal in the treated non-Bt entries similar to results in Bt soybean. Soybean 100-seed weights and harvested yields were similar between the Bt and non-Bt entries each year of this study. It seems that Bt transgenic soybean provides excellent season-long control of lepidopteran pests and have yields equal to the standard cultivars examined in this study. Once available to producers, this Bt technology has the potential to provide an effective insect pest management option similar to that being used in Bt cotton, *Gossypium hirsutum* L., and Bt corn, *Zea mays* L., and enhance the sustainability and profitability of soybean production in the southern region where lepidopteran pests cause annual economic losses to the crop.

KEY WORDS *Bacillus thuringiensis*, resistance management, transgenic soybean, *Anticarsia gemmatalis*, *Pseudoplusia includens*

Soybean, *Glycine max* (L.) Merr., is a major agricultural commodity in the United States, with 30.6 million ha planted in 2006, producing a record 86.8 million metric tons, with a total crop value in access of US$19.7 billion (American Soybean Association 2007). Several arthropod pests cause economic losses to the crop annually (Higley and Boethel 1994, Funderburk et al. 1999). Many of these soybean pests are lepidopterans, including soybean looper, *Pseudoplusia includens* (Walker), and velvetbean caterpillar, *Anticarsia gemmatalis* Hübner (Funderburk 1994, Sullivan and Boethel 1994). Other lepidopteran species that have been reported to cause sporadic losses to soybean include corn earworm, *Helicoverpa zea* (Boddie); green cloverworm, *Hypena scabra* (F.); lesser cornstalk borer, *Elasmopalpus lignosellus* (Zeller); and several *Spodoptera* species (armyworms) (Funderburk et al. 1999, MacRae et al. 2005). Insecticides are commonly used throughout the southern and southeastern United States for controlling economic infestations of these lepidopteran pests. Chemical control has generally provided an effective and economical means of pest suppression. However, narrow windows for effective application timing, the development of insecticide resistance, public concerns about reducing overall pesticide use, removal of several of the commonly used standard materials from the market, and increased production costs have lead to a more integrated pest management (IPM) approach that integrates insecticidal control with other tactics and production practices to lessen the dependence on pesticides (Todd et al. 1994, MacRae et al. 2005, Miklos et al. 2007).

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Biological and cultural control options for managing lepidopteran pests on soybean have been investigated but are not widely used as primary control tactics (Pitre 1983, McPherson and Bondari 1991, Yeargan 1994, Luttrell et al. 1998). Developing soybean lines with resistance to lepidopteran feeding also has seen some limited success but not widely incorporated into soybean IPM programs, due primarily to lower yields and undesirable agronomic qualities (Boethel 1999, McPherson and Buss 2007). Recent advances in crop biotechnology have enabled the development of transgenic plants as an efficient and environmentally sound alternative tactic for controlling lepidopteran pests (Parrott et al. 1994, Williams et al. 1998, MacRae et al. 2005, Miklos et al. 2007).

A common soil bacterium found throughout the environment, *Bacillus thuringiensis* (Bt), has been used to control certain insect pests for many years (Adang et al. 1985, Tabashnik et al. 1994). Bt δ-endotoxins (crystal proteins) are highly specific in their insecticidal activity to certain insect orders, including Lepidoptera, and are nontoxic to mammals, making them more environmentally sound than conventional insecticides (McGaughey and Whalon 1992). Bt crystal proteins are activated under alkaline conditions of the insect midgut resulting in the formation of cation channels that lead to osmotic imbalance and lysis of the midgut cells, causing gut paralysis and eventually insect death (Knowles 1994). The transgenic expression of Bt proteins is reportedly very effective for controlling insect pests in several major crop plants, including corn (*Zea mays* L.), rice (*Oryza sativa* L.), cotton (*Gossypium hirsutum* L.), potato (*Solanum* spp.), tomato (*Lycopersicon* spp.), tobacco (*Nicotiana* spp.), soybean, and canola (*Brassica napus* L.) (Miklos et al. 2007), with little or no negative effect on predatory arthropods (French et al. 2004, Head et al. 2005, Torres and Ruberson 2005, Marvier et al. 2007).

The commercial adoption of Bt cotton and Bt corn has significantly reduced lepidopteran injury to these crops (Armstrong et al. 1995, Perlak et al. 2001). Bt soybean varieties are not commercially available at the present time; however, some experimental lines have been developed (Parrott et al. 1994, Walker et al. 2000, MacRae et al. 2005, McPherson and MacRae 2009). This study was conducted in 2003–2007 to examine several Bt soybean lines for season-long suppression of naturally occurring populations of velvetbean caterpillar, soybean looper, and green cloverworm, and the resultant crop injury that they caused. Comparisons also were made in lepidopteran abundance, crop injury, and yields between Bt soybean lines, non-Bt lines, and insecticide-treated non-Bt lines, to assess the effectiveness of Bt transgene technology as an effective soybean IPM tactic.

Materials and Methods

The transgenic Bt soybean lines used in this study were created using *Agrobacterium*-mediated DNA transfer into selected soybean cultivars by using techniques described by Hinchee et al. (1988). All transgenic lines were determined to contain one intact copy of the Bt gene *tic107*, a synthetic cry*1A* construct similar to Cry1Ac (Fischhoff and Perlak 1996), as confirmed by Southern blot analysis (data not shown). No marker genes or other plasmid DNA sequences outside of the transfer DNA region were present in any of the transgenic lines, again as confirmed by Southern blot analysis. Isogenic Bt-negative lines were derived for each line through Mendelian segregation (MacRae et al. 2005).

In 2003, five maturity group V soybean entries were evaluated, three containing homozygous T_4 plants of Bt lines GM_A19487, GM_A19459, and GM_A19478 plus two Bt negative lines (isoline GM_A19459 and the parental 'Asgrow A5547′) as negative controls. All entries were planted on 28 May at the University of Georgia Lang Research Farm in Tift County, GA. The entries were planted in a randomized complete block design with four replications in plots four rows wide (0.3-m row spacing) by 9.15 m in length. Before planting, the ground was turned and bedded and a preplant incorporation of pendimethalin herbicide (Prowl 3.3, 1.12 kg [AI] ha^{-1}, BASF Corp., Research Triangle Park, NC) was applied on 23 May. Fertilizer (3% N, 9% P_2O, and 18% K_2O, Fletcher Limestone, Tifton, GA) was applied broadcast in mid-May at a rate of 561 kg ha^{-1}. Companion randomized plots with the same five soybean entries were planted adjacent to the test site and these plots were treated with l-cyhalothrin insecticide (Karate Z, 0.018 kg [AI] ha^{-1}, Syngenta, Greensboro, NC) on 14 August and bifenthrin insecticide (Capture 2 EC, 0.11 kg [AI] ha^{-1}, FMC Corp., Philadelphia, PA) on 5 September when lepidopteran pest populations approached economic threshold levels in the untreated non-Bt entries. The insecticide sprays were applied with a CO_2-powered backpack sprayer that delivered 187 liters ha^{-1} at 276 kPa with two TX-12 nozzles per row. A split-plot arrangement of the five soybean entries could have been used in this experiment; however, the overall treatment effects (i.e., do insecticides reduce lepidopteran) and the overall soybean entry effects (does Bt soybean have fewer lepidopteran than non-Bt soybean) in a split-plot arrangement would not provide specific soybean entry comparisons under naturally occurring, untreated, lepidopteran populations. The two insecticide applications, in the treated test, were applied to determine whether the five soybean entries responded similarly when lepidopteran populations and resultant crop injury were suppressed and/or when the lepidopteran pest complex was changed due to insecticidal impacts.

Each plot was sampled every 7–10 d for lepidopteran pests once the soybean reached the V5 growth stage (four uncurled trifoliolates; Teare and Hodges 1994) and continued until the R7 growth stage (beginning maturity; Teare and Hodges 1994) by taking two 1-m ground cloth samples from a single row (Kogan and Pitre 1980). All lepidopteran caterpillars collected in each sample were identified to species and counted. At maturity, all plots were harvested with a plot combine, and seed weights and yields were determined. During the July–September sampling dates, all plants in each plot were visually rated for percentage of defoliation, in increments of 5%, and a plot estimate was determined for percentage of foliage removed. Once all defoliation estimates were obtained, each plot was examined a second time and the defoliation estimates were compared between plots to ensure that the estimates were relative to the other plots. This method of visual defoliation has proven to be a reliable estimate of relative defoliation among treatments compared with measured leaf area removed using an area meter (McPherson et al. 1996) or compared with photographs of similar leaves with known percentage of defoliation (Kogan and Kuhlman 1982, All et al. 1989). All insect count, percentage of defoliation, seed weight, and yield data were subjected to analysis of variance (ANOVA) statistical procedures of SAS, and significant treatment means ($P < 0.05$) were separated using the Waller–Duncan K-ratio *t*-test (SAS Institute 1990). Before analysis, all defoliation percentages were transformed to the square root of the arcsine, and both the percentages and the transformed percentages were analyzed. Voucher specimens of the lepidopteran caterpillars were deposited in the University of Georgia Tifton Campus Arthropod Collection.

In 2005, four maturity group V soybean entries were evaluated. The three Bt-positive entries consisted of F_6 progeny of crosses between the three Bt lines GM_A19459, GM_A19478, and GM_A19487, and the genotypically similar soybean Agrow5602 containing the commercial Roundup Ready (RR) transgene, with all progeny homozygous positive for both Bt and RR. A5602 soybean was used as a negative control. All entries were planted on 20 May at the Lang Research Farm. Plots were four rows wide (0.3-m row spacing) by 9.15 m long planted in a randomized complete block design with four replications. Seed bed preparation, preplant herbicide treatments, and fertilizer applications were identical to those reported for the 2003 experiment. Companion randomized plots were planted adjacent to the test site and treated with insecticides using a CO_2-powered sprayer as reported in 2003. However, in the 2005 test, esfenvalerate (Asana XL, 0.04 kg [AI] ha^{-1}, DuPont Crop Protection, Newark, DE) was applied on 29 July, spinosad (Tracer 4 SC, 0.07 kg [AI] ha^{-1}, Dow AgroSciences, Indianapolis, IN) was applied on 16 August, and gamma-cyhalothrin (Prolex 1.25, 0.01 kg [AI] ha^{-1}, Dow AgroSciences) was applied on 26 August in the treated test site. Plot sampling, percentage of defoliation estimates, seed weights, and yields also were obtained as reported for the 2003 trial. All insect count, defoliation (transformed and nontransformed data), seed weight, and yield data were analyzed with ANOVA, and significant ($P < 0.05$) treatment means were separated with the Waller–Duncan K-ratio *t*-test (SAS Institute 1990).

In 2006, four maturity group V soybean entries were evaluated. The Bt-positive entry consisted of F_7 progeny of a cross between the Bt line GM_A19459 and A5602, with progeny homozygous positive for both the Bt and RR transgenes. The remaining three entries were the Bt-negative A5602 cultivar treated with insecticides whenever larval populations of target leipdopteran reached economic threshold (trt ET), whenever target lepidopterans were present (trt present), or left untreated. No insecticides for target lepidopterans were applied to the Bt soybean entry. All entries planted on 24 May at the Lang Research Farm. Plots were four rows wide (0.3-m row spacing) by 9.15 m long planted in a randomized complete block design with four replications. A second experiment with these same soybean entries and planting design was planted on 7 July 2006. The seed bed preparation, preplant herbicide and fertilizer applications for these two plantings were identical to those reported previously. The A5602 plots treated at economic threshold (eight caterpillars per 0.3 row m, Jones and McPherson 2003) were treated with spinosad (0.07 kg [AI] ha^{-1}) on 30 August in the early planting and 8 September in the late planting. The A5602 plots treated whenever caterpillars were present were treated five times in the May planting and four times in the July planting. The insecticides included spinosad (0.07 kg [AI] ha^{-1}), thiodicarb (Larvin 3.2, 0.67 kg [AI] ha^{-1}, Bayer CropScience, Indianapolis, IN), indoxacarb (Steward 1.25 EC, 0.065 kg [AI] ha^{-1}, DuPont Crop Protection), spinosad (0.07 kg [AI] ha^{-1}), and thiodicarb (0.67 kg [AI] ha^{-1}), in this order, when up to five sprays were made. These foliar insecticide applications were applied with a CO_2-powered backpack sprayer that delivered 187 liters ha^{-1} at 276 k Pa with two TX-12 nozzles per row. Only border rows between the four soybean test entries were sampled every 7 d in both planting date trials by taking eight 1-m random samples from each planting date each week. Thus, no lepidopteran population density comparisons could be made between entries within planting date. However, the mean populations (seven weekly samples between 17 August and 28 September) and peak populations on 21 September were compared between the two planting dates by using a *t*-test. Plot sampling, percentage of defoliation, seed weights, yield, and statistical analyses for each planting date in 2006 were identical to the protocols reported for the 2003 test.

In 2007, two soybean experiments were conducted. One trial contained two maturity group V entries consisting of BC_2F_4 progeny of crosses between the GM_A19459 or A5547 recurrent parents and the Asgrow A3244 containing an experimental RR transgene. Thus, the homozygous Bt-positive entry (designated GM_A19459/RR) and the Bt-negative control (designated A5547/RR) were generated from separate backcrossing paths, resulting in similar but not isogenic entries. The second trial contained two maturity group VII entries (more southern adapted soybean maturity group with a longer growing season, Teare and Hodges 1994), consisting of BC_2F_4 progeny of a cross between the GM_A19459 recurrent parent and the commercial RR soybean 'AW7110'. One homozygous Bt-positive entry and one Bt-negative control were isolated from a single backcrossing path, resulting in near isogenicity

Table 1. Mean ± SEM lepidopteran larvae and peak larvae (3 September) per row-meter, percentage of defoliation, 100-seed weights, and yield (13% moisture) of five Bt-positive (+) or Bt-negative (−) soybean entries either treated with foliar insecticides for target lepidopteran pests or left untreated, Tift County, GA, 2003

Soybean entry	Lepidoptera per row-m[a]		% defoliation		100-seed wt (g)	Yield (kg/ha)
	Mean	Peak	5 Sept.	23 Sept.		
Untreated						
GM_A19487 (+)	0.3 ± 0.4b	1.0 ± 0.5b	0.3 ± 0.5b	0.8 ± 1.0b	15.7 ± 0.7a	2,405 ± 397a
GM_A19459 A (−)	11.6 ± 9.0a	29.0 ± 5.3a	26.3 ± 2.5a	52.5 ± 5.0a	15.6 ± 0.6a	2,553 ± 606a
GM_A19459 B (+)	0.3 ± 0.4b	1.0 ± 0.3b	0.1 ± 0.3b	0.1 ± 0.3b	16.0 ± 1.5a	2,243 ± 943a
GM_A19478 (+)	0.4 ± 0.3b	0.5 ± 0.3b	0.6 ± 0.5b	1.4 ± 0.5b	16.3 ± 0.9a	2,701 ± 701a
A5547 (−)	6.5 ± 6.0a	18.8 ± 3.5a	22.5 ± 5.0a	53.8 ± 4.8a	15.1 ± 1.2a	2,245 ± 451a
Treated[b]						
GM_A19487 (+)	0.6 ± 0.4b	1.3 ± 0.3b	0.0 ± 0.0c	0.3 ± 0.5b	16.6 ± 0.9a	2,466 ± 303a
GM_A19459 A (−)	4.5 ± 3.3a	8.8 ± 2.1a	4.0 ± 1.2b	6.3 ± 1.4a	16.3 ± 1.2a	2,614 ± 566a
GM_A19459 B (+)	0.4 ± 0.3b	0.8 ± 0.3b	0.0 ± 0.0c	0.1 ± 0.3b	17.4 ± 0.9a	2,216 ± 263a
GM_A19478 (+)	0.5 ± 0.4b	0.8 ± 0.5b	0.0 ± 0.0c	0.0 ± 0.0b	17.0 ± 0.6a	2,695 ± 613a
A5547 (−)	3.7 ± 4.0a	8.5 ± 1.0a	5.0 ± 0.0a	6.9 ± 2.4a	16.2 ± 1.1a	2,560 ± 411a

Column means within each treatment category followed by the same letter are not significantly different, Waller–Duncan K-ratio *t* test ($P = 0.05$).

[a] Mean from seven weekly samples taken between 8 August and 24 September, when populations were present.

[b] Foliar insecticide treatments included Karate Z applied on 14 August and Capture 2EC applied on 5 September.

outside of the Bt transgene. Each of the two entries in both tests also had an insecticide-treated plot to suppress naturally occurring populations of target pests as well; thus, both tests had a total of four soybean entries. Plots were four rows wide (0.3-m row spacing) by 9.15 m long, planted on 11 June in a randomized complete block design with four replications, in both trials. Three foliar insecticide sprays were applied to the treated plots in both tests on 9 August (zeta-cypermethrin, 0.22 kg [AI] ha^{-1}, FMC Corp.), 16 August (spinosad, 0.07 kg [AI] ha^{-1}) and 11 September (indoxacarb, 0.065 kg [AI] ha^{-1}) to suppress the naturally occurring populations of lepidopteran pests. These sprays were applied with a CO_2-powered backpack sprayer that delivered 187 liters ha^{-1} at 276 k Pa. The protocols for sampling, percentage of defoliation, seed weights, yields, and statistical analyses for both soybean maturity group trials in 2007 were identical as those reported for the 2003 test.

Results and Discussion

In the 2003 trial, mean lepidopteran larvae per row-meter was significantly lower ($F = 4.81$; df = 4, 24; $P < 0.01$) on the three untreated Bt soybean entries than on the two untreated non-Bt entries during the seven weekly samples taken between 8 August and 24 September, when population densities were two or more larvae per row-meter in the non-Bt entries (Table 1). Peak population densities on 3 September also were significantly lower in the three Bt entries ($F = 30.79$; df = 4, 12; $P < 0.01$). The percentage of defoliation also was significantly lower in the Bt entries on 5 September ($F = 44.23$; df = 4, 12; $P < 0.01$) and 23 September ($F = 67.0$; df = 4, 12; $P < 0.01$). Seed weights and yields were not different between the five untreated soybean entries (Table 1). The two insecticide applications, applied in the treated plots on 14 August and 5 September, when lepidopteran larvae were increasing in the non-Bt soybean entries, reduced the mean and peak larval populations in the two non-Bt entries. The three treated Bt entries had significantly lower mean larvae ($F = 3.10$; df = 4, 24; $P < 0.034$), peak larvae on 3 September ($F = 18.10$, df = 4, 12; $P < 0.01$), defoliation on 5 September ($F = 93.0$; df = 4, 12; $P < 0.01$), and defoliation on 23 September ($F = 27.66$; df = 4, 12; $P < 0.01$) than the treated non-Bt entries (Table 1). Seed weights and yields were not different between the five treated soybean entries (Table 1). The mean population density (seven sampling dates) of lepidopteran larvae in the untreated plots in 2003 consisted primarily of soybean looper (60.3%), but some velvetbean caterpillar (35.5%) and green cloverworm (4.2%) also were counted. During the population peak on 3 September, the lepidopteran complex consisted of 49.0% soybean looper and 51% velvetbean caterpillar. In the treated plots, the mean larval population density (seven dates) consisted of 98.7% soybean looper and 1.3% velvetbean caterpillar, whereas the population peak on 3 September was 100% soybean looper. It is apparent that the two insecticide applications not only suppressed the lepidopteran larvae (29.0 larvae per row-m in the untreated GM_A19459 isoline versus 8.8 larvae per row-m in the treated GM_A19459 isoline; Table 1), but also greatly influenced the composition of lepidopteran species in the complex. The complex consisted almost exclusively of soybean looper in the treated plots but only 49.0% soybean looper in the untreated plots at the population peak on 3 September. The three Bt entries were very effective in suppressing the lepidopteran species present in both the untreated plots (soybean looper and velvetbean caterpillar) and the treated plots (soybean looper).

In the 2005 trial, mean lepidopteran larvae ($F = 34.36$; df = 3, 18; $P < 0.01$) from seven sampling dates and peak larvae ($F = 24.92$; df = 3, 9; $P < 0.01$) on 31 August were significantly lower on the three untreated Bt soybean entries than on the untreated A5602 soybean (Table 2). Results of statistical analyses on lepidopteran larvae on the treated soybean entries were similar to those in the untreated test; however,

Table 2. Mean ± SEM lepidopteran larvae and peak larvae (31 August) per row-meter, percentage of defoliation, 100-seed weights, and yield (13% moisture) of four Bt-positive (+) or Bt-negative (−) soybean entries either treated with foliar insecticides for target lepidopteran pests or left untreated, Tift County, GA, 2005

Soybean entry	Lepidoptera per row-m[a]		% defoliation		100-seed wt (g)	Yield (kg/ha)
	Mean	Peak	5 Sept.	23 Sept.		
Untreated						
GM_A19459(+)	0.1 ± 0.1b	0.3 ± 0.1b	0.1 ± 0.3b	0.0 ± 0.0b	13.8 ± 0.9a	2,371 ± 283a
GM_A19478 (+)	0.2 ± 0.2b	0.6 ± 0.2b	0.0 ± 0.0b	0.0 ± 0.0b	12.5 ± 0.8bc	2,364 ± 202a
GM_A19487 (+)	0.0 ± 0.0b	0.0 ± 0.0b	0.1 ± 0.3b	0.0 ± 0.0b	13.4 ± 0.5ab	2,787 ± 385a
A5602 (−)	6.2 ± 2.5a	11.8 ± 4.5a	26.3 ± 6.3a	42.5 ± 2.9a	11.8 ± 0.6c	2,513 ± 397a
Treated[b]						
GM_A19459 (+)	0.1 ± 0.1b	0.1 ± 0.1b	0.0 ± 0.0b	0.0 ± 0.0b	14.4 ± 0.4a	2,647 ± 377a
GM_A19478 (+)	0.2 ± 0.2b	0.5 ± 0.2b	0.0 ± 0.0b	0.0 ± 0.0b	14.3 ± 0.1a	2,661 ± 300a
GM_A19487 (+)	0.1 ± 0.2b	0.4 ± 0.2b	0.0 ± 0.0b	0.0 ± 0.0b	13.4 ± 1.4b	2,796 ± 377a
A5602 (−)	1.7 ± 0.5a	3.8 ± 1.5a	8.8 ± 3.0a	10.0 ± 4.1a	12.9 ± 0.6b	2,696 ± 458a

Column means within each treatment category followed by the same letter are not significantly different, Waller–Duncan K-ratio *t* test (P = 0.05).

[a] Mean from seven weekly samples taken between 4 August and 29 September, when larval populations were present.

[b] Foliar insecticide treatments included Asana XL applied on 29 July, Tracer 4 SC applied on 16 August, and Prolex 1.25 applied on 26 August.

the mean larvae (F = 26.82; df = 3, 18; $P < 0.01$) and peak larvae (F = 4.63; df = 3, 9; P = 0.032) were much lower when the three insecticides were applied (Table 2). Percentage of defoliation also was significantly lower in the untreated Bt entries than in the untreated A5602 soybean (5 September: F = 67.53; df = 3, 9; $P <$ 0.01; 23 September: F = 467.0; df = 3, 9; $P < 0.01$) and also in the treated test (5 September: F = 33.47; df = 3, 9; $P < 0.01$; 23 September: F = 27.05; df = 3, 9; $P <$ 0.01). Defoliation was essentially nonexistent in both the treated and nontreated Bt entries in 2005, whereas defoliation in the A5602 soybean reached 42.5% in the untreated plots and 10% in the treated plots. The mean lepidopteran larval population in the untreated test consisted of 50.1% velvetbean caterpillar, 47.7% soybean looper, and 2.2% green cloverworm. During the population peak on 31 August, the population consisted of 67.8% velvetbean caterpillar and 32.2% soybean looper. In the treated test, mean lepidopteran larval density consisted of 30.8% velvetbean caterpillar, 67.4% soybean looper, and 1.8% green cloverworm, whereas the population peak was 90.9% soybean looper and 9.1% velvetbean caterpillar. As in the 2003 experiment, the insecticide sprays applied to all four entries (three Bt and one non-Bt) greatly reduced the lepidopteran populations and shifted the population complex to include more soybean looper than velvetbean caterpillar. The three Bt entries effectively suppressed both population complexes, i.e., the untreated natural population of soybean looper plus velvetbean caterpillar and the treated population of primarily soybean looper. Seed weights in the Bt entries GM_A19459 and GM_A19487 were higher than for A5602 soybean (F = 6.94; df = 3, 9; P = 0.01) in the untreated test, whereas those of the Bt entries GM_A19459 and GM_A19478 were higher than the Bt A19487 and the A5602 soybean (F = 5.09; df = 3, 9; P = 0.025) in the treated test (Table 2). Yields were not significantly different between the Bt entries and the A5602 soybean in either the untreated or treated test (Table 2).

In 2006, the mean lepidopteran larvae (7 wk in August and September) on soybean border rows of the A5602 soybean planted in May was higher than in the A5602 border rows planted in July (t = 3.38, df = 3, P = 0.024); however, the peak densities on 21 September were not significantly different between planting dates (Table 3). Overall, lepidopteran populations were low in both planting dates throughout the 2006 season. The mean lepidopteran larval population in the May planting consisted of 63.4% velvetbean caterpillar, 29.5% soybean looper, and 7.4% green cloverworm, whereas in the July planting it was 62.9% soybean looper, 34.8% velvetbean caterpillars, and 2.3% green cloverworm. The population peaks on 21 September consisted of 50.7% velvetbean caterpillar and 49.3% soybean looper in the May planting and 82.6% soybean looper and 17.4% velvetbean caterpillar in the July planting. The date-of-planting in the 2006 trials thus had a significant impact on the mean population of lepidopteran larvae and also the composition of species in the complex, similar to the insecticidal effects noted in the 2003 and 2005 experiments. Percentage of defoliation was significantly lower in the Bt entry and the treated A5602 soybean when larvae were present in both the May planting (5 September: F = 38.08; df = 3, 12; $P < 0.01$; 23 September: F = 195.05; df = 3, 12; $P < 0.01$) and in the July planting (8 September: F = 24.88; df = 3, 12; $P < 0.01$; 29 September: F = 149.27; df = 3, 12; $P < 0.01$) (Table 3). Seed weights were higher in the Bt GM_A19459 entry in both the May (F = 13.78; df = 3, 12; $P < 0.01$) and July (F = 10.38; df = 3, 12; $P < 0.01$) plantings (Table 3). Yields were not different between the four soybean entries in either planting date, although there was a trend for higher yields in the A5602 soybean treated five times (lepidopteran larvae present) in the May planting (F = 3.04; df = 3, 12; P = 0.07). The overall yields in the July planting were reduced by ≈33% compared with yields in the May planting. This is not surprising because the 7 July planting was much later than the 10 May–10 June optimum dates for planting soybean in Georgia (Woodruff et al. 2009).

In the maturity group V soybean trial in 2007, the mean lepidopteran population (F = 19.04; df = 3, 18; $P < 0.01$) and the peak population (F = 35.33; df = 3,

Table 3. Mean ± SEM lepidopteran larvae and peak larvae (21 September) per row-meter in field borders, plus percentage of defoliation, 100-seed weights, and yield (13% moisture) of four Bt-positive (+) or Bt-negative (−) soybean entries produced under different insect management regimes when planted on either 24 May or 3 July, Tift County, GA, 2006

Soybean entry[a]	Lepidoptera per row-m[b]		% defoliation[c]		100-seed wt (g)	Yield (kg/ha)
	Mean	Peak	5 Sept.	23 Sept.		
Planted on 24 May						
GM_A19459 untrt (+)			0.0 ± 0.0c	0.0 ± 0.0c	17.4 ± 0.9a	4,123 ± 242a
A5602 trt ET (−)			11.5 ± 3.6b	13.0 ± 2.7b	15.7 ± 0.8b	4,190 ± 115a
A5602 trt present (−)			1.0 ± 0.0c	1.1 ± 0.2c	15.3 ± 0.6b	4,433 ± 121a
A5602 untreated (−)	6.0 ± 2.6a	8.6 ± 3.3a	17.0 ± 4.5a	27.0 ± 2.7a	14.9 ± 0.6b	4,210 ± 88a
Planted on 7 July						
GM_A19459 untrt (+)			0.0 ± 0.0b	0.0 ± 0.0c	14.5 ± 0.5a	2,856 ± 155a
A5602 trt ET (−)			6.0 ± 1.4a	12.5 ± 2.5b	13.3 ± 0.3b	2,721 ± 290a
A5602 trt present (−)			1.9 ± 0.5b	1.3 ± 0.4c	13.5 ± 0.6b	2,843 ± 357a
A5602 untreated (−)	3.1 ± 2.0b	5.8 ± 6.0a	7.5 ± 3.1a	24.0 ± 4.2a	13.1 ± 0.3b	2,540 ± 168a

Column means for each planting date with the same letter are not significantly different, Waller–Duncan K-ratio t test ($P = 0.05$).

[a] Insect management regimes included no insecticides applied (untrt), treated with foliar insecticide at economic threshold (trt ET) for lepidopteran larvae, or treated when lepidopteran larvae were present (trt present) to prevent defoliation.

[b] Mean from seven weekly samples taken from border rows between the soybean entries during the period of 17 August through 28 September, when larval populations were present. Mean densities were significantly different between the two planting dates ($P = 0.05$; t-test).

[c] Percentage of defoliation estimates for the 3 July planting were taken on 8 and 29 September.

9; $P < 0.01$) were higher on the untreated A5547 soybean (Bt-negative control) than on the other soybean entries (Table 4). The untreated Bt entry (also the treated Bt entry) had very low lepidopteran populations on all sampling dates. The mean lepidopteran larval population (seven weekly samples) consisted of 66.9% soybean looper and 33.1% velvetbean caterpillar, whereas the peak lepidopteran population density on 12 September consisted of 51.5% velvetbean caterpillar and 48.5% soybean looper. These population densities caused 7.5% defoliation in the untreated A5547 soybean on 6 September ($F = 60.47$; df = 3, 9; $P < 0.01$) and 17.5% on 20 September ($F = 137.21$; df = 3, 9; $P < 0.01$), significantly higher levels than in the two Bt entries and the treated A5547 entry (Table 4). Seed weights and yields were not significantly different between the four soybean entries.

In the maturity group VII soybean trial in 2007, the mean lepidopteran population density ($F = 23.64$; df = 3, 18; $P < 0.01$) and the peak population density ($F = 387.0$; df = 3, 9; $P < 0.01$) were significantly higher on the untreated non-Bt entry than on the other three soybean entries (Table 5). Very few lepidopteran larvae were observed on the Bt entry on any sampling date, whereas densities were light on the treated non-Bt entry. Soybean looper accounted for 64.3% of the mean lepidopteran population during the seven weekly samples taken between 15 August and 26 September, whereas 34.5% were velvetbean caterpillar and 1.2% were green cloverworm. During the population peak, 70.7% of the lepidopteran larvae were soybean looper and 29.3% were velvetbean caterpillar. Percentage of defoliation was significantly higher in the untreated non-Bt entry than in the other three soybean entries on 13 September ($F = 156.7$; df = 3, 9; $P < 0.01$) and 27 September ($F = 485.8$; df = 3, 9; $P < 0.01$), but seed weights and crop yields were not different between the four soybean entries (Table 5).

The results obtained from this series of experiments conducted in 2003–2007 document the performance of Bt soybean, developed by Monsanto Company, to effectively suppress larval populations of velvetbean caterpillar, soybean looper, and green cloverworm on the crop throughout the entire growing season. Lepidopteran populations rarely exceeded one larva per row-m in the Bt plots on any sampling date during this multiyear project, even during population peaks when untreated non-Bt entries contained from 10 to 29 larvae per row-m. The cumulative percentage of defoliation also was very low in every Bt soybean entry (from 0.0 to 1.4% across all trials) compared with the untreated non-Bt entries ranging from 17.5% in 2007 to

Table 4. Mean ± SEM lepidopteran larvae and peak larvae (12 September) per row-meter, percentage of defoliation, 100-seed weights, and yield (13% moisture) in maturity group five Bt-positive (+) or Bt-negative (−) soybean entries either treated with foliar insecticides for target lepidopteran pests (T) or left untreated (UT), Tifton, GA, 2007

Soybean and treatment	Lepidoptera per row-m		% defoliation		100-seed wt (g)	Yield (kg/ha)
	Mean	Peak	6 Sept.	20 Sept.		
GM_A19459/RR_2 (+) UT	0.1 ± 0.1b	0.3 ± 0.3c	0.0 ± 0.0c	0.0 ± 0.0b	17.0 ± 0.5a	2,937 ± 121a
A5547/RR_2 (−) UT	5.4 ± 2.8a	8.3 ± 2.7a	7.5 ± 0.5a	17.5 ± 2.9a	15.9 ± 0.6a	2,890 ± 411a
GM_A19459/RR_2 (+) T	0.2 ± 0.7b	0.4 ± 0.2c	0.0 ± 0.0c	0.0 ± 0.0b	16.4 ± 0.7a	2,977 ± 478a
A5547/RR_2 (−) T	1.1 ± 1.3b	3.4 ± 0.8b	1.4 ± 0.1b	2.4 ± 0.5b	15.9 ± 0.7a	3,166 ± 445a

Lepidopteran larvae include velvetbean caterpillar, soybean looper, and green cloverworm, combined. Mean from seven weekly samples taken between 15 August and 26 September, when larval populations were present. Column means with the same letter are not significantly different, Waller–Duncan K-ratio t-test ($P = 0.05$). Soybean entries that were treated (T) received foliar insecticide sprays on 9 and 16 August and 11 September.

Table 5. Mean ± SEM lepidopteran larvae and peak larvae (12 September) per row-meter, percentage of defoliation, 100-seed weights, and yield (13% moisture) in maturity group seven Bt-positive (+) or Bt-negative (−) soybean entries either treated with foliar insecticides for target lepidopteran pests (T) or left untreated (UT), Tifton, GA, 2007

Soybean and treatment	Lepidoptera per row-m		% defoliation		100-seed wt (g)	Yield (kg/ha)
	Mean	Peak	13 Sept.	27 Sept.		
GM_A19459/RR (+) UT	0.3 ± 0.3b	0.8 ± 1.5c	0.0 ± 0.0b	0.0 ± 0.0c	16.3 ± 0.2a	2,445 ± 168a
GM_A19459/RR (−) UT	10.4 ± 3.6a	14.8 ± 4.6a	11.9 ± 2.4a	32.5 ± 2.9a	16.2 ± 0.3a	2,203 ± 673a
GM_A19459/RR (+) T	0.2 ± 0.2b	0.3 ± 0.5c	0.0 ± 0.0b	0.0 ± 0.0c	15.4 ± 0.8a	2,371 ± 222a
GM_A19459/RR (−) T	2.5 ± 2.9b	7.5 ± 1.3b	2.0 ± 0.8b	2.6 ± 0.5b	15.2 ± 0.8a	2,445 ± 135a

Lepidopteran larvae include velvetbean caterpillar, soybean looper, and green cloverworm, combined. Mean from seven weekly samples taken between 15 August and 26 September, when larval populations were present. Column means with the same letter are not significantly different, Waller–Duncan K-ratio *t*-test ($P = 0.05$). Soybean entries that were treated (T) received foliar insecticide sprays on 9 and 16 August and 11 September.

53.8% in 2003. Yields of the transgenic Bt soybean lines were equal to the non-Bt cultivars examined alongside them in these replicated field trials. Non-Bt soybean entries treated with insecticides from 2 to 5 times during the season did have lower populations of lepidopteran larvae and percentage of defoliation than their untreated non-Bt counterparts, similar to the results observed in the Bt entries. However, these repeated insecticide applications are costly, hazardous to humans and the environment, and can lead to reduced effectiveness due to insecticidal resistance development (Todd et al. 1994), especially in soybean looper populations exposed to insecticides (Felland et al. 1990, Mascarenhas and Boethel 1997).

The naturally occurring lepidopteran pest infestations observed in every year of this study were sufficient to cause significant differences in defoliation levels between the Bt and non-Bt entries and between the insecticide treated and nontreated entries. However, soybean yield differences were not significant between the Bt and non-Bt or the insecticide and no-insecticide comparisons. This lack of yield response to lower pest injury in the Bt and insecticide-treated entries is not surprising due to the overall low defoliation levels that were observed most years of this study (the highest defoliation levels ranged from 17.5 to 53.8% in the 4 yr), combined with the fact that most of the defoliation occurred during late season when pods were already filled with seeds. Minimal yield reductions are expected in such circumstances (Beard et al. 1994). Nevertheless, all of the Bt entries examined during this study effectively controlled the lepidopteran species observed and the defoliation they caused, specifically velvetbean caterpillar, soybean looper, and green cloverworm.

The excellent efficacy of these Bt soybean lines against multiple lepidopteran pests confirms earlier reports that this technology is an effective pest management tactic for the soybean crop (Parrott et al. 1994, Walker et al. 2000, Baur et al. 2003, MacRae et al. 2005, Miklos et al. 2007, McPherson and MacRae 2009). Multiyear field studies on arthropod abundance in Bt and non-Bt cotton and corn production systems also have reported on the efficacy of Bt transgenes for controlling arthropod pests (Storer et al. 2001, Sisterson et al. 2004, Head et al. 2005, Ali and Luttrell 2007). Confirming yield performance and other desirable agronomic characteristics within the soybean Bt lines will be required if these entries are to become commercially acceptable. The impact of Bt soybean on the requirements for non-Bt lepidopteran refugia already established for commercial production of Bt cotton and Bt corn in the southeastern United States also will need to be assessed. If made available to producers, Bt soybean transgenic lines have the potential to provide the economic and environmental benefits already being realized by other commercialized Bt crops (MacRae et al. 2005).

Acknowledgments

We are grateful to Del Taylor and Neal Roberson for technical support and to Monsanto Company and the University of Georgia Agricultural Experiment Stations for financial support.

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Received 10 February 2009; accepted 30 April 2009.