Assessing Lepidopteran Abundance and Crop Injury in Soybean Lines Exhibiting a Synthetic *Bacillus thuringiensis* cry1A Gene[1]

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J. Entomol. Sci. 44(2): 120-131 (April 2009)

Abstract Transgenic lines of soybean, *Glycine max* (L.) Merrill, expressing a synthetic *cry 1*A gene from *Bacillus thuringiensis* Berliner *(Bt)*, were evaluated in replicated field trials in 1999-2002 for suppression of lepidopteran pests and the resultant crop injury. Velvetbean caterpillar, *Anticarsia gemmatalis* Hübner, soybean looper, *Pseudoplusia includens* (Walker), and green cloverworm, *Hypena scabra* (F.), population densities were essentially absent throughout the growing season in each year of the study in each of the *Bt* lines evaluated compared with moderate (5-10 larvae per row-m) to heavy (15-20 larvae per row-m) populations in the isogenic and parental lines serving as soybean controls. Significant *A. gemmatalis* larval population reductions were observed in the *Bt* entries compared with the non*Bt* entries in each year of this study. The *P. includens* larval densities were significantly lower in *Bt* entries in the 3 yrs of this study when population densities were abundant, whereas *H. scabra* were lower in the *Bt* lines in the 2 yrs of this study when this species was present. Cumulative defoliation in non*Bt* soybean entries exceeded 95% in some years compared with 0.0-1.6% in the transgenic lines containing *Bt*. Yields of the transgenic soybean lines were equal to or higher than the non*Bt* lines examined in each year of this study. It appears that these *Bt* transgenic soybeans provide superior season-long control of the common lepidopteran pests on soybeans in the southern U.S., resulting in reduced defoliation levels and potentially higher yields compared with equivalent cultivars that lack the *Bt* trait.

Key Words *Bt* soybean, *Anticarsia gemmatalis, Pseudoplusia includens, Hypena scabra*, transgenic soybean

Soybean, *Glycine max* (L.) Merrill, is a major agricultural crop produced in the U.S., with 29.9 million ha planted in 2002 worth over $15 billion to the producers (American Soybean Association 2003). Soybean crops in the southern region of the U.S. are attacked by numerous arthropod pests causing economic losses annually (Funderburk et al. 1999). The velvetbean caterpillar, *Anticarsia gemmatalis* Hübner, and soybean looper, *Pseudoplusia includens* (Walker), are annual economic insect pests in Georgia (Guillebeau et al. 2004) and throughout the southern U.S. (Sullivan and Boethel 1994, Funderburk 1994). Other lepidopteran species that can cause economic losses in the southern U.S. include soybean podworm, *Helicoverpa zea* (Boddie), green cloverworm, *Hypena scabra* (F.), several armyworm species, and

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lesser cornstalk borer, *Elasmopalpus lignosellus* (Zeller) (Funderburk et al. 1999, MacRae et al. 2005).

Insecticidal control is the primary management tactic used to consistently prevent economic crop losses when outbreaks of insect pests occur on soybean (Todd et al. 1994). However, several effective chemistries have been removed from the marketplace in recent years due to label revisions or cancellations associated with environmental concerns or the costly reregistration process (Todd et al. 1994). Also, some lepidopteran pests have developed resistance to some labeled insecticides (Thomas and Boethel 1994, Felland et al. 1990, Leonard et al. 1990). Thus, more costly alternative insecticides and insecticide combinations are now necessary to achieve acceptable control of these insecticide resistant pests. The overall increased public awareness of water quality, air pollution and food processing also mandates the judicious use of widespread applications of insecticides on the soybean crop (Todd et al. 1994). Alternative biological and cultural control options for managing lepidopteran pests on soybeans have been developed but are not widely used due to economic and practical limitations (Luttrell et al. 1998, Yeargan 1994, Fuxa 1994). Developing insect-resistant soybean lines with acceptable yields and agronomic qualities also has not been sufficiently successful to enable widespread adoption within soybean insect pest management programs (Boethel 1999, McPherson and Buss 2007).

Recent advances in crop biotechnology report on transgenic crops that express δ-endotoxins (*Cry* proteins) from *Bacillus thuringiensis* Berliner (*Bt*), a common soil bacterium found throughout the environment (MacRae et al. 2005, Marking 2001, Miklos et al. 2007, Parrott et al. 1994). The adoption of *Bt* cotton, *Gossypium hirsutum* L., and *Bt* corn, *Zea mays* (L.), has reduced the impact of lepidopteran injury to those crops (Perlak et al. 2001, Armstrong et al. 1995). *Bt* soybean varieties are not commercially available at this time; however, some experimental lines have been developed (Miklos et al. 2007, MacRae et al. 2005, Parrott et al. 1994, Walker et al. 2000). Benefits that are expected from Bt technology include lowered insecticide usage, better control of key pests with less field scouting, reduced risks of pest losses due to poorly timed pesticide applications, and improved safety to the applicator (Conner et al. 2004). The objective of this study was to examine several transgenic *Bt* soybean lines for their ability to provide season-long suppression of lepidopteran soybean pests including velvetbean caterpillars, soybean loopers, and green cloverworms and to evaluate the yields of *Bt* lines in the presence of these pest populations against isogene and parental lines lacking the *Bt* trait.

Materials and Methods

The transgenic *Bt* soybean lines used in this study were created using *Agrobacterium*–mediated DNA transfer into selected soybean cultivars using techniques described by Hinchee et al. (1988). The transgenic lines were determined to contain one intact copy of the *Bt* gene *tic 107*, a synthetic *Cry1*A construct similar to *Cry1*Ac (Fischhoff and Perlak 1995). Isogenic *Bt*-negative lines were derived for each line through Mendelian segregation (MacRae et al. 2005). Nine soybean entries, 7 containing *Bt* transgenes and 2 without *Bt* transgenes, were evaluated for lepidopteran abundance and defoliation in replicated field plots in 1999. One *Bt*-positive entry (862) was delivered from 'Thorne' variety soybean, whereas the remaining 6 *Bt*-positive and 2 *Bt*-negative entries were derived from 'A3237' variety soybean. Both of these parental varieties are maturity group 3.2. Plots were established at the University of Georgia

Coastal Plain Experiment Station in Tift Co., GA, and were 4 rows × 10 m in length, spaced 0.3 m apart. The entries were planted on 13 July in a randomized complete block design with 4 replications following the Georgia Cooperative Extension Service soybean production guidelines (Jost 2006). The relatively late planting was included in an attempt to achieve a sensitive stage of plant growth in the early-maturing plants during the expected occurrence of target pests. Prior to 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) plus vernolate herbicide (Vernam 7E, 2.02 kg [AI] ha^{-1}, Zeneca Ag Products, Wilmington, DE) was applied on 12 July. Fertilizer (3% N, 9% P_2O, and 18% K_2O, Fletcher Limestone, Tifton, GA) was applied broadcast in late June at a rate of 561 kg ha^{-1}.

Each plot was sampled every 7-10 d for insect pests beginning in mid-August at the V5 vegetative stage (unifoliate leaves plus 4 uncurled trifoliolate leaves [Teare and Hodges 1994]) and continuing until late September at the beginning of maturity, or the R7 reproductive stage (Teare and Hodges 1994). Two 1-m ground cloth samples were randomly taken down a single row from each plot on each sampling date (Kogan and Pitre 1980). All lepidopteran larvae observed in each sample were identified to species and counted. Also, on each sampling date all plots were examined for plant growth stage (Teare and Hodges 1994). At maturity, the 2 middle rows of each plot were harvested on 8 October with a small plot combine (Almaco Model SPC 20, Nevada, IA), and plot yields were determined. In mid-August, early September and mid-September, all plants in each plot were visually examined for percentage of defoliation, in increments of 5%, and a plot estimate was determined for percentage of foliage removed. Once all plot defoliation estimates were obtained, each plot was examined a second time, and the estimates were compared with the other plots to ensure that the percentages of defoliation were relative to each other. This method of visual defoliation has proven to be a reliable estimate of relative defoliation among treatments compared with measured leaf area removal with 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, defoliation, and yield data were subjected to analysis of variance (ANOVA) (SAS Institute 2004), and significant treatment means were separated using the Waller-Duncan K-ratio *t*-test (SAS Institute 2004). Prior to analysis, all defoliation percentages were transformed to the square root of the arcsine, and both the transformed and nontransformed percentages were analyzed.

In 2000, four soybean entries were planted, 1 containing *Bt* transgenes and 3 without transgenes, at the same Tift Co. site. All entries were of maturity group 3.2, with the transgenic entry and its *Bt*-negetative isoline derived from A3237 soybean. Nontransformed A3237 and the genetically similar 'AG3201' variety soybean were included in the trial as additional *Bt*-negative controls. Plots were 4 rows × 9.2 m in length, spaced 0.3 m apart and planted on 18 July in a randomized complete block design with 4 replications. Seed bed preparation, preplant herbicide and fertilizer applications were made as reported for the 1999 test with the relatively late planting date used to maximize exposure to target pests. Plot sampling for arthropods, plant growth stages, and percentage defoliation estimates were as reported for the 1999 trial. The 2000 test plots were harvested on 10 October, using a small-plot combine to harvest the two center rows of each plot. All insect count, defoliation, and yield data were analyzed with ANOVA (SAS Institute 2004), and treatment means were separated with the Waller-Duncan K-ratio *t*-test.

In 2001, two separate trials were conducted. The first test was planted on 8 June and contained 6 soybean entries, 3 containing *Bt* transgenes and 3 without *Bt*. Entries in this trial included BC_1F_5 progeny of a cross between the *Bt* line 781 and 'F6-8' soybean variety (maturity group 6.0). Near-isogenic *Bt*-postive and *Bt*-negetative lines were generated through segregation, and F6-8 was included in the trial as a negative check. The second test contained 6 different soybean entries, 2 containing *Bt* transgenes and 4 without transgenes, and was planted on 11 July. Entries in this trial included BC_1F_5 progeny of a cross between the *Bt*-positive transgenic line 781 in its parental A3237 background and soybean variety 'Estrella' (maturity group 7.0), in which near-isogenic *Bt*-positive and *Bt*-negative lines were generated through segregation. Estrella and another late-maturity group cultivar ('MS8000' soybean, maturity group 8.0) were included in the trial as additional negative controls. Progeny from this cross were expected to be later maturing than the A3237 parental background. Both test sites contained plots 4 rows × 9.2 m long, spaced 0.3 m apart, and arranged in a randomized complete block design with 3 replications. Seed bed preparation, a preplant incorporated application of pendimethalin herbicide, 1.12 kg [AI] ha^{-1}, and 561 kg ha^{-1} of fertilizer were conducted as previously reported. Plot sampling for arthropods, plant growth stages, percentage defoliation estimates, plot harvesting and statistical analysis of the data, were conducted as previously outlined. The earlier planted trial was harvested (2 center rows) on 8 October, and the later planted trial was harvested on 5 November.

In 2002, four soybean entries were evaluated for insect pest abundance and crop damage, 3 containing *Bt* transgenes and 1 without *Bt*. All entries were derived from 'A5547' soybean variety (maturity group 5.5), with the *Bt*-negative line representing untransformed A5547. The entries were planted on 11 June, in plots 4 rows × 9.2 m wide, spaced 0.3 m apart, and arranged in a randomized complete block design with 4 replications. The seed bed preparation, pendimethalin herbicide, and fertilizer application were similar to the methods reported for the 2001 trials. Plot sampling for arthropods, plant growth stages, defoliation, yield determinations, and statistical analyses were conducted as previously described. The yield samples were harvested on 7 October. Voucher specimens of the lepidopteran caterpillars collected during this study are deposited in the University of Georgia Tifton Campus Arthropod Collection.

Results and Discussion

Velvetbean caterpillar populations were almost completely absent in all 7 soybean lines containing *Bt* transgenes in the 1999 field trial (Table 1). In contrast, the 2 soybean lines without *Bt* transgenes had significantly higher populations of larvae on 31 August ($F = 20.44$; df = 8,24; $P < 0.01$), 8 September ($F = 26.74$; df = 8,24; $P < 0.01$), and 14 September ($F = 34.87$; df = 8,24; $P < 0.01$) (Table 1). Prior to 31 August, larval densities ranged from 0-0.5/row-m in all soybean entries, whereas on 21 September there were only 1.0 and 1.5 per row-m in the A3237 parental variety and A3237-781(–) *Bt*-negative entries, respectively, and no larvae were observed in any of the *Bt* entries. The resulting percentage defoliation from velvetbean caterpillar feeding was significantly higher ($F = 40.03$; df = 8, 24; $P < 0.01$) in the 2 non*Bt* entries (97.5 and 95.0%) than in the 7 *Bt* entries (0.0-0.6%) on 22 September (Table 1). No other lepidopteran larvae were commonly observed at this test site in 1999, thus, plant defoliation could be attributed primarily to velvetbean caterpillar feeding. The plot yields were low in all 9 entries evaluated

Table 1. Larval densities (± SEM) of velvetbean caterpillar (and plant growth stage on each sampling date), percentage defoliation and yield (13% moisture) on nine soybean lines either with *Bt* transgenes (+) or without *Bt* transgenes (–), Tifton, GA, 1999

Soybean Line	Larvae per row-meter			% Defol. 22 Sept.	Yield kg / ha
	31 Aug (R5)	8 Sept (R5)	14 Sept (R6)		
A3237-726 (+)	0.0 ± 0.0c	0.3 ± 0.1b	0.3 ± 0.1b	0.3 ± 0.1b	526 ± 155a
A3237-781 (+)	0.0 ± 0.0c	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0b	579 ± 222a
A3237-851 (+)	0.3 ± 0.1c	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0b	512 ± 168a
A3237-859 (+)	0.0 ± 0.0c	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0b	593 ± 329a
Thorne-862 (+)	0.3 ± 0.2c	0.6 ± 0.3b	0.6 ± 0.3b	0.6 ± 0.3b	411 ± 134a
A3237-863 (+)	0.0 ± 0.0c	0.1 ± 0.1b	0.1 ± 0.1b	0.1 ± 0.1b	573 ± 236a
A3237-1085 (+)	0.0 ± 0.0c	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0b	626 ± 219a
A3237-781 (–)	5.5 ± 1.8a	33.3 ± 5.8a	31.5 ± 10.3a	97.5 ± 1.1a	424 ± 160a
A3237 variety (–)	3.0 ± 1.0b	30.0 ± 3.5a	17.0 ± 8.3a	95.0 ± 5.8a	323 ± 173a

Column means followed by the same letter are not significantly different, Waller-Duncan K-ratio *t*-test, $P = 0.05$. Larval densities were 0.5 and 1.5 per row-m on the A3237 variety on 24 August and 21 September, respectively, and < 0.5 per row-m on all other sampling dates. The R5 growth stage had pods filling with seeds and the R6 growth stage had pods containing full-size seeds (Teare and Hodges 1994).

in 1999 (Table 1) due to the later than normal planting date (13 July compared with the recommended planting dates of 10 May to 10 June; [Jost 2006]) and the dry growing conditions during August and September. Although yields were not significantly different between the entries ($F = 1.25$; df = 8, 24; $P = 0.31$), all the soybean lines with *Bt* transgenes (except Thorne-862) produced mean yields of 100-200 kg ha^{-1} higher than the 2 lines without *Bt* transgenes. The yields in the 2 non*Bt* entries were undoubtedly reduced due to larval defoliation. However, this defoliation occurred when the soybean entries were in the late seed-filling (R5 growth stage) or full-size seed (R6) growth stages, thus yield reductions were minimal compared with yield reductions associated with lepidopteran defoliation that occurs when soybean pods are beginning to form and fill with seeds (Board et al. 1994, Board 2004, McPherson and Buss 2007).

Both velvetbean caterpillar and soybean looper larvae were present at relatively high densities on the 3 non*Bt* soybean entries evaluated in 2000, but infrequently observed on any sampling date on the *Bt* entry (Table 2). Velvetbean caterpillars were significantly higher on the non*Bt* entries than on the *Bt* entry on 7 September ($F = 8.95$; df = 3, 9; $P = 0.01$), 14 September ($F = 5.93$; df = 3, 9; $P = 0.02$), 21 September ($F = 15.48$; df = 3, 9; $P < 0.01$), and 27 September ($F = 15.56$; df = 3, 9; $P < 0.01$) (Table 2). Soybean looper larval densities also were significantly higher on the non *Bt* entries on 7 September ($F = 4.90$; df = 3, 9; $P = 0.028$), 14 September ($F = 11.96$; df = 3, 9; $P < 0.01$), and 21 September ($F = 3.89$; df = 3, 9; $P = 0.05$). Green cloverworm larvae also were more abundant on the non*Bt* entries on 14 September ($F = 5.75$; df = 3, 9; $P = 0.022$) (Table 2). The relatively high densities of velvetbean caterpillar larvae, soybean looper larvae, and to a limited extent green cloverworm larvae, combined to cause

Table 2. Larval densities (± SEM) of velvetbean caterpillars, soybean loopers (SBL), and green cloverworms (GCW) (and plant growth stage on each sampling date) plus the percentage defoliation and yield (13% moisture) on four soybean lines with *Bt* transgenes (+) or without *Bt* transgenes (–), Tifton, GA, 2000

	Velvetbean caterpillar larvae per row-meter					
Soybean Line	7 Sept (R5)	14 Sept (R6)	21 Sept (R6)	27 Sept (R7)	% Defol 27 Sept*	Yield kg / ha
781 AGR (+)	0.1 ± 0.3b	0.3 ± 0.1b	0.0 ± 0.0b	0.1 ± 0.3b	1.6 ± 1.9b	919 ± 195a
781 AGR (–)	9.4 ± 4.8a	11.1 ± 3.5a	8.8 ± 2.5a	4.0 ± 1.5a	66.3 ± 13.0a	769 ± 132a
AG 3237 (–)	10.3 ± 3.8a	12.1 ± 3.0a	9.6 ± 3.0a	4.6 ± 2.3a	76.3 ± 4.2a	754 ± 52a
AG 3201 (–)	8.4 ± 4.5a	8.0 ± 2.8a	7.8 ± 3.8a	3.6 ± 1.8a	77.5 ± 10.3a	815 ± 289a
	Other lepidopteran larvae per row-meter					
	SBL 7 Sept	SBL 14 Sept	SBL 21 Sept	GCW 14 Sept		
781 AGR (+)	0.1 ± 0.3b	0.1 ± 0.3c	0.0 ± 0.0b	0.1 ± 0.36b		
781 AGR (–)	8.5 ± 2.8a	10.9 ± 4.3a	1.3 ± 0.3a	5.0 ± 2.0a		
AG 3237 (–)	6.0 ± 2.5a	9.0 ± 2.9a	1.6 ± 0.8a	4.8 ± 1.5a		
AG 3201 (–)	3.8 ± 2.0ab	2.4 ± 1.1b	0.9 ± 0.3a	0.4 ± 0.3b		

Column means followed by the same letter are not significantly different, Waller-Duncan K-ratio *t*-test, $P = 0.05$. Lepidopteran larval densities were low in all soybean lines during July and August (mean total larvae < 2 per row-m). The R5 growth stage had pods filling with seeds, the R6 growth stage had pods containing fill-size seeds, and the R7 growth stage was beginning to mature (Teare and Hodges 1994).

66-77% defoliation in the non*Bt* entries compared with only 1.6% in the *Bt* line (F = 120.83; df = 3, 9; P < 0.01) (Table 2). Yields did not differ among the 4 soybean lines evaluated (F = 1.67; df = 3, 9; P > 0.05), but the mean yield for 781AGR (+) was higher than for some of the non*Bt* entries (Table 2). Undoubtedly, the high defoliation levels in the non*Bt* entries had a negative impact on yields in these lines, but by the time this defoliation occurred these early-maturing plants had already reached the R5 and R6 plant growth stages.

In the 2001 soybean trials, velvetbean caterpillar and soybean looper larvae were the commonly observed lepidopteran species in both the 8 June and 11 July plantings (Table 3). In the 8 June trial, velvetbean caterpillars were significantly more abundant in the non*Bt* entries on 6 September (F = 10.03; df = 5, 10; P < 0.01), 13 September (F = 9.32; df = 5, 10; P < 0.01), and 20 September (F = 9.76; df = 5, 10; P < 0.01), and soybean looper larvae were more abundant in the non*Bt* entries on 6 September (F = 3.19; df = 5, 10; P = 0.05). In the 11 July planting, velvetbean caterpillar densities were higher in the non*Bt* lines on 6 September (F = 9.74; df = 5, 10; P < 0.01), 13 September (F = 3.51; df = 5, 10; P = 0.043) and 20 September (F = 6.34; df = 5, 10; P < 0.01), and soybean looper larvae were more abundant on the non*Bt* lines on 6 September (F = 7.94; df = 5, 10; P < 0.01). Green cloverworm larval densities were low in 2001; however, in the 8 June trial, there were significantly more green cloverworm larvae (F = 3.35; df = 5, 10; P = 0.05) in the 3 non*Bt* entries (1.3-0.7 larvae per row-m) than in the three *Bt* entires (which had no green cloverworm larvae). Also in the 11 July planting, green cloverworm larval densities were approaching a significant difference (F = 3.07; df = 5, 10; P = 0.061), with more in the 4 non*Bt* entires (2.2-1.7 per row-m) than in the 2 *Bt* entries (0.2-0.0 per row-m). The percentage defoliation also was significantly higher in the non*Bt* soybean entries in both the 8 June planted trial (F = 156.10; df = 5, 10; P < 0.01) and the 11 July planted trial (F = 99.32; df = 5, 10; P < 0.01) (Table 3). Yields were significantly higher in the *Bt* lines than in some of the non*Bt* lines in the 8 June trial (F = 6.20; df = 5, 10; P < 0.01), but no yield differences were noted among the soybean entries in the 11 July trial (Table 3). Yields were much higher in the earlier maturity group soybean entries planted on 8 June (2177-2582 kg ha^{-1}) compared with the later maturity group entries planted on 11 July (1444-1706 kg ha^{-1}). The 11 July planting was a month later than the recommended latest planting date of 10 June for soybeans produced in Georgia (Jost 2006).

In the 2002 trial, velvetbean catpillar densities were significantly higher on the non*Bt* entry than in the 3 *Bt*-entries on 20 August (F = 15.21; df = 3, 9; P < 0.01), 27 August (F = 37.28; df = 3, 9; P < 0.01), and 3 September (F = 17.59; df = 3, 9; P < 0.01) (Table 4). Soybean looper larval densities also were significantly higher on the non *Bt* entry on 20 August (F = 8.25; df = 3, 9; P < 0.01) and 3 September (F = 6.55; df = 3, 9; P = 0.015) (Table 4). Green cloverworm larval population densities were low in all soybean entries (<1.0 per row-m) on all sampling dates, and no differences among entries were observed. The percentage defoliation in the non*Bt* entry (83.3%) was significantly higher (F = 588.0; df = 3, 9; P < 0.01) than in the 3 *Bt* entries (Table 4). Yields were similar between the *Bt* and non*Bt* soybean entries being evaluated in 2002 (Table 4). This is likely due to the fact that significant defoliation of the *Bt*-negative line did not occur until after these maturity groups 5 soybeans had reached full-sized seed (R6) growth stage (Board et al. 1994, Board 2004, McPherson and Buss 2007).

The results obtained from these studies conducted in 1999-2002 suggest that all of the *Bt* soybean entries evaluated, developed by Monsanto Company, provide nearly

Table 3. Larval densities (± SEM) of velvetbean caterpillars (VBC) and soybean loopers (SBL) (and plant growth stage on each sampling date) plus percentage defoliation and yield (13% moisture) in six soybean lines with *Bt* transgenes (+) or without *Bt* transgenes (–), Tifton, GA, 2001

Soybean Line	Larvae per row–meter VBC 6 Sept	VBC 13 Sept	VBC 20 Sept	SBL 6 Sept	% Defol. 20 Sept	Yield kg / ha
8 June Planting						
F6-8RR 43.00_32 (+)	0.5 ± 0.2b	0.0 ± 0.0c	0.3 ± 0.2b	0.0 ± 0.0b	0.5 ± 0.0c	2582 ± 56ab
F6-8RR 43.00_12 (–)	7.7 ± 2.5a	11.3 ± 4.1a	5.5 ± 2.1a	2.5 ± 1.3ab	50.0 ± 4.1b	2177 ± 305c
F6-8RR 29.00_34 (+)	0.5 ± 0.1b	0.0 ± 0.0c	0.3 ± 0.1b	0.0 ± 0.0b	0.2 ± 0.2c	2747 ± 207a
F6-8RR 29.00_35 (+)	0.2 ± 0.1b	0.0 ± 0.0c	0.0 ± 0.0b	0.3 ± 0.1b	0.2 ± 0.3c	2794 ± 111a
F6-8RR 27.00_08 (–)	9.0 ± 3.5a	8.2 ± 3.3ab	4.2 ± 1.2a	1.7 ± 1.1ab	48.3 ± 6.2b	2430 ± 120bc
F6-8RR (–)	7.7 ± 4.1a	5.3 ± 4.3b	5.7 ± 2.3a	3.5 ± 2.1a	60.0 ± 4.1a	2424 ± 92bc
Plant Growth Stage	(R6)	(R6)	(R7)	(R6)		
11 July Planting					17 Oct	
Estrella RR 44.0027 (–)	5.3 ± 2.7a	1.7 ± 0.4ab	3.0 ± 0.7a	1.8 ± 0.8bc	16.7 ± 2.4b	1575 ± 78a
MS 8000 (–)	5.7 ± 2.1a	3.0 ± 1.7ab	3.8 ± 1.1a	3.2 ± 2.1ab	26.7 ± 2.4a	1444 ± 259a
Estrella RR 42.0053 (+)	0.2 ± 0.3b	1.0 ± 0.7ab	0.0 ± 0.0b	0.3 ± 0.2c	0.2 ± 0.1c	1464 ± 120a
Estrella RR 09.0020 (–)	8.5 ± 1.6a	4.8 ± 1.7a	3.0 ± 1.4a	4.0 ± 2.4a	30.0 ± 4.1a	1517 ± 101a
Estrella RR (–)	5.2 ± 1.7a	4.8 ± 2.1a	3.0 ± 0.7a	0.5 ± 0.4c	20.0 ± 4.1b	1675 ± 145a
Estrella RR 44.0044 (+)	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0c	0.3 ± 0.1c	1706 ± 288a
Plant Growth Stage	(R3 - 4)	(R5)	(R5)	(R3 - 4)		

Column means with the same letter are not significantly different, Waller-Duncan K-ratio *t*-test, $P = 0.05$. Mean VBC and SBL larval densities were low (< 2 per row-m) in July, August, and after 20 September in both the 8 June and 11 July Plantings. The R3-R4 growth stages had pods beginning to form, the R5 growth stage had pods filling with seeds, and the R6 growth stage had pods containing full-size seeds (Teare and Hodges 1994).

Table 4. Larval densities (± SEM) of velvetbean caterpillars and soybean loopers (and plant growth stage on each sampling date), percentage defoliation and yield (13% moisture, kg/ha) in four soybean lines with *Bt* transgenes (+) or without *Bt* transgenes (–), Tifton, GA, 2002

	Velbetbean caterpillar larvae per row-meter			
Soybean Line	20 Aug	27 Aug	3 Sept	% Defol 19 Sept
GM_A19487 (+)	0.0 ± 0.0b	0.3 ± 0.2b	0.3 ± 0.2b	0.2 ± 0.2b
GM_A19459A (+)	0.0 ± 0.0b	0.3 ± 0.2b	1.0 ± 1.4b	0.2 ± 0.2b
GM_A19459B (+)	0.0 ± 0.0b	0.0 ± 0.0b	0.3 ± 0.2b	0.0 ± 0.0b
A5547 (–)	6.1 ± 0.4a	15.9 ± 0.8a	10.6 ± 0.6a	83.3 ± 10.2a
	Soybean looper larvae per row-meter			Yield
GM_A19487 (+)	0.0 ± 0.0b	0.0 ± 0.0a	0.0 ± 0.0b	1653 ± 101a
GM_A19459A (+)	0.3 ± 0.2b	0.0 ± 0.0a	0.7 ± 0.9b	1686 ± 112a
GM_A19459B (+)	0.0 ± 0.0b	0.0 ± 0.0a	0.0 ± 0.0b	1627 ± 88a
A5547 (–)	7.1 ± 0.6a	1.3 ± 0.4a	4.3 ± 0.3a	1556 ± 74a
	(R5)	(R5)	(R6)	

Column means with the same letter are not significantly different, Waller-Duncan K-ratio *t*-test, $P = 0.05$. Lepidopteran larvae were low (< 1.5 per row-m) on all sampling dates before 20 August and after 3 September. The R5 growth stage had pods filling with seeds and the R6 growth stage had pods containing full-size seeds (Teare and Hodges 1994).

complete control of velvetbean caterpillars, soybean loopers, and green cloverworms throughout the entire growing season. The cumulative percentage of defoliation was extremely low in every *Bt*-soybean entry (ranging from 0.0-1.6% across all years) compared with the non*Bt* entries (ranging from 95.0-97.5% in 1999, 66.3-77.5% in 2000, 16.7-60.0% in 2001, and 83.3% in 2002). Yields of the transgenic soybean lines with *Bt* were equal to the standard soybean cultivars being evaluated in these replicated field trials. However, yields in all the trials were lower than expected due to the later than recommended planting dates (Jost 2006). However, these later planting dates were selected to maximize the likelihood of late-season lepidipteran infestations (McPherson and Bondari 1991). In one trial, the 8 June 2001 planting date, yields for the *Bt* lines were actually significantly higher than the non*Bt* entries. Thus, it appears that *Bt* transgenic soybean lines are available that provide season-long control of the common lepidopteran pests on soybeans produced in the southern states and that these *Bt* lines yield as good or better than some of the currently used soybean cultivars.

The lepidopteran pest infestations were sufficient to cause significant differences in the percentages of defoliation between the *Bt* entries and the non*Bt* entries in all 4 years of this study. However, soybean yields were significantly lower in the non*Bt* entries in only one trial (2001), results confirming earlier reports that heavy defoliation during late season (pods already filled with seeds) causes minimal yield reductions (Board et al. 1994, Board 2004). The lepidopteran species that were effectively controlled in all the *Bt* transgene soybean entries examined in this study included *A. gemmatalis, P. includens,* and *H. scabra*. Other lepidopteran pests observed at low densities during this study included *Spodoptera exigua* (Hübner), S. *ornithogalli* (Guenée), and *Helicoverpa zea* (Broddie), but populations were too low to make comparisons between the *Bt* and non*Bt* soybean entries.

The high degree of efficacy that these *Bt* soybean lines exhibited in this study throughout the season against multiple lepidopteran pests confirms previous reports that this technology is an effective pest management tactic for the soybean crop (MacRae et al. 2005, Miklos et al. 2007, Parrott et al. 1994, Baur et al. 2003). Confirming yield performance and other desirable agronomic characteristics within the *Bt* lines will be necessary for these entries to become commercially acceptable. Once available to producers, these *Bt* transgenic lines have the potential to provide the economic and environmental benefits already being realized by the commercialization of *Bt* cotton and *Bt* corn (MacRae et al. 2005).

Acknowledgments

The authors thank Del Taylor and Neal Roberson for technical support and Monsanto Company and the University of Georgia Agricultural Experiment Stations for financial support.

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