Field Evaluation of Soybean Engineered with a Synthetic *cry1Ac* Transgene for Resistance to Corn Earworm, Soybean Looper, Velvetbean Caterpillar (Lepidoptera: Noctuidae), and Lesser Cornstalk Borer (Lepidoptera: Pyralidae)

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ABSTRACT A transgenic line of the soybean 'Jack', *Glycine max* (L.) Merrill, expressing a synthetic *cry1Ac* gene from *Bacillus thuringiensis* variety *kurstaki* (Jack-Bt), was evaluated for resistance to four lepidopteran pests in the field. Jack-Bt and genotypes serving as susceptible and resistant controls were planted in field cages and artificially infested with larvae of corn earworm, *Helicoverpa zea* (Boddie), and velvetbean caterpillar, *Anticarsia gemmatalis* (Hübner), in 1996, 1997, and 1998, and also with soybean looper, *Pseudoplusia includens* (Walker), in 1996. Susceptible controls included Jack (1996–1998), 'Cobb' (1996), and Jack-HPH (1996). GatIR 81–296 was used as the resistant control in all 3 yr. Compared with untransformed Jack, Jack-Bt showed three to five times less defoliation from corn earworm and eight to nine times less damage from velvetbean caterpillar. Defoliation of GatIR 81–296 was intermediate between that of Jack and Jack-Bt for corn earworm, and similar to that of Jack for velveltbean caterpillar. Jack-Bt exhibited significant, but lower resistance to soybean looper. Jack-Bt also showed four times greater resistance than Jack to natural infestations of lesser cornstalk borer, *Elasmopalpus lignosellus* (Zeller), in conventional field plots at two locations in 1998. Data from these experiments suggest that expression of this *cry1Ac* construct in soybean should provide adequate levels of resistance to several lepidopteran pests under field conditions.

KEY WORDS *Bacillus thuringiensis*, Lepidoptera, Noctuidae, Pyralidae, resistance management, soybean

RECENT ADVANCES IN plant biotechnology, such as the use of transgenic cultivars and marker-assisted breeding to better exploit host plant resistance, have provided new approaches for the management of insect pests. One of the most promising of these is the development of cultivars that express one of the δ-endotoxin genes from *Bacillus thuringiensis* subsp. *kurstaki* (Roush 1997). A 1997 review article listed 18 species of economic importance that have been successfully transformed with a Bt gene (Mazier et al. 1997). This number is continuously increasing, and several transgenic cotton, maize, and potato cultivars have been commercialized since 1995 (Jouanin et al. 1998). Bt cultivars were planted on ≈1.2 million hectares in the United States in 1996, and by 1997 plantings had increased to an estimated 1.05 million hectares of Bt corn, 1.0 million hectares of Bt cotton, and 10,000 hectares of Bt potato, as well as plantings in Australia and Canada (All et al. 1999).

Baccilus thuringiensis transgenes in commercial cultivars target one or a few major pests, such as heliothine species and the pink bollworm, *Pectinophora gossypiella* (Saunders), in cotton, the European corn borer, *Ostrinia nubilalis* (Hübner), and the southwestern corn borer, *Diatraea grandiosella* (Dyar), in corn, or the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), in potato. A wide variety of lepidopteran pests can attack soybean. Velvetbean caterpillar, *Anticarsia gemmatalis* (Hübner), corn earworm, *Helicoverpa zea* (Boddie), and soybean looper, *Pseudoplusia includens* (Walker), are major pests of soybean in the southeastern United States. All three of these noctuids are defoliators, and *H. zea* causes additional damage to flowers, pods, and developing seeds. *H. zea* was once considered the major soybean insect pest in much of the southeastern Coastal Plain (Stinner et al. 1980), and although this no longer appears to be the case, it remains one of the five most important soybean pests in Georgia (McPherson et al. 1999). In some years, *P. includens* has been the most important insect pest of soybean (Adams et al. 1988), and has become the most costly insect pest to control on soybean in the Gulf Coast states (Mascarenhas and Boethel 1997). *A. gemmatalis* causes extremely high levels of defoliation when infestation is heavy, and can severely damage

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axillary meristems. A single larva can consume up to 110 cm^2 of soybean foliage (Aragón et al. 1997). The lesser cornstalk borer, *Elasmopalpus lignosellus* (Zeller), is another serious pest of soybean in many years when the weather conditions are hot and dry immediately after planting and is difficult to control with conventional insecticide applications (Funderburk and Mack 1994).

The development of elite insect-resistant, high-yielding soybean cultivars through conventional plant breeding has proven difficult (All et al. 1989). Breeding efforts have resulted in the release of four insect-resistant cultivars, but their late maturity, inferior yield, and tendency to lodge have discouraged acceptance by producers (Boethel 1999, Lambert and Tyler 1999). Furthermore, Rowan et al. (1993) found only moderate resistance in four commercial cultivars among 46 cultivars evaluated. This limited availability of elite cultivars with resistance prompted attempts to engineer soybean with a Bt insecticidal protein gene.

The first report of the successful expression of a δ-endotoxin gene in soybean involved a truncated native *cry1Ab* gene from *B. thuringiensis* subsp. *kurstaki* HD-1 (Parrott et al. 1994). Although protein expression was undetectable in T_1 plants from one cell line, these plants showed a level of resistance to *A. gemmatalis* similar to that of GatIR 81–296, a resistant breeding line derived from the plant introduction PI 229358 (Beach and Todd 1987, 1988). Subsequent attempts to obtain improved expression of a Bt toxin gene in soybean resulted in the development of a line expressing a truncated synthetic *cry1Ac* gene based on the *B. thuringiensis* subsp. *kurstaki cry1Ac* sequence and designed to be highly expressed in plants (Stewart et al. 1996b). Protein expression in homozygous T_1 plants of one line (7b) was 46 ng mg^{-1}, and these plants showed resistance to *H. zea*, *P. includens*, and *A. gemmatalis* in detached leaf feeding bioassays (Stewart et al. 1996b).

The objective of our studies was to investigate the resistance of a transgenic line to *H. zea*, *P. includens*, *A. gemmatalis*, and *E. lignosellus* under field conditions to better evaluate the potential for using this *cry1Ac* transgene in the development of insect-resistant soybean cultivars.

Materials and Methods

Soybean Genotypes. The transgenic line 7b of the cultivar Jack (hereafter referred to as Jack-Bt) used in these studies was developed by Stewart et al. (1996a), and carries one intact copy and one rearranged copy of a synthetic *cry1Ac* construct in which the 1.8-kb coding sequence is driven by the cauliflower mosaic virus (CaMV) 35S promoter (Adang et al. 1987). Homozygous transgenic seed from the T_2, T_3, and T_4 generations were planted in the field at The University of Georgia Plant Sciences Farm, near Athens, GA, in 1996, 1997, and 1998, respectively. T_5 plants were also grown at The University of Georgia Coastal Plain Experiment Station in Tifton, GA, in 1998.

In 1996, Jack-Bt was compared in cage studies, which are described below, with the following four other entries: (1) untransformed Jack (Nickell et al. 1990), a late maturity group (MG) II cultivar; (2) a transgenic Jack line expressing a hygromycin phosphotransferase transgene (Jack-HPH); (3) 'Cobb', an insect-susceptible MG VIII cultivar (All et al. 1989); and (4) GatIR 81–296 (hereafter referred to as IR 81–296), an insect-resistant breeding line derived from a 'GaSoy17' × PI 229358 cross (Beach and Todd 1987, 1988). The 1997 and 1998 cage experiments excluded Jack-HPH and Cobb. Additional studies in 1998 were conducted in conventional field plots for the purpose of evaluating Jack-Bt resistance to natural infestations of *E. lignosellus* at Athens and to various insect pests at the Tifton site.

Insects. Three different noctuid pests were included in the 1996 cage experiments. *H. zea* eggs were obtained from Ronald Meyer of the Insect Biology and Population Management Research Laboratory (USDA-ARS, Tifton, GA), and eggs from *P. includens* and *A. gemmatalis* were obtained from the Southern Field Crop Insect Management Laboratory (USDA-ARS, Stoneville, MS). The 1997 and 1998 cage experiments involved only *H. zea* and *A. gemmatalis.* Freshly hatched larvae were incorporated into a corncob grits mixture and applied to moistened plant foliage using a "bazooka" mechanical infestation device to facilitate uniform infestations (Wiseman et al. 1980). The conventional field experiments were conducted at Athens and Tifton in 1998 with endemic lepidopteran populations.

Cage Studies. Cage studies were conducted under conditions similar to those described by Rector et al. (1998), and all tests were carried out according to the biosafety requirements of the USDA-APHIS and The University of Georgia IBC for conducting field tests with transgenic soybean. In 1996, three studies were conducted in a 10 m wide by 20 m long steel-framed quonset-style cage covered with a 0.9 mm by 0.9-mm saran mesh. In the 1997 and 1998 cage studies, an *A. gemmatalis* test was conducted in the same cage used in 1996, and an *H. zea* test was carried out in an adjacent cage of a similar size, but constructed from lumber and 3.8-cm PVC pipe (Rowan et al. 1991). Artificial lighting provided by floodlights was used to extend the photoperiod to 17 h to delay flowering of the MG II entries (i.e., Jack and its transgenic derivatives) while the tests were being conducted. Each cage was illuminated with 16 120-W bulbs mounted on posts.

The experimental unit consisted of a "hill" with six plants of the same genotype, and hills were planted in a 46 by 76-cm spacing. In 1996, plant genoypes were randomized at the level of individual hills, but in 1997 and 1998 the randomization unit consisted of three rows of three hills each, in which the outer rows served as border rows to intercept larvae migrating from adjacent plots in the same replication. Each hill was infested with ≈140 larvae two to fives times per test, beginning when plants were in the V3 to V4 stages of development (Fehr et al. 1971). Resistance was

evaluated through visual estimates of percent defoliation of the plants in a hill, and estimates were made at 2- to 3-d intervals beginning 7–8 d after the initial infestation and continuing for ≈6 wk. In 1996, the sampling unit was an individual hill; but in 1997 and 1998, the sampling unit consisted of the middle row of three hills in each nine-hill plot.

A randomized complete block design was used, and following an arcsine $\sqrt{\text{percent}}$ transformation to reduce heterogeneity of variances (Steel and Torrie 1980), data were subjected to analysis of variance (ANOVA) using PC-SAS (SAS Institute 1987). Significant differences ($P \leq 0.05$) were determined by Fisher protected least significant difference (LSD) of the transformed data, though the means and standard errors presented here were calculated from untransformed data.

1996 Cage Studies. There were 12 replications for each of the three insect species. All tests were conducted in the same cage, and the three studies were separated by 30-cm high barriers to discourage larval migration between studies. These barriers consisted of aluminum flashing partially buried in the ground and painted along the top with an insect trap adhesive. Hills were planted 17 June, and those in the *H. zea* experiment were infested three times (11, 18, and 25 July), those in the *P. includens* experiment twice (11 and 20 July), and those in the *A. gemmatalis* experiment twice (11 and 19 July).

1997 Cage Studies. The switch from single-hill plots to nine-hill plots was a response to the substantial migration of older larval instars observed during the 1996 studies. A single insect species was used to infest plants in each cage, and because of the larger plot size, the number of replications was reduced to seven and only three genotypes were planted. Replications were separated by the type of barriers described above. Defoliation data were taken on the three hills in the center row of each plot, and ANOVA was conducted on the mean percent defoliation of these hills, following transformation. Seed yield was also recorded and analyzed for significant differences ($P \leq 0.05$) among the soybean genotypes.

The 1997 field studies were planted 7 July and infested with *H. zea* (30 July) or *A. gemmatalis* (1 August) when most plants were at the V3 stage (Fehr et al. 1971). There were four subsequent infestations with *H. zea* (1, 4, 6, and 8 August), and one additional *A. gemmatalis* infestation (8 August). Defoliation data were taken in the *H. zea* cage 6, 8, 10, 12, 14, 16, 18, 20, 22, 26, and 30 d after infestation, and in the *A. gemmatalis* cage 4, 6, 8, 10, 12, 14, 16, 18, 20, and 24 d after infestation.

1998 Cage Studies. The 1998 studies were similar in design to the 1997 experiments, and were planted 30 June. Two cages were used again, with one for each insect species. The *H. zea* study was infested four times (22, 24, and 31 July, and 7 August), and the *A. gemmatalis* study was infested three times (24 and 31 July, and 9 August). Percent defoliation by *H. zea* was estimated at 9, 12, 14, 16, 19, 22, 26, and 30 d after infestation, and at 7, 10, 12, 14, 17, 20, 24, 28, and 32 d after infestation for *A. gemmatalis*. The 1998 data were analyzed as described for the previous years. Because of the differences in infestation times and frequencies, data from 1997 and 1998 were not subjected to a combined analysis.

1998 Conventional Field Studies (Endemic Infestations). The Athens *E. lignosellus* study and the Tifton study were planted in two-row plots ≈6.1 m in length and with a 0.8-m spacing between rows. No supplemental lighting was used. At Athens, the only entries were Jack and Jack-Bt, and the study consisted of a series of plantings over time, beginning 29 June, with four replications per planting date in an randomized complete block design. Subsequent plantings on 13 August, 18 September, and 17 October were made to ensure that plants at various stages of development would be available for oviposition when colonizing *E. lignosellus* moths arrived at the site. Plants showing symptoms of *E. lignosellus* damage (i.e., dead or wilting leaves) were more closely examined, and those with live larvae, larval silk, or a puncture wound just below the soil surface were counted as having *E. lignosellus* damage. Extent of *E. lignosellus* damage in each plot was calculated as a percent of the total number of plants.

The Tifton study was planted 22 June, and included Jack, Jack-Bt and 'Asgrow 2704 LL'. The two-row plots were planted in a randomized complete block design with eight replications. After an infestation by *E. lignosellus*, data on the number of dead plants per plot whose death could unambiguously be attributed to *E. lignosellus* infestation were taken 3 August and analyzed following log (Y +1) transformation to reduce heterogeneity of variances. Percentage of defoliation after an infestation by lepidopterans, predominantly *A. gemmatalis*, was recorded 11 August and subjected to an arcsine $\sqrt{\text{percent}}$ transformation before ANOVA. Means were again declared statistically different ($P \leq 0.05$) based on Fisher protected LSD.

Results and Discussion

1996 Cage Study. Of the three pest species used in the experiments, *H. zea* caused the least defoliation. By 8 d after infestation, both Jack-Bt and IR 81–296 were showing significantly less *H. zea* damage ($F = 8.40$; df = 4, 11; $P < 0.001$) than the other three genotypes (Fig. 1A). At 15 d after infestation, Jack-Bt was less defoliated than any of the other entries, though IR 81–296 was showing more resistance than the remaining genotypes ($F = 23.36$; df = 4, 11; $P < 0.001$). At this point, damage to Cobb, Jack, and Jack-HPH was at a similar level. This pattern remained essentially unchanged over the next week.

In the *P. includens* study, Jack-Bt and GatIR 81–296 showed good resistance relative to the other genotypes at 8 d after infestation ($F = 20.05$; df = 4, 11; $P < 0.001$), at which time mean percent defoliation of Jack, Jack-HPH, and Cobb already exceeded 50% (Fig. 1B). By 15 d after infestation, defoliation by *P. includens*

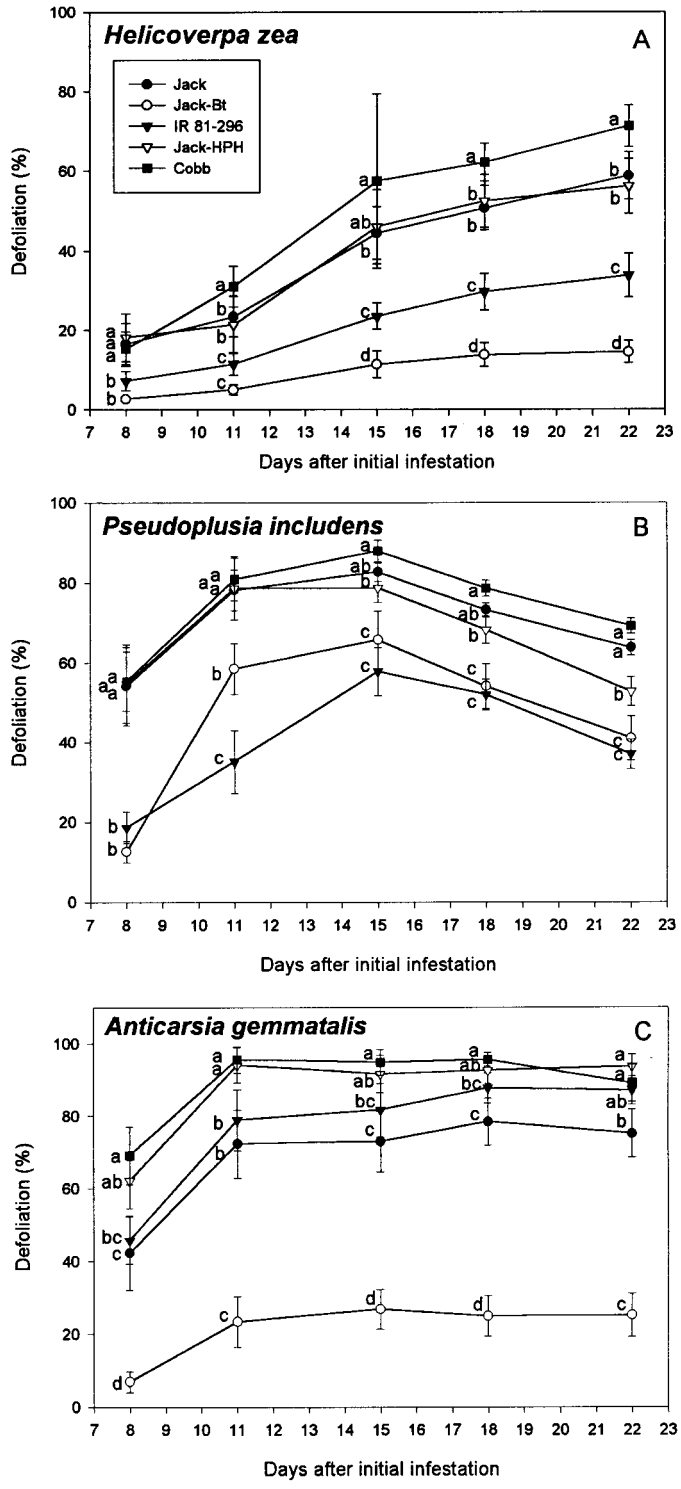

Fig. 1. Percent defoliation (±SE) over time by *H. zea* (A), *P. includens* (B), and *A. gemmatalis* (C) in 1996. Data from individually randomized hills (n = 12). Different lower case letters indicate significant differences between means, based on analysis of transformed data. Note that these graphs show untransformed data.

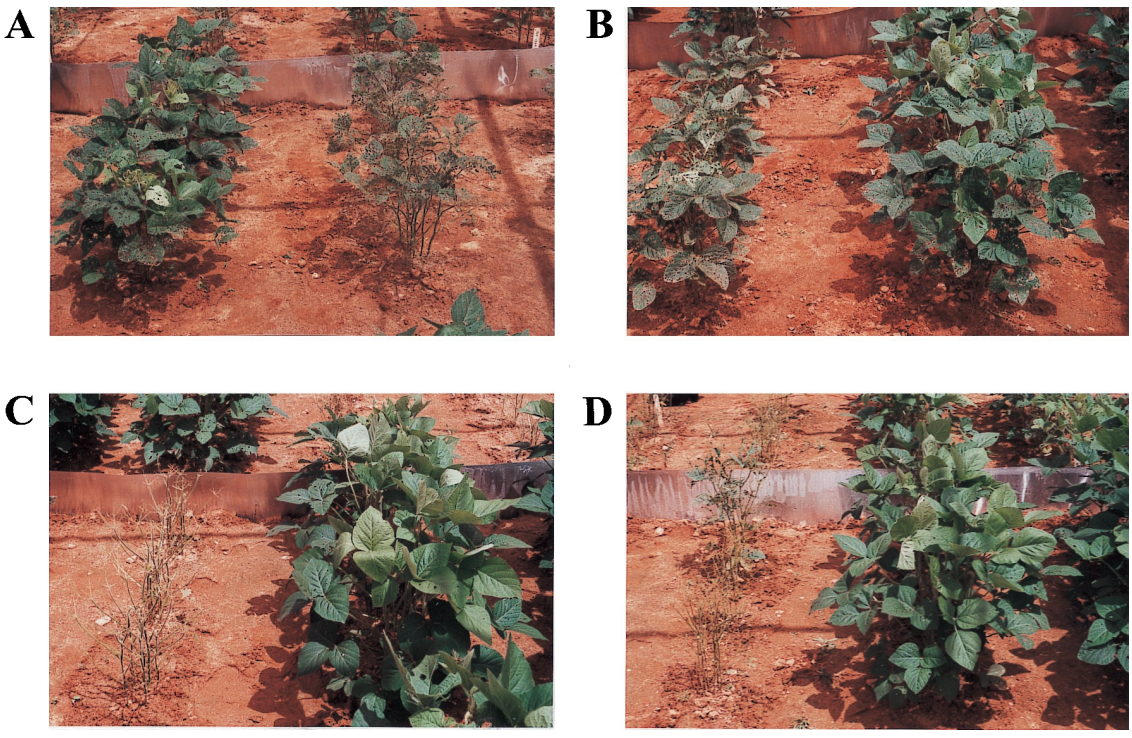

Fig. 2. Defoliation of entries planted in adjacent rows in 1997, shown 19 d after initial infestation with larvae of *H. zea* or *A. gemmatalis.* The top photos show *H. zea* damage to (A) Jack-Bt (left) and Jack (right), and to (B) IR 81–296 (left) and Jack-Bt (right). The bottom photos show *A. gemmatalis* damage to (C) Jack (left) and Jack-Bt (right), and to (D) IR 81–296 (left) and Jack-Bt (right).

was equivalent for Jack and Cobb, and significantly lower for Jack-Bt and IR 81–296 ($F = 18.85$; df = 4, 11; $P < 0.001$), which had sustained equivalent amounts of damage. These levels of defoliation would be expected to reduce yields of all genotypes, especially if they were to occur during the reproductive stages (Gazzoni and Moscardi 1998). At 22 d after infestation, IR 81–296 and Jack-Bt still showed significant resistance ($F = 7.19$; df = 4, 11; $P < 0.001$), but all entries had begun to regrow foliage after pupation of *P. includens* larvae from the first infestation. The comparatively low resistance of Jack-Bt to *P. includens* was also observed by Stewart et al. (1996b) in detached leaf bioassays, and various commercial preparations of *B. thuringiensis* have failed to provide adequate control of *P. includens* on cotton in Alabama and South Carolina (Sullivan 1992). Although soybean is the preferred host of *P. includens*, cotton serves as an occasional host, so there is a concern that the widespread use of Bt cotton cultivars could accelerate the development of *P. includens* strains resistant to δ-entodoxins in the Southeast, where the two crops are grown in close proximity (Mascarenhas et al. 1998).

Jack-Bt was more resistant to *A. gemmatalis* than the other genotypes at all observation dates ($F = 19.59$–40.43; df = 4, 11; $P < 0.001$). Most Cobb and Jack-HPH plants were almost completely defoliated within the first 11 d, and IR 81–296 and Jack also were badly damaged during this period (Fig. 1C). Jack remained significantly less defoliated than Cobb, suggesting that Jack may have some endogenous resistance to this pest. Jack also was significantly more resistant than Jack-HPH at both 8 d ($F = 19.59$; df = 4, 11; $P < 0.001$) and 22 d after infestation ($F = 40.43$; df = 4, 11; $P < 0.001$), which is difficult to explain unless somaclonal variation or transgene positional effects are involved. These phenomena are not uncommon in transgenic plants regenerated from tissue culture (Benedict et al. 1992, 1993).

Larval migration was a common phenomenon in all studies. Larvae on susceptible genotypes tended to remain on those plants until high levels of defoliation had occurred, after which the older instars would invade plants in neighboring hills. These latter instars were apparently more tolerant to the δ-endotoxin in Jack-Bt foliage and could feed longer without consuming lethal doses. Zehnder and Gelernter (1989) found that third-instar Colorado potato beetle, *Leptinotarsa decemlineata* (Say), were more tolerant than second-instar larvae to *B. thuringiensis* variety *san diego*, and larvae are usually most susceptible to δ-endotoxins immediately after eclosion (Roush 1994).

1997 Cage Studies. In general, defoliation patterns of Jack, Jack-Bt, and IR 81–296 were similar in 1997 and 1998 (Figs. 2-4). In 1997, differences in damage by both *H. zea* and *A. gemmatalis* were already apparent at 8 d after infestation ($F = 25.39$; df = 2, 6; $P < 0.001$) and remained throughout the period during which

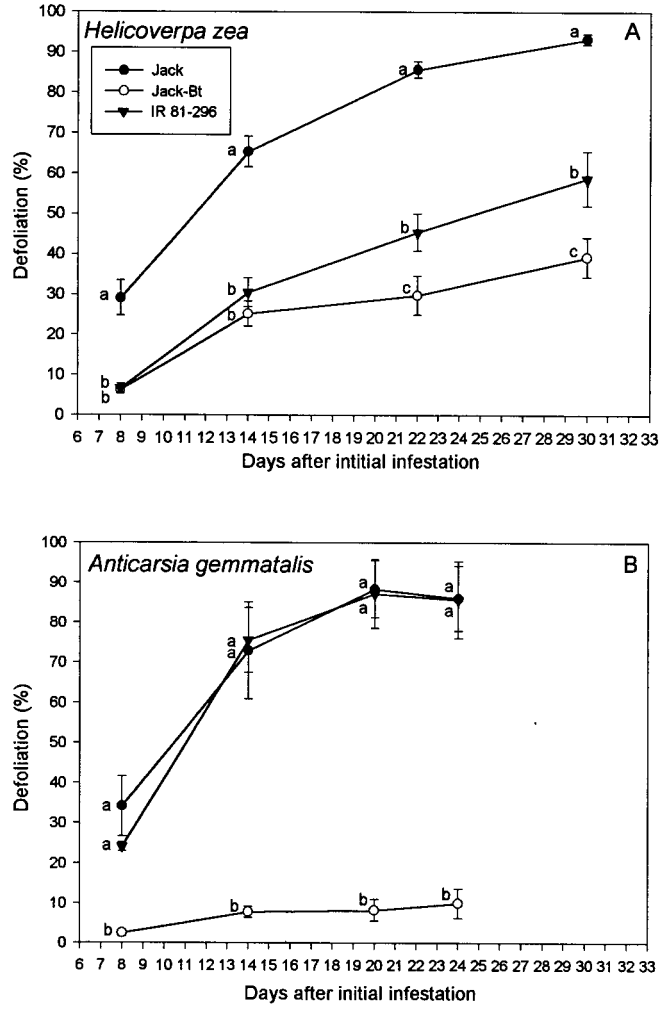

Fig. 3. Percent defoliation (±SE) over time by *H. zea* (A) and *A. gemmatalis* (B) in 1997. Untransformed data from center three hills in nine-hill plots ($n = 7$). Different lower case letters indicate significant differences between means, based on analysis of transformed data.

data were collected (Figs. 2 and 3). Defoliation of Jack proceeded rapidly during the first 3 wk of the study compared with the defoliation rate of the other entries, and leveled off during the fourth week (Fig. 3). Damage from *H. zea* continued to increase throughout the period of observation, reflecting the multiple infestations (Fig. 3A). IR 81–296 damage remained intermediate between that of Jack and Jack-Bt (Figs. 2 and 3).

Feeding by *A. gemmatalis* instars from the first infestation caused extensive defoliation to both Jack and IR81–296 by 14 d after infestation, and damage to these genotypes peaked at close to 90% around 20 d after infestation (Fig. 3B). The defoliation means for the susceptible genotypes were reduced by a replication in one corner of the *A. gemmatalis* cage, but some Jack and IR81–296 hills were already 100% defoliated at 12 d after infestation, and the meristems had been destroyed as well on most plants in these hills (Fig. 2). The larval migrations seen in 1996 also were evident in 1997 in the form of visibly greater damage to Jack-Bt border rows in replications where Jack and Jack-Bt were adjacent to one another, especially in the *A. gemmatalis* cage.

The levels of damage to Jack and IR 81–296 were above an economic injury threshold of 35% defoliation for vegetative-stage plants (Sullivan and Boethel 1994), and Jack and IR 81–296 yields were lower than that of Jack-Bt in both the *H. zea* ($F = 10.29$; df = 2, 6; $P = 0.002$) and *A. gemmatalis* ($F = 6.79$; df = 2, 6; $P = 0.011$) studies (Fig. 5). Although the possibility of a genotype effect cannot be ruled out in comparing the yields of Jack-Bt and IR 81–296, one would expect negligible genotypic differences between Jack and Jack-Bt for the same level of defoliation.

1998 Cage Studies. The general results in the 1998 cage studies resembled those of the earlier studies, though overall defoliation was lower than in the other

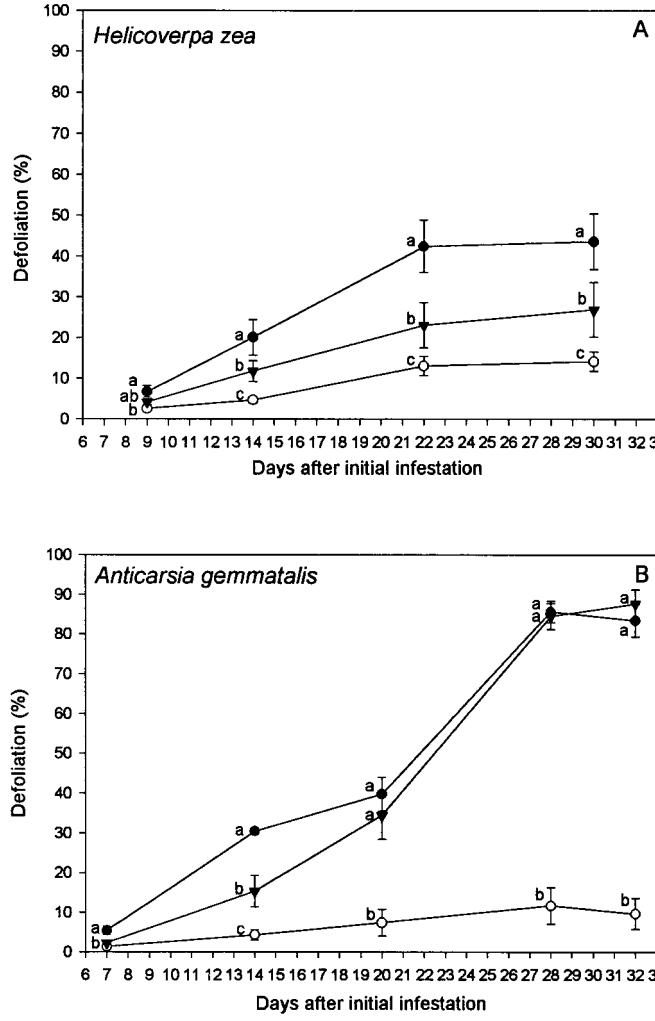

Fig. 4. Percent defoliation (±SE) over time by *H. zea* (A) and *A. gemmatalis* (B) in 1998. Untransformed data from center three hills in nine-hill plots ($n = 7$). Different lower case letters indicate significant differences between means, based on analysis of transformed data.

years (Fig. 4). This might have been caused by heavy natural infestations of ants (Hymenoptera: Formicidae) within the plots. In 1998 the cages were not pretreated with malathion, which had been used in 1996 and 1997 to eliminate insects already present in the cages when they were covered.

Defoliation by *H. zea* increased until reaching a plateau at 22–30 d after infestation, and never attained the levels seen in 1997 (Fig. 4A). Damage to IR 81–296 was intermediate between that of Jack and Jack-Bt. Other researchers also have reported that *H. zea* is less sensitive to Bt toxins than some other Lepidoptera, such as *Heliothis virescens* (MacIntosh et al. 1990). Defoliation by *A. gemmatalis* increased substantially between 20 and 28 d after infestation, suggesting that larval survival in the second and third infestations was higher than in the first (Fig. 4B).

Unlike the results from the 1997 experiments, there were no significant differences among seed yields of the three genotypes in either of the 1998 studies ($F = 0.55$–2.13; df = 2, 6; $P > 0.05$; Fig. 5). In the *H. zea* study, this was probably due to the lower levels of defoliation in 1998, and the ability of soybean to compensate for these levels of defoliation during the vegetative stages by regrowth and delayed senescence (Haile et al. 1998). Defoliation by *A. gemmatalis* in 1998, however, reached equivalent levels to those of the 1997 experiment, albeit at a slower rate (28 d after infestation versus 20 d after infestation). The minimal impact on yield was surprising, because damage peaked as the plants were entering reproductive stages of development, where they should have been increasingly sensitive to defoliation (Gazzoni and Moscardi 1998). One factor that may partially explain the difference is the observation that in 1997 many of the susceptible plants had severe damage to stems and meristems, whereas in 1998 the extent of damage to these or-

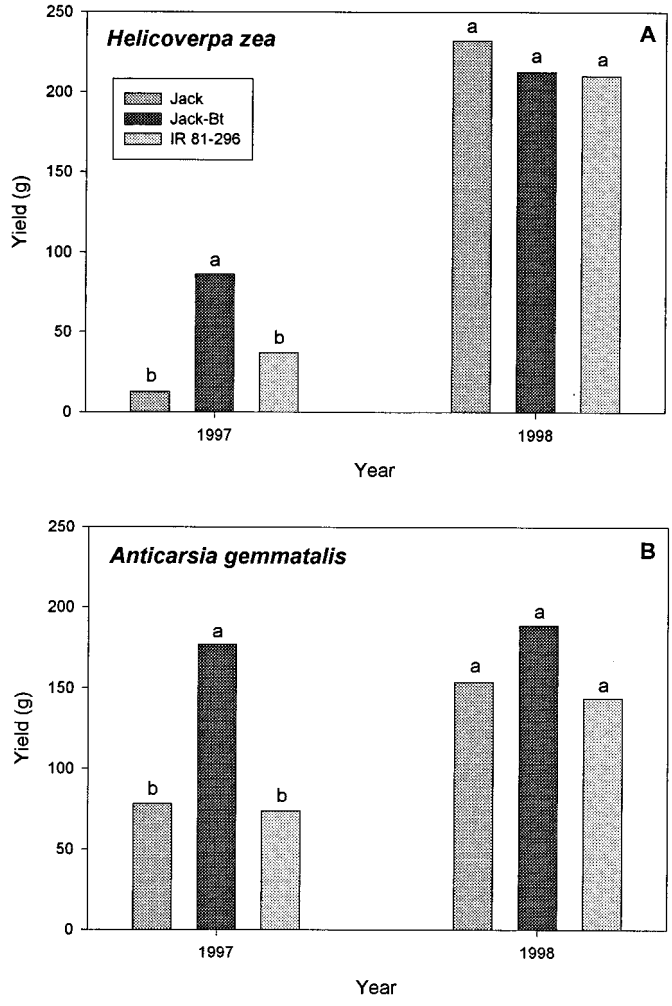

Fig. 5. Seed yields (g) from entries following defoliation by *H. zea* (A) and *A. gemmatalis* (B). Values represent mean yields of center three hills in nine-hill plots of each genotype. Different letters indicate a significant difference between genotypes ($P \leq 0.05$) in the same year based on Fisher Protected LSD.

gans was less severe, facilitating regrowth of foliage after pupation of the larvae.

1998 Endemic Infestation Studies. Infestations by *E. lignosellus* occurred in the seedlings planted at Tifton, GA, and in the second and third plantings at Athens. In the second planting at Athens, a higher percentage of Jack plants was killed (2.0%) than of Jack-Bt plants (0.5%) ($F = 26.24$; df = 1, 4; $P = 0.007$), whereas in the third planting the difference between Jack (1.8%) and Jack-Bt (1.5%) was not significantly different ($F = 0.14$; df = 1, 4; $P > 0.05$). Singsit et al. (1997) reported that this same transgene also provided protection against *E. lignosellus* when expressed in peanut, *Arachis hypogaea* L.

Analysis of transformed data for the number of plants killed by *E. lignosellus* in each plot at Tifton showed significant differences among all three genotypes ($F = 37.34$; df = 2, 7; $P < 0.001$), with Jack (8.3 dead plants per row) intermediate between Jack-Bt (0.5 plants), and Asgrow 2704 LL (16.4 plants). At the time the data were recorded, plants were at the R5 stage (i.e., beginning seed growth), so infestation must have occurred during the late vegetative or very early reproductive stages. Defoliation of Jack (13%) and Asgrow 2704 LL (16%) was not significantly different ($P > 0.05$) at 9 wk after planting, whereas Jack-Bt (1%) was significantly less damaged ($F = 42.27$; df = 2, 7; $P < 0.001$) than either of the other genotypes.

In conclusion, the results of these tests demonstrate both the utility and limitations of a single Bt toxin in providing resistance to lepidopteran pests, and confirm earlier laboratory observations regarding the differential control of noctuid species through expression of the *cry1Ac* gene in soybean (Stewart et al. 1996b). The specificity of Bt endotoxins limits the ability to extrapolate the results of a bioassay involving only one lepidopteran species.

Although expression of the *cry1Ac* transgene provided some level of resistance to all of the pests tested, it was especially effective against *A. gemmatalis*, which is also a major pest in Argentina and Brazil, where it is the target of almost 80% of the insecticides used on soybean in that country (Aragón et al. 1997). Resistance to *A. gemmatalis* remained effective throughout the periods of observation and through three generations, suggesting that transgene expression did not significantly diminish as the plants matured, and that expression was stable across generations. The protection against *E. lignosellus* also is noteworthy, because the feeding habits of this pest make it difficult to control with conventional insecticide applications. However, because of the limited range of pest species controlled by a single δ-endotoxin and the potential for the evolution of resistant pest populations, δ-endotoxin genes may be most useful in soybean if they are deployed together with resistance genes that have different modes of action.

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