Insect Populations on *Bacillus thuringiensis* Transgenic Sweet Corn[1]

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Abstract Insect populations and damage in three plantings (early, mid-, and late-season) of *Bacillus thuringiensis* (Bt) transgenic sweet corn were compared to a non-transgenic isoline at Charleston, SC, during 2000. The transgenic corn was expressing the cryIA(b) gene from *B. thuringiensis* var. *kurstaki*. No fall armyworms, *Spodoptera frugiperda* (J. E. Smith), were present in the early-planted crop, and numbers of corn earworms, *Heliocoverpa zea* (Boddie), were significantly reduced in the Bt-transgenic corn compared to the non-Bt isoline. The non-Bt isoline had 65% *H. zea* infested ears compared to only 10% ears from the Bt-transgenic line. Damage and numbers of *H. zea* and *S. frugiperda* also were significantly higher in the non-Bt sweet corn planted in mid- and late-season plantings. Percent damaged ears by *H. zea* was 77% in the non-transgenic sweet corn and 21% in the Bt-sweet corn during the mid-season crop. In the late-planted crop, populations of *H. zea* averaged 15 per 120 ears in the non-BT isoline compared to less than 2 per 120 ears in the Bt-transgenic crop. Whorl damage by fall armyworms significantly reduced plant height in both mid-season and late-season non-transgenic crops but not in Bt-transgenic sweet corn. Numbers of other insects and spiders were low and not significantly different between the two treatments. Bt transformed sweet corn will play a major role in reducing populations of *H. zea, S. frugiperda* and limiting pesticides in this crop.

Key Words *Bacillus thuringiensis,* Bt-transgenic sweet corn, corn earworm, *Helicoverpa zea,* fall armyworm, *Spodoptera frugiperda,* beneficial species

The major pest of sweet corn, *Zea mays* L., in the southeastern United States is the corn earworm, *Helicoverpa zea* (Boddie). In late-planted sweet corn, the fall armyworm, *Spodoptera frugiperda* (J. E. Smith), also attacks the whorls and, to a lesser extent, the ears. Frequent applications of chemical insecticides are normally required to produce a crop with an acceptable level of damage (Lynch et al. 1998). Corn earworm is a particularly troublesome pest in that the neonate larvae move inside the ear soon after hatching, thereby avoiding contact with conventional insecticides. Managing these pests with insecticides is not only expensive but may cause health and environmental problems, development of pesticide resistance, and have negative impacts on pollinating species and natural enemies.

Bacillus thuringiensis (Bt) transgenic field corn expressing the Bt endotoxin Cry1A(b) or Cry9c, significantly reduced damage by the European corn borer, *Ostrinia nubilalis* (Hübner), and southwestern corn borer, *Diatraea grandiosella* Dyar

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(Archer et al. 2000). Williams et al. (1998) used a laboratory bioassay to determine that husks and silks from Bt transgenic corn either killed or reduced the growth of *H. zea* and *S. frugiperda* larvae. Earlier studies had shown that Bt transgenic corn hybrids reduced *S. frugiperda* feeding in whorl-stage plants (Williams et al. 1997). Wiseman et al. (1999) incorporated leaves, silks and kernels from a Bt-transgenic sweet corn hybrid with the *cry1A*(*b*) gene into a pinto bean diet. Bioassays using the diet revealed that all plant parts were highly resistant to *H. zea,* and diets containing leaves and silks were resistant to *S. frugiperda.* Ear damage by *H. zea* was negligible when Bt-transgenic sweet corn containing the gene from *B. thuringiensis* ssp. *kurstaki* (Berliner) was tested in the field (Lynch et al. 1999a). In Minnesota, Burkness et al. (2001) found that sweet corn hybrids expressing the Bt toxin significantly reduced populations of both *O. nubilalis* and *H. zea.* The general conclusion from these studies was that Bt-transgenic sweet corn offered environmentally compatible possibilities for managing insect pests in this crop (Lynch et al. 1998). However, under heavy *H. zea* population pressure, Bt-sweet corn alone was not sufficient to suppress this pest to levels where damage was acceptable so that applications of insecticides still would be required. Lynch et al. (1999b) suggested that a minimum number of insecticide applications was necessary to effectively manage *H. zea* and *S. frugiperda* in Bt-transgenic sweet corn in Georgia.

In addition to assessing the impact of Bt-transgenic sweet corn on pest species, it is important to consider the possible effects on non-target species. This study was designed to evaluate the effects of sweet corn expressing the Bt toxin on *H. zea, S. frugiperda* and other plant feeding and beneficial arthropods.

Materials and Methods

Bt-transgenic sweet corn and a companion non-Bt isoline was planted on 15 March, 12 July, and 8 August 2000 at Clemson University's Coastal Research and Education Center, Charleston, SC. Sweet corn seeds (Attribute®, GH 0937) expressing the *cryIA*(*b*) gene from *B. thuringiensis* var. *kurstaki,* were obtained from Syngenta Seeds (Boise, ID). The experimental design was a randomized complete block with 6 replications. Each plot was 8 rows by 6.1 m long with 1.5 m alleys between blocks. Four rows of the non-Bt isolines were planted around the field as guard rows. Rows were spaced at 91.4 cm with 20.3 cm between plants within rows. Standard sweet corn cultural practices were followed throughout the season.

The harvest samples were taken from the early-planted crop on 13 June, from the mid-season crop on 19 September and from the later-planted crop on 19 October. Numbers of *H. zea* larvae in 20 ears from each plot were recorded and their sizes were approximated as follows: small (first to third instars), medium (fourth instars), and, large (fifth instars or older). No *S. frugiperda* were present in the early-planted crop. The height of 10 plants from each plot was measured and recorded on 15 August from the mid-season crop and on 20 September from the late-season crop. *Spodoptera frugiperda* larvae were collected from the whorls of plants on 4 August and 11 August from the second crop and on 11 September for the late-planted crop. Larvae were placed on artificial diet (Burton 1969), held in a rearing room at 26°C and 60 to 70% RH, and monitored daily until they died from parasitism, entomopathogens or emerged as adults. D-Vac (Dietrick 1961) suction samples were taken from 36.6 m row from each treatment from the early-planted crop on 19 May and from the late-planted plots on 4 October 2000, when plants were at the early-ear formation stage.

Insects and spiders were sorted, counted and recorded. Comparisons were made between mean numbers of insects, spiders, plant heights and damaged ears in the Bt transgenic sweet corn and non-Bt isoline using the Student's t-test at $P = 0.05$.

Results and Discussion

Early season crop. Populations of *H. zea* were lowest during the early-planted field test, and no *S. frugiperda* were present in this crop. Numbers of small *H. zea* larvae were low (2 per 20 plants) in both Bt-transgenic and non-Bt isoline, and these differences were not significant ($P < 0.05$) (Table 1). However, both medium and large *H. zea* larvae from the Bt transgenic sweet corn plots were significantly ($P < 0.05$) lower than in the non-Bt isoline (Table 1). We found no large (fifth instar or larger) *H. zea* larvae in the Bt transgenic crop. Sweet corn with the Bt gene had the greatest impact on early instars. This was reported earlier by Lynch et al. (1999a). Ears from non-Bt isoline corn had 23.3% *H. zea* damage with live larvae compared to only 6.7% in the Bt sweet corn (Table 2). When total damage to ears was compared, 65.8% of the non-Bt isoline was damaged by *H. zea* compared to 10.8% from the Bt transgenic sweet corn plots.

Differences in mean numbers of other insects or spiders collected from the Bt transgenic corn compared to the non-transgenic crop were not significantly different (Table 3). However, numbers of these arthropods were low and variable.

Table 1. Mean (± standard deviation) number of *H. zea* larvae per ear from early, mid-season and late-season crops of Bt transgenic and non-Bt isolines of sweet corn. Larval samples taken on 13 June, 19 September and 19 October 2000 from early, mid-season and late-planted crops, respectively. Charleston, SC

	Small (1^{st}-3^{rd} instar)	Medium (4^{th} instar)	Large ($5^{th} \geq$ instar)
Early planting (15 March 2000)			
Non-Bt isoline	0.11 ± 0.36 a	0.18 ± 0.44 a	0.05 ± 0.22
Bt	0.08 ± 0.29 a	0.02 ± 0.13 b	0.00
Mid-season planting (12 July 2000)			
Non-Bt isoline	0.29 ± 0.56 a	0.44 ± 0.78 a	0.79 ± 0.89 a
Bt	0.09 ± 0.39 b	0.13 ± 0.13 b	0.04 ± 0.20 b
Late-season planting (8 August 2000)			
Non-Bt isoline	0.15 ± 0.40 a	0.41 ± 0.65 a	0.31 ± 0.55 a
Bt	0.02 ± 0.13 b	0.04 ± 0.24 b	0.02 ± 0.13 b

Numbers in a column followed by the same letter are not significantly different according to the Student's t-test ($P < 0.05$).

Table 2. Mean (± standard deviation) plant height, percent whorls damaged by *S. frugiperda* and percent ears damaged by *H. zea* from early, mid-season and late-season crops of Bt transgenic and non-Bt isolines of sweet corn. Charleston, SC

	S. frugiperda damage		*H. zea* damage	
	Plant height (cm)	Percent whorls damaged	Total percent ears damaged	Percent ears damaged with larvae present
Early planting (15 March 2000)				
Non-Bt isoline	—	0	65.8 ± 7.4 a	23.3 ± 10.8 a
Bt	—	0	10.8 ± 8.0 b	6.7 ± 5.2 b
Mid-season planting (12 July 2000)				
Non-Bt isoline	57.5 ± 12.5 a	70.4 ± 12.3 a	98.3 ± 2.6 a	77.5 ± 12.1 a
Bt	88.2 ± 10.4 b	3.8 ± 4.2 b	88.3 ± 11.3 a	20.8 ± 10.7 b
Late-season planting (8 August 2000)				
Non-Bt isoline	79.3 ± 15.0 a	98.8 ± 2.9 a	95.8 ± 4.6 a	70.3 ± 17.2 a
Bt	101.7 ± 17.6 b	10.5 ± 6.1 b	29.2 ± 15.6 b	5.8 ± 4.9 b

Numbers in a column followed by the same letter are not significantly different according to the Student's t-test ($P < 0.05$), after arcsin transformation of percentage data.

Mid- and late-season crops. Sweet corn planted at mid- and late-season was infested by both *S. frugiperda* and *H. zea. Helicoverpa zea* population pressure was greater than in the first planting and the greatest difference between treatments was reflected in numbers of large *H. zea* larvae (Table 1). *Helicoverpa zea* was present in 77.5% of the ears in the non-Bt isoline whereas only 20.8% of the ears in the Bt transgenic sweet corn had *H. zea* larvae (Table 2). Overall ear damage was higher (98.3%) in ears of non-Bt sweet corn compared to the non-transgenic plants (88.3%) (Table 2). However, damage to the ears in the transgenic sweet corn was mostly due to only a slight amount of feeding by small instars and significantly fewer live larvae present compared to the non-Bt isoline. There were fewer than one *H. zea* (all instars) per 20 ears in the Bt sweet corn in the late-season crop, but medium-sized larvae were found in 70.3% of the ears in the non-Bt isoline (Table 2). Percent damaged ears (with and without live *H. zea* larvae) in the late-season crop was significantly ($P < 0.05$) higher in the non-Bt isoline (Table 2).

The mid-season Bt-transgenic crop, planted on 12 July had only 3.8% of the plants with whorls damaged by *S. frugiperda,* whereas 70.4% of the non-Bt genotype had serious whorl damage (Table 2). This difference was even more exaggerated in the late-season crop where nearly 100% of the plants were damaged in the non-Bt isoline, but *S. frugiperda* had fed on the whorls of only about 11% of the Bt-transgenic plants. Severe whorl feeding caused the plants to be stunted and significantly shorter than those in the non-Bt isoline in both mid- and late-season plantings (Table 2).

Table 3. Mean numbers of arthropods suction sampled from 6.1 m of row in Bt transgenic sweet corn and a non-Bt isoline. Planted 15 March sampled by D-Vac suction sampler on 19 May 2000. Charleston, SC

	Herbivores						
Genotype	Chrysomelidae	Miridae	Thripidae	Cicadellidae	Lygaeidae	Alydidae	Aphididae
Bt	16a	2a	1a	5a	0.5a	0.5a	2a
Non-Bt	24a	4a	2a	3a	0.5a	1a	4a

	Beneficials						
Genotype	Anthocoridae	Coccinellidae	Lygaeidae	Spiders	Ants	Parasitoids	Nabidae
Bt	3a	2a	1a	1a	0.5a	1.5a	1a
Non-Bt	6a	2a	1a	3a	1.5a	3a	1a

Numbers in a column followed by the same letter are not significantly different according to the Student's t-test ($P < 0.05$).

Table 4. Mean numbers of arthropods suction sampled from 6.1 m of row in Bt transgenic sweet corn and a non-Bt isoline. Planted 8 August sampled by D-Vac suction sampler on 4 October 2000. Charleston, SC

	Herbivores				
Genotype	Chrysomelidae	Miridae	Cicadellidae	Lygaeidae	Curculionidae
Bt	36a	1.3	7.2a	0.8a	0
Non-Bt	27a	0	9.7a	0.7a	0.2

	Beneficials						
Genotype	Anthocoridae	Coccinellidae	Lygaeidae	Spiders	Ants	Parasitoids	Nabidae
Bt	1.3a	2.8a	0.5a	0.2a	4.5a	0.5a	0.2a
Non-Bt	4.8a	2.3a	0.2a	0.3a	3.0a	2.7a	0.3a

Numbers in a column followed by the same letter are not significantly different according to the Student's t-test ($P < 0.05$).

Numbers of other insects and spiders suction sampled from the two treatments were not significantly different (Table 4).

Detection of possible differences in parasitism was not possible due to low numbers of *H. zea* and *S. frugiperda* larvae collected. Bernal et al. (2002) discussed the indirect effects of Bt-transgenic corn on parasitoids of stem borers. They concluded that host quality may influence by sublethal effects of the Bt toxin which, in turn, could affect parasitoid performance.

In summary, Bt transgenic sweet corn significantly reduced ear-feeding by both *H. zea* and *S. frugiperda* and whorl-feeding by *S. frugiperda*. The impact on *H. zea* in the ears was especially evident with younger larvae because almost no large larvae were present in the early-planted crop of Bt transgenic sweet corn. Mostly small to medium-sized *H. zea* larvae were found in the mid- and late-season crops. Sweet corn containing the Bt toxin was able to withstand heavy populations of *S. frugiperda.*

Heliocoverpa zea larvae, and their damage in mid- and late-planted Bt transgenic crops, still would have required additional controls to meet the stringent market standards for sweet corn in commercial production. However, our findings agree with those of Lynch et al. (1999a) who reported that Bt transgenic sweet corn can play a major role in reducing numbers of insecticide applications that are currently applied to the crop. We found no evidence to suggest that Bt transgenic sweet corn negatively impacted non-target species although numbers were low and variable.

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