EVALUATION OF YIELDGARD TRANSGENIC RESISTANCE FOR CONTROL OF FALL ARMYWORM AND CORN EARWORM (LEPIDOPTERA: NOCTUIDAE) ON CORN

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ABSTRACT

Fall armyworm, *Spodoptera frugiperda* (J. E. Smith), and corn earworm, *Helicoverpa zea* Boddie, perennially cause leaf and ear damage to corn in the southeastern USA. Development of transgenic hybrids expressing insecticidal endotoxin from *Bacillus thuringiensis* (Bt) offers a new approach to managing these insects in field corn. Transgenic Bt hybrids with either the Bt11 or MON810 event, collectively known as YieldGard Technology, were evaluated for control fall armyworm and corn earworm in southern Georgia during 1998, which coincided with a severe outbreak of fall armyworm. YieldGard Bt resistance consistently reduced whorl infestation and damage to low levels and also reduced ear infestations and larval numbers per ear. However, larval establishment did occur on many ears of resistant plants, but once established in ears, larvae of both species developed more slowly and caused much less kernel damage on resistant than susceptible plants. We found no relationship between YieldGard Bt resistance and corn grain aflatoxin concentrations. Yield responses were variable with the prevention of yield loss being proportional to the severity of insect damage. These results indicate that YieldGard resistance is effective in preventing significant losses to field corn by fall armyworm and corn earworm. Further, evaluation under a variety of growing conditions and insect infestation levels is needed to clearly assess the value of YieldGard technology to corn growers in the Southeast.

Key Words: Plant resistance, *Spodoptera frugiperda*, *Helicoverpa zea*, Transgenic crops, Bt resistance

RESUMEN

Spodoptera frugiperda (J.E. Smith), y *Helicoverpa zea* Boddie, perennemente causan daño de hoja y mazorca al maíz en el sureste de los Estados Unidos de América. El desarrollo de híbridos transgénicos que expresan la endotoxina insecticida de *Bacillus thuringiensis* (Bt) ofrece una nueva practica para controlar estos insectos en campos de maíz. Híbridos transgénicos Bt, ya sea con evento Bt11 o MON810, colectivamente conocidos como tecnología YieldGard, fueron evaluados para control de *S. frugiperda* y *H. zea* en el sur de Georgia durante el 1998, lo cual coincidió con una epidemia severa de *S. frugiperda*. Resistencia YieldGard Bt consistentemente redujo infestación de cogollo y daños a niveles bajos y también redujo infestación de mazorca y el numero de larvas por mazorca. Sin embargo, establecimiento larval si ocurrió en numerosas mazorcas de plantas resistentes, pero una vez establecidas el la mazorca, larvas de ambas especies se desarrollaron mas lentamente y causaron mucho menos daño de grano en plantas resistentes que en las susceptibles. No encontramos ninguna relación entre resistencia YieldGard Bt y concentraciones de aflatoxina en granos de maíz. Producción de cosechas fueron variables con la prevención de perdida de producción siendo proporcional a la severidad del daño por insecto. Estos resultados indican que resistencia YieldGard es efectiva en prevenir perdidas significativas de maíz de campo por *S. frugiperda* y *H. zea*.

Fall armyworm, *Spodoptera frugiperda* (J. E. Smith), and corn earworm, *Helicoverpa zea* Boddie, are the most important insect pests of corn in the southeastern U.S. Both insects infest whorl stage plants causing leaf injury, but more importantly they infest ears causing direct loss of grain. Insecticidal control is difficult and generally not cost effective in field corn. Typically, early planting times are recommended in the Southeast partly to avoid damaging levels of both insects which often occur later in the season. Germplasm with moderate levels of leaf feeding resistance to fall armyworm has been released (Williams et al. 1997, 1998). High levels of resistance to fall armyworm and corn earworm in silks of the tropical corn 'Zapalote Chico' also have been identified (Wiseman & Windstrom 1986). However, these natural sources of plant resistance have not been effectively deployed.

Transgenic corn hybrids expressing the insecticidal protein Cry1Ab from *Bacillus thuringiensis* (Bt) var. *kurstaki* were originally developed to control European corn borer, *Ostrinia nubilalis* (Hübner) and offer the potential for reducing losses by fall armyworm and corn earworm. Several events of transgenic Bt corn have been developed with different modes of toxin expression (Ostlie et al. 1997). The most promising events are Bt11 (Novartis Seeds) and MON810 (Monsanto Co.) where endotoxin is expressed in vegetative and reproductive structures throughout the season (Armstrong et al. 1995; Williams et al. 1997). Hybrids containing either of these events are collectively referred to as having 'YieldGard Technology' (Ostlie et al. 1997). Laboratory feeding trials and small controlled field trials have shown that hybrids containing the Bt11 event reduce fall armyworm and corn earworm growth and survival (Williams et al. 1997, 1998). YieldGard resistance also is very effective against Southwestern corn borer (Archer et al. 2000; Williams et al. 1998), but this insect either does not occur or is not economically important in the coastal plain region of the southeastern U.S.

Because of concerns about the potential for corn earworm to develop virulence to Bt technology in transgenic cotton, YieldGard transgenic corn was not commercially deployed in the Southeast until 1998. In a series of studies at five locations in Alabama in 1998 with corn planted at the recommended time and 1 and 2 months later, DeLamar et al. (1998a-e) demonstrated that YieldGard resistance (events MON810 and Bt11) prevented whorl damage, kernel damage, and yield loss by lepidopterans, primarily fall armyworm and corn earworm, in later plantings at all locations. YieldGard resistance generally did not improve the performance of corn planted at recommended times, because these plantings generally escaped severe lepidopteran damage. YieldGard resistance has not been evaluated in the field under natural infestations of fall armyworm or corn earworm in Georgia. Furthermore, lepidopteran infestations of ears have been linked with increased levels of fungal infection and contamination of grain by mycotoxins such as aflatoxin produced by *Aspergillus flavus* (e.g., Windstrom 1979; McMillian 1983; McMillian et al. 1985; Smith & Riley 1992). Effective reduction in lepidopteran ear infestations with transgenic Bt resistance may also help reduce aflatoxin contamination of grain (Williams et al. 1998).

Our objective was to evaluate the effect of both YieldGard Technology events on fall armyworm and corn earworm infestations and damage and on grain aflatoxin contamination of field corn. Trails were conducted in southern Georgia during the summer of 1998 and coincided with a severe outbreak of fall armyworm.

MATERIALS AND METHODS

Trials were conducted on a Greenville sandy loam soil at the Univ. of Georgia Southwest Branch Experiment Station near Plains, and on a Tifton sandy loam soil at the Attapulgus Research Center near Attapulgus and the Coastal Plain Experiment Station near Tifton. The study area at each location was fertilized, chisel plowed or subsoiled twice, and disk harrowed. Before disking 440 kg/ha of 3-18-9 (N-P-K) granular fertilizer was applied and an additional 112 kg of nitrogen was applied as ammonium nitrate about 20 d after planting. Seed at all locations was planted with an air-planter at the rate of 66,700 plants per ha. Pendimethalin (Prowl) at 0.71 L/ha and atrazine (Aatrex) at 0.57 L/ha were applied to control weeds. No other pesticides were applied. Natural rainfall was supplemented by irrigating weekly with 6 cm/ha of water as needed at all locations.

The experimental design within each planting date and location was a split plot design with whole plots being brand (manufacturer) and split plots being hybrid pairs within manufacturer. At Attapulgus a single planting occurred on 23 April 1998. Planting dates were conducted as separate side-by-side trails with two dates at Tifton (13 and 23 April 1998) and three dates at Plains (14 April, 12 May and 3 June 1998). Hybrid pairs were a Bt hybrid and a non-Bt isoline or near isoline hybrid. Susceptible and Bt-resistant hybrid pairs at Plains were Pioneer Brand 3223 and 31B13 (Bt), Golden Harvest (Monsanto) 2530 and 2530Bt, Dekalb DK 591 and DK 591BtY, and Novartis N79-P4 and N79L3 (Bt). Pairs at Tifton were Pioneer Brand 3223 and 31B13 (Bt), Pioneer Brand 3394 and 33V08 (Bt), and Novartis N79-P4 and N79L3 (Bt). Pairs at Attapulgus were Pioneer Brand 3223 and 31B13 (Bt), Dekalb DK 591 and DK 591BtY, and Novartis N79-P4 and N79L3 (Bt). Whole plots were arranged in a randomized complete block design with four replications at Attapulgus and the first and second planting dates at Plains and 3 replications at both plantings at Tifton and the third date at Plains. Plots measured 15.2 m by 6 rows (76-cm rows) at Plains, 21.3 m by 4 rows (91-cm rows) at Attapulgus and 9.1 m by 4 rows (91-cm rows) at Tifton.

Whorl defoliation was assessed by rating 30 plants (all plants at Tifton) in the two center rows per plot about 6 wk after planting at the 8-10 leaf stage for each planting date. Plants were rated for damage using a 0-9 scale (Davis et al. 1992) where 0 is no damage and 9 is whorl and furl almost completely defoliated. The damage scale is not linear with ratings of ≥4 indicating substantially more damage than ratings of ≤3. Twenty to 30 larvae were collected for species identification from infested whorls in rows at the edge of plots. Ear damage of 12 ears per plot was assessed by counting the number of live larvae and larval feeding cavities and measuring the total length of all feeding cavities for each ear (Windstrom 1967). Ear infestations were sampled twice at Attapulgus with live larvae in the first sample being identified to species and categorized as small, medium or large and final ear damage being assessed at the second sample.

The two center rows of each plot were harvested with a Hege two-row corn combine on August 12, September 1, and September 12 for the three respective planting dates at Plains and 20 August at Tifton. At Attapulgus, plots (all 4 rows) were harvested on 10 August using a John Deere 4420 combine with a 4-row corn head modified for small plot harvesting. Grain yields were adjusted to 15.5% moisture content. A 2-kg subsample of grain was collected from each plot in trials at Plains and Attapulgus for determination of grain aflatoxin levels. Kernels were ground to pass a 20 mesh screen, well mixed, and 100 g subsample were extracted. Aflatoxin contamination was determined using the Vicam immunoaffinity column method (Truckness et al. 1991) and is reported as total aflatoxin (B_1 + B_2 + G_1 + G_2) in parts per billion of seed.

Results were analyzed within each planting date and location with an analysis of variance for a split plot design. Before analysis, percentage data were transformed by square-root arcsine, and numeric data were transformed by $\log_{10}(x + 1)$. Significance of main effects for brand (manufacturer) and hybrid resistance (i.e., Bt verses non-Bt) were determined using F test at $P = 0.05$. Brand × hybrid-resistance interactions were not significant (P = 0.05) for any parameter. Therefore, only hybrid resistance main effects (i.e., average across all brands) are presented for the combined analyses.

RESULTS

Species Composition

Fall armyworm populations reached damaging outbreak levels earlier than normal in 1998 resulting in the worst damage to corn in Georgia in the last decade. Whorl infestations in all trials consisted almost entirely of fall armyworm. Ear infestations at Attapulgus (N = 430 larvae) were 89% fall armyworm and 11% corn earworm. At Tifton, ear infestations were 90% fall armyworm and 10% corn earworm in both plantings (N = 178). At Plains, fall armyworms accounted for 11%, 48% and 73% of total live larvae observed in ears of all hybrids in the first (N = 75), second (N = 131), and third planting (N = 33) dates, respectively, with the balance being corn earworm.

Whorl Infestations and Damage

Hybrid brand did not significantly (P = 0.05) affect the percentage of infested whorls or mean damage rating per plant and per infested plant in any trial. Whorl infestations and damage increased substantially from the first to third plantings at Plains, but were similar between plantings at Tifton (Table 1). YieldGard Bt resistance greatly reduced whorl infestations, whorl damage ratings per plant and whorl damage rating per infested plant at Attapulgus and in all plantings at Plains (Table 1). Whorl infestations and whorl damage ratings also were smaller in resistant than susceptible hybrids in both plantings at Tifton. However whorl infestations were much lower at Tifton than at the other locations, and differences were not significant in either planting.

Ear Infestations and Damage

Hybrid brand main effects were not significant (P = 0.05) in any trial for the percentage of infested ears or mean damage rating per ear and per infested ear. Ear infestations in susceptible hybrids were uniformly high in all trials, but ear damage became progressively more severe in later plantings at Tifton and Plains (Table 2). The percentage of infested ears was reduced in resistant hybrid in all plantings at Tifton and Plains, although 30% to 70% of ears of resistant hybrids were infested. However, at Attapulgus all ears of susceptible hybrids and almost every ear of resistant hybrids were infested. YieldGard resistance significantly reduced the number of larval cavities which is a direct measure of the number of larvae per ear in all trials (Table 2). Furthermore, resistance also greatly reduced the amount of damage per ear and per infested ear in all trials, with the exception of the damage per infested ear in the first planting at Tifton.

Many larvae were present in ears during the first ear sample at Attapulgus. The size distribution of larvae reveals that the majority of fall armyworms and corn earworms were medium sized (i.e., instars 3 and 4) in ears of Bt resistant plants, but most larvae were large sized (i.e., instars 5 and 6) in ears of susceptible plants (Fig. 1).

Grain Yield and Aflatoxin Levels

Grain yield and aflatoxin level were not significantly different between brands in any trial, except at Attapulgus where both Pioneer brand hybrids yielded more than the other hybrids. This difference in yield presumably is due to differences in agronomic characteristics and not to differences in insect resistance.

TABLE 1. MEAN (±SE) WHORL INFESTATION AND WHORL DAMAGE RATING CAUSED BY FALL ARMYWORM IN SUSCEPTIBLE AND 'YIELDGARD' RESISTANT CORN IN GEORGIA, 1998.

Location	Planting date	Bt resistance	Infested whorls (%)	Damage rating[1] per plant	Damage rating[1] per infested plant
Attapulgus	23 March	-	83.3 ± 2.2	4.0 ± 0.3	4.9 ± 0.2
		+	13.8 ± 1.8	0.5 ± 0.1	3.2 ± 0.4
		F	675.83***	303.82***	16.81**
Tifton	13 April	-	12.3 ± 2.2	0.23 ± 0.06	1.77 ± 0.13
		+	6.9 ± 1.5	0.10 ± 0.02	1.42 ± 0.11
		F	2.03 ns	2.14 ns	2.09 ns
	23 April	-	8.8 ± 0.4	0.14 ± 0.03	1.75 ± 0.49
		+	4.4 ± 1.9	0.05 ± 0.02	1.16 ± 0.06
		F	2.33 ns	2.42 ns	1.19 ns
Plains	14 April	-	23.3 ± 3.2	0.5 ± 0.1	2.1 ± 0.2
		+	9.2 ± 1.8	0.1 ± 0.1	1.0 ± 0.2
		F	19.64**	24.56**	19.75**
	12 May	-	49.4 ± 5.4	2.5 ± 0.3	5.1 ± 0.2
		+	14.1 ± 3.6	0.5 ± 0.2	3.8 ± 0.3
		F	42.79**	37.22***	18.35**
	3 June	-	96.1 ± 1.4	5.4 ± 0.2	5.6 ± 0.1
		+	35.0 ± 2.6	1.1 ± 0.1	3.4 ± 0.1
		F	283.41***	1068.67***	45.08***

ns, **, *** indicate not significant and significant at $P = 0.01$ and $P = 0.001$, respectively.
[1]Rating scale of Davis et al. (1992) where 0 is no damage and 9 is whorl and furl destroyed.

TABLE 2. MEAN (±SE) EAR INFESTATION, CAVITY NUMBER AND LENGTH CAUSED BY FALL ARMYWORM AND CORN EARWORM IN SUSCEPTIBLE AND 'YIELDGARD' RESISTANT CORN IN GEORGIA, 1998.

Location	Planting date	Bt resistance	Infested ears (%)	Larval cavities per ear	Damage rating[1] per ear	Damage rating[1] per infested ear
Attapulgus	23 March	-	100.0 ± 0	2.4 ± 0.1	8.9 ± 0.6	8.9 ± 0.6
		+	96.5 ± 1.6	1.1 ± 0.1	2.7 ± 0.2	2.8 ± 0.2
		F	4.48 ns	90.89***	135.79***	131.21***
Tifton	13 April	-	81.5 ± 4.0	0.9 ± 0.1	2.3 ± 0.2	2.7 ± 0.2
		+	28.7 ± 7.9	0.3 ± 0.1	0.7 ± 0.2	2.0 ± 0.3
		F	45.73***	67.73***	37.82***	2.42 ns
	23 April	-	93.5 ± 4.0	1.1 ± 0.1	4.0 ± 0.2	4.3 ± 0.1
		+	53.7 ± 4.9	0.6 ± 0.1	1.3 ± 0.4	2.3 ± 0.6
		F	30.61**	34.65***	25.40**	15.25**
Plains	14 April	-	91.0 ± 2.4	1.21 ± 0.18	4.3 ± 1.2	4.7 ± 0.1
		+	36.8 ± 7.2	0.38 ± 0.25	0.9 ± 0.2	2.2 ± 0.2
		F	63.77**	124.22***	392.57***	97.81***
	12 May	-	95.3 ± 2.0	1.43 ± 0.32	5.7 ± 0.6	5.9 ± 0.6
		+	68.2 ± 4.7	0.73 ± 0.22	1.9 ± 0.3	2.7 ± 0.2
		F	54.89***	37.17***	37.56***	23.69**
	3 June	-	94.4 ± 2.0	1.77 ± 0.34	7.5 ± 0.7	7.9 ± 0.6
		+	40.7 ± 6.0	0.41 ± 0.18	0.9 ± 0.1	2.1 ± 0.2
		F	288.74***	142.16***	104.88***	104.48***

ns, **, *** indicate not significant and significant at $P = 0.01$ and $P = 0.001$, respectively.
[1]Rating scale of Windstrom (1967) where 0 is no damage.

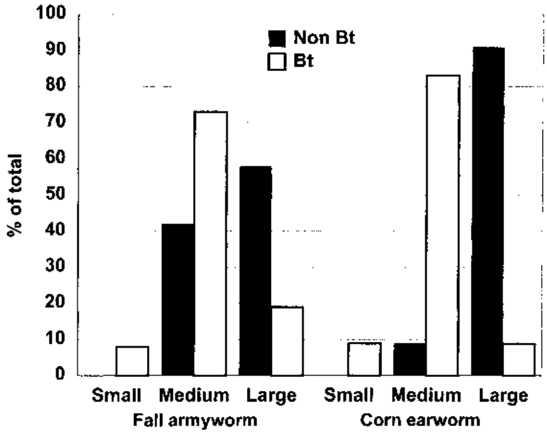

Fig. 1. Size distribution fall armyworm and corn earworm larvae in ears of susceptible and Bt resistant corn hybrids at Attapulgus, GA, 1998.

YieldGard resistance at Attapulgus prevented significant yield ($F = 113.29$; df = 1, 9; $P = 0.0001$) losses in all brands of hybrids (Fig. 2). Resistance in this trial prevented an average yield loss of 28% which equaled 2141 kg/ha (=34.1 bu/acre). Grain yields at Tifton were not significantly different between Bt resistant types in either planting (Date 1: $F = 0.14$; df = 1, 9; $P = 0.72$; Date 2: $F = 0.08$; df = 1, 9; $P = 0.79$).

Grain yields at Plains were low for all planting dates with yields being greatest on the second planting date (Fig. 2). Average grain yields were not significantly ($F = 0.01$; df = 1, 12; $P = 0.98$) different between susceptible and resistant hybrids on the first planting date. YieldGard resistance prevented significant grain yield losses of 21.8% during the second planting date ($F = 15.27$; df = 1, 12; $P = 0.0021$) and 74.5% during the third planting date ($F = 128.41$; df = 1, 9; $P = 0.0001$).

Grain aflatoxin concentrations were extremely high at Attapulgus and in the first planting at Plains. Aflatoxin progressively declined to low levels with later plantings at Plains (Fig. 3). Grain aflatoxin concentrations were not significantly different between susceptible and Bt-resistant hybrids in any trial (Attapulgus: $F = 2.58$, df = 1, 9, $P = 0.14$; Plains PD1: $F = 0.50$, df = 1, 12, $P = 0.50$; Plains PD2: $F = 0.20$, $P = 0.66$; Plains PD3: $F = 0.81$, df = 1, 9, $P = 0.40$).

Discussion

YieldGard Bt resistance consistently prevented whorl infestation and damage by fall armyworm. Even when larvae established on resistant plants, whorl damage of infested plants was substantially reduced. YieldGard resistance also reduced lepidopteran infestations and the number of larvae in ears, but larval establishment did occur on many ears of resistant plants. Indeed, at Attapulgus, where large numbers of fall armyworm predominated, Bt resistance did not reduce the percentage of infested ears. However, once established in ears, larvae of both species developed more slowly and caused much less kernel damage on ears of resistant than susceptible plants. The lack of significant brand by resistance interactions for any variable measured also verifies that hybrids with the Bt11 and MON810 events were similar in efficacy controlling in whorl and ear infestations for both species.

Despite reports showing an association between lepidopteran damage and aflatoxin contamination of corn grain (e.g., Windstrom 1979; McMillian 1983; McMillian et al. 1985; Smith & Riley 1992), we found that YieldGard Bt resistance

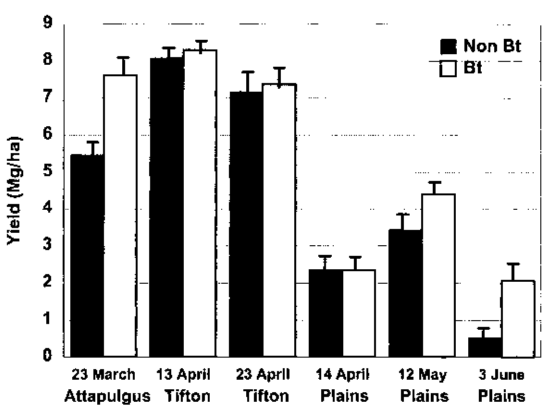

Fig. 2. Grain yield (±SE) of susceptible and Bt resistant corn hybrids planted at three location in southern Georgia in 1998.

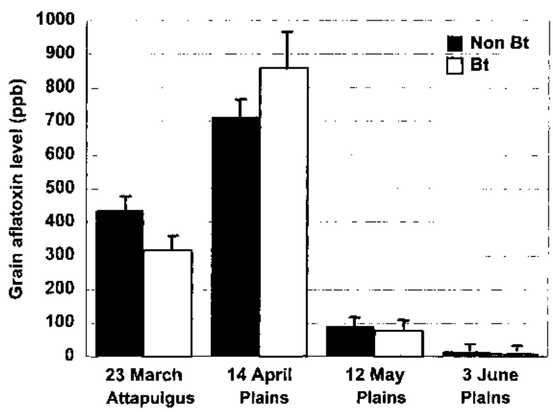

Fig. 3. Grain aflatoxin concentration (±SE) of susceptible and Bt resistant corn hybrids at Attapulgus and three planting times at Plains, GA in 1998.

did not affect aflatoxin concentrations in grain. Although YieldGard Bt resistance prevented most kernel damage, larvae frequently established on resistance ears. Newer events with high levels of toxin expression that virtually prevent larval establishment may be needed to effectively test the hypothesis that lepidopterans infestations enhance aflatoxin contamination of corn grain.

Growing conditions in 1998 were hot and very dry causing most dryland corn production to be destroyed. Yield of irrigated corn also was reduced because of high temperatures during most of the season and especially during pollination and silking. Hybrid yields of plantings at Plains were low and do not permit useful economic comparisons of the value of YieldGard technology. However, grain yields at Attapulgus were typical of this location in 1998. Assuming no large differences in grain quality and at a grain price of $76.52 per Mg (= $2.00 per bu), the average yield loss of 2141 kg/ha (= 34.1 bu/acre) produced a $168.45 per ha (= $68.20 per acre) gross return from YieldGard technology in this one trial. However, economic benefit must be more extensively evaluated under a variety of growing conditions and insect infestations levels to clearly assess the value of YieldGard technology to corn growers in the Southeast.

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