ENHANCING INHERENT FALL ARMYWORM (LEPIDOPTERA: NOCTUIDAE) RESISTANCE OF CORN WITH *BACILLUS THURINGIENSIS* GENES

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ABSTRACT

Fall armyworm, *Spodoptera frugiperda* (J. E. Smith), is a serious pest of corn, *Zea mays* L., in the southern United States. Larvae feed extensively on leaves and other parts of the plant. Germplasm exhibiting a moderate level of resistance to leaf feeding damage has been identified and released. This germplasm has been used in breeding programs for developing corn hybrids with resistance to fall armyworm and other Lepidoptera. In recent years, much effort has also been devoted to developing corn hybrids with genes from *Bacillus thuringiensis* (*Bt*) that encode insecticidal proteins. Some of these hybrids have exhibited moderate resistance to fall armyworm damage. In this investigation hybrids with both native genetic resistance and genes from *Bt* encoding insecticidal proteins were evaluated for resistance to fall armyworm in field tests and laboratory bioassays. Hybrids with both types of resistance sustained less fall armyworm damage than hybrids that had only native genetic resistance or genes from *Bt* encoding insecticidal proteins alone. Larvae that fed on hybrids with both types of resistance were significantly smaller after feeding on plants in the field or on lyophilized whorl leaf tissue in a laboratory bioassay for 10 d than larvae fed on susceptible hybrids or hybrids with only one type of resistance. Both traditional host plant resistance and transformation of corn with genes from *Bt* provide hybrids with moderate levels of resistance, but when used together, they are complementary. Deployment of hybrids with both types of resistance should reduce losses to fall armyworm and also reduce the rate of buildups of resistance to *Bt* in fall armyworm populations.

Key words: Host plant resistance, insect resistance, maize, transgenic hybrids, transformation

RESUMEN

El gusano cogollero del maíz, *Spodoptera frugiperda* (J. E. Smith), es una peste seria del maíz, *Zea mays* L., en el sur de los Estados Unidos. Las larvas se alimentan extensivamente de las hojas y otras partes de la planta. Germoplasma que exhibe un nivel moderado de resistencia contra daño en sus hojas se ha identificado y plantado. Este germoplasma se ha usado en programas para el desarrollo de maíz híbrido con resistencia al gusano cogollero y a otros lepidópteros. En años recientes se ha dedicado mucho esfuerzo también al desarrollo de maíz híbrido con genes de *Bacillus thuringiensis (Bt)* que codifican a proteínas insecticidas. Algunos de estos híbridos han exhibido resistencia moderada al daño causado por el gusano cogollero del maíz. En esta investigación, híbridos con resistencia genética nativa y con genes de *Bt* que codifican a proteínas insecticidas se evaluaron en cuanto a su resistencia contra el gusano cogollero en bioensayos de campo y laboratorio. Híbridos con ambos tipos de resistencia sostuvieron menos daño que híbridos que sólo tenían resistencia genética nativa o que sólo tenían genes de *Bt* que codifican a proteínas insecticidas. Las larvas que se alimentaron sobre híbridos con ambos tipos de resistencia resultaron ser significativamente más pequeñas después de alimentarse de plantas en el campo o de tejido foliar liofilizado en un bioensayo de laboratorio por 10 d comparado con las larvas que se alimentaron de híbridos susceptibles o híbridos con sólo un tipo de resistencia. La resistencia tradicional de la planta hospedera y la transformación del maíz con genes de *Bt* proporcionan niveles moderados de resistencia a los híbridos, pero cuando se usan juntos, se complementan. El desarrollo de híbridos con ambos tipos de resistencia debe de reducir la pérdida causada por el gusano cogollero al igual que debe de reducir la tasa del incremento de la resistencia al *Bt* en poblaciones del gusano cogollero del maíz.

Fall armyworm, *Spodoptera frugiperda* (J. E. Smith), is a major pest of corn, *Zea mays* L., in the southern United States. Larvae feed extensively on leaves and other above-ground parts of the plant. Heavy feeding can cause devastating yield losses (Sparks 1986). For 30 years USDA-ARS scientists at Mississippi State, MS have conducted research on resistance in corn to maize insect and diseases that attack it. A primary objective of this research has been the development and release of corn germplasm with resistance to fall armyworm (Williams & Davis 1997). After germplasm was released, requests for seed generally came from mainly entomologists and plant breeders with commercial seed corn companies. DEKALB Genetics Corporation has reported extensive use of this germplasm in a breeding program for developing corn hybrids with resistance to multiple species of Lepidoptera, including fall armyworm (Overman 1987, 1997).

The germplasm has exhibited only a moderate level of resistance to leaf feeding by fall armyworm (Williams & Davis 1997); therefore, efforts to identify new sources and higher levels of resistance have continued. Since the development of transformation techniques for corn, much effort has been expended on developing corn plants expressing genes that encode insecticidal proteins isolated form the bacterium *Bacillus thuringiensis* (*Bt*) (Boulter 1993). Transgenic corn plants expressing *Bt* δ-endotoxin insecticidal proteins have been evaluated in field tests for resistance to European corn borer first brood (whorl stage attack) and second brood (reproductive stage attack) have been observed (Armstrong et al. 1995, Koziel et al. 1993). Transgenic hybrids evaluated for resistance to southwestern corn borer and fall armyworm in 1994 and 1995 at Mississippi State exhibited a high level of resistance to southwestern corn borer both years, but only a moderate resistance, especially in 1995, to fall armyworm (Williams et al. 1997).

The current investigation was undertaken to evaluate inherently resistant and susceptible corn hybrids including some with an added *Bt* gene. A primary objective was to compare the effectiveness of native genetic resistance, *Bt* insecticidal proteins, and a combination *of* the two in reducing fall armyworm in corn.

MATERIALS AND METHODS

Eight proprietary experimental dent corn hybrids were provided by DEKALB Genetics Corporation, 3100 Sycamore Drive, DeKalb, IL 60115. These included two hybrids, MBR line × F-line A-Mon810 and MBR line × F-line B-Mon810, in which native genetic and *Bt* resistance were combined; the corresponding two near isogenic hybrids that lacked resistance from *Bt*, MBR line × F-line A and MBR line × F-line B; DK 591, a susceptible hybrid; DK 591-Mon810, the corresponding hybrid with resistance from *Bt* added; MBR line × M-line, a single cross hybrid with native genetic resistance in one parental inbred line; and DK 626, a susceptible hybrid. Two additional hybrids were included as checks: Mp704 × Mp707, a cross between two inbred lines with native genetic resistance, and Ab24E × SC229, a cross between two susceptible inbred lines (Williams et al. 1997).

These hybrids were evaluated for leaf feeding damage and survival and growth of fall armyworm larvae in an experiment planted at Mississippi State, MS on 17 April 1997. The experimental design was a randomized complete block with five replications of two-row plots. Rows were approximately 5 m long and spaced 1 m apart. Each plot was bordered by two rows of N7639*Bt*. Except for two hybrids that were segregating for *Bt* expression, rows were over planted at a rate of 35 kernels per row and thinned to 20 plants. The two hybrids that were segregating, MBR line × F-line A-Mon810 and MBR line × F-line B-Mon810, were planted at a double rate. Non-*Bt* expressing segregates were determined by an enzyme-linked innumosorbent assay (ELISA) strip test (GeneCheck™ √ B.t.k. Corn Lab Test Kit, Part number 70755, Monsanto Co., St Louis, MO 63198) and removed before plots were thinned. On 23 May, when plants were in the V6 to V7 stage of growth (Richie et al. 1982), plants were infested with two applications of approximately 35 fall armyworm neonates each. The larvae were taken from a laboratory colony, mixed with corn cob grits, and applied with a plastic dispenser (Mihm 1989). Leaf feeding damage was visually rated 7 and 14 d later (Davis et al. 1992) on a scale of 0, no damage, to 9, extensive damage. On 2 June, 10d after plant infestation, 10 plants were removed from each plot and dissected. The surviving larvae were counted and weighted.

Three additional rows of each hybrid were grown to provide leaf tissue for laboratory bioassays. On 29 May when plants were in the V8 stage of growth, whorls were removed, trimmed to approximately 15 cm in length, placed in plastic freezer bags, and frozen at -18°C. The frozen tissue was later lyophilized and ground to a fine powder using a laboratory mill with a 1-mm mesh screen.

Laboratory bioassays were conducted as described by Williams & Buckley (1992). Test diets were prepared form each hybrid by combining 250 ml distilled water, 2.4 g agar, and 12.5 mg gentamicin sulfate, 132 mg sorbic acid, and 528 mg ascorbic acid. The mixture was heated to 82°C while stirring, and 10 g of leaf tissue was then added. The mixture was dispensed in 10 ml aliquots into 30-ml plastic cups. Twenty cups of diet were prepared from each hybrid, and each cup was infested with one fall armyworm neonate and covered with a paperboard lid. Cups were arranged in a randomized complete block design with five replications of four cups each in an environmental chamber at 28°C and a photoperiod of 12:12 (L:D). The larvae were weighed after 10 d.

Plot means were calculated for the 7 and 14-d leaf feeding ratings, number of surviving larvae per plant, larval weight, and larval biomass (total weight of larvae per plant) for the field data. Mean larval weights were calculated from the laboratory data. An analysis of variance of data for each trait was performed (SAS Institute Inc. 1987). Hybrid means were compared by Fisher's Protected LSD Test (Steel & Torrie, 1980).

Results and Discussion

Visual leaf feeding ratings made at 7 and 14 d are given in Table 1. The numerical ratings made at 14 d were slightly higher, except for MBR line × F-line B, than those made at 7 d. The two hybrids that had both native genetic resistance and a *Bt* gene sustained the least damage. They were significantly less damaged than the corresponding near-isogenic hybrids or DK 591-Mon810, which possessed only resistance

Table 1. Fall armyworm leaf feeding damage sustained by resistant and susceptible corn hybrids evaluated at Mississippi State in 1997 ($\bar{x}$ ± SD).

		Leaf feeding damage[2]	
Hybrid	Classification[1]	7 d	14 d
MBR line × F-line A-Mon810	R × S*Bt*	3.2 ± 0.4[3]	3.6 ± 0.7[4]
MBR line × F-line B-Mon810	R × S*Bt*	3.2 ± 0.3	3.9 ± 0.5
MBR line × F-line A	R × S	6.9 ± 0.7	7.1 ± 0.7
MBR line × F-line B	R × S	6.1 ± 0.7	5.7 ± 1.3
DK 591-Mon810	S × S*Bt*	4.1 ± 0.2	4.8 ± 0.3
DK 591	S × S	8.2 ± 0.6	8.9 ± 0.2
MBR line × M-line	R × S	6.3 ± 0.8	6.8 ± 0.8
DK 626	S × S	7.2 ± 0.7	8.1 ± 0.7
Mp704 × Mp707	R × R	4.4 ± 0.4	4.7 ± 0.6
Ab24E × SC229	S × S	8.8 ± 0.3	9.0 ± 0.0
LSD (0.05)		0.7	0.8

[1]R indicates fall armyworm resistant inbred; S indicates susceptible inbred; S*Bt* indicates susceptible inbred with added *Bt* gene.
[2]Fall armyworm damage was visually rated at 7 and 14 d after plants were infested with 70 neonates each on a scale of 0 (no damage) to 9 (extensive damage).
[3]$F = 75.28$, df = 9, 36, $P \leq 0.01$.
[4]$F = 46.41$, df = 9, 36, $P \leq 0.01$.

TABLE 2. FALL ARMYWORM LARVAL SURVIVAL AND WEIGHT ON RESISTANT AND SUSCEPTIBLE CORN PLANTS AFTER 10 D IN A FIELD TEST OR LABORATORY BIOASSAY ($\bar{x}$ ± SD).

	Field[1]			Laboratory[2]
Hybrid	Larvae/plant	Larval wt. (mg)	Biomass/plant (mg)	
MBR line × F-line A-Mon810	0.7 ± 0.5[3]	2 ± 1[4]	2 ± 1[5]	93 ± 33[6]
MBR line × F-line B-Mon810	0.8 ± 0.8	2 ± 1	2 ± 1	93 ± 24
MBR line × F-line A	8.8 ± 1.5	17 ± 3	146 ± 16	406 ± 21
MBR line × F-line B	5.2 ± 3.7	12 ± 4	57 ± 33	391 ± 33
DK 591-Mon810	3.0 ± 1.6	2 ± 1	6 ± 4	179 ± 62
DK 591	13.0 ± 4.9	22 ± 5	277 ± 57	409 ± 43
MBR line × M-line	6.7 ± 3.0	11 ± 3	71 ± 26	304 ± 33
DK 626	10.3 ± 3.6	19 ± 5	189 ± 70	395 ± 29
Mp704 × Mp707	2.3 ± 1.6	7 ± 1	15 ± 10	170 ± 16
Ab24E × SC229	9.8 ± 2.7	30 ± 6	286 ± 56	400 ± 30
LSD (0.05)	2.9	4	44	44

[1]Fall armyworm larvae were recovered, counted, and weighed 10 d after plants were infested with 70 neonates each.
[2]Larvae were fed for 10 d on diets containing lyophilized leaf tissue.
[3]$F = 18.80$; df = 9, 36; $P < 0.01$.
[4]$F = 54.84$; df = 9, 36; $P < 0.01$.
[5]$F = 55.30$; df = 9, 36; $P < 0.01$.
[6]$F = 77.82$; df = 9, 36; $P < 0.01$.

from *Bt*, or Mp704 × Mp707, which had the highest level of native genetic resistance. Mp704 × Mp707 was superior to all hybrids except those with a *Bt* gene.

Fewer than one larva per plant survived to 10 d on the two MBR × S*Bt* hybrids, and the mean weight of the surviving larvae was only 2 mg (Table 2). This is consistent with the types of leaf feeding damage, primarily pinholes and small circular and elongated lesions, sustained by these hybrids. On susceptible hybrids, this type of damage is generally associated with feeding of the early instars. More than 10 larvae per plant survived on the three susceptible hybrids, DK 591, DK 626, and Ab24E × SC229. The mean weight of larvae recovered from these hybrids was approximately 23 mg.

Both larval survival and growth are components of larval biomass per plant so differences in each are reflected in this single measure of resistance. Using biomass as an indication of level of resistance, the three hybrids with a *Bt* gene did not differ from each other nor did they differ from Mp704 × Mp707, the hybrid with native genetic resistance in both parents. These hybrids were superior, however, to the three hybrids with native genetic resistance in only one inbred parent. With the exception of MBR line × F-line A and DK 626, all hybrids with either native genetic resistance or a gene from *Bt* were significantly better than the susceptible hybrids.

Larvae reared for 10 d in the laboratory bioassay were much heavier than those that fed on plants in the field for the same period of time. The smallest larvae were those that fed on diets containing leaf tissue from hybrids with both native genetic resistance and resistance from *Bt*. Larvae fed on DK 591-Mon810, with only *Bt* resistance, and Mp704 × Mp707, with native genetic resistance in both parents, were significantly heavier than those fed on the two MBR × S*Bt* hybrids, but weighed significantly less than those fed on the R × S and S × S hybrids.

Regardless of which trait is used as a measure of resistance, the insecticidal proteins from *Bt* contribute to more resistant hybrids. In combination with native genetic resistance in one parental inbred, the *Bt* gene is even more effective. Because the one hybrid resulting from a cross between two inbred lines with native genetic resistance was significantly more resistant than those with genetic resistance in only one inbred parent, it seems reasonable to speculate that if a gene from *Bt* could be inserted into such a hybrid, the combination of native genetic resistance and *Bt* would be even more effective. It is possible that the level of resistance exhibited by the best hybrids in this investigation would be sufficient to eliminate or substantially reduce yield losses associated with fall armyworm damage in farmers' fields. Another potential benefit from deploying *Bt* genes in combination with native resistance is likely to be greater stability of the resistance.

ENDNOTE

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