Field Resistance of *Bacillus thuringiensis* Berliner Transformed Maize to Fall Armyworm (Lepidoptera: Noctuidae) and Southwestern Corn Borer (Lepidoptera: Crambidae) Leaf Feeding[1]

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Abstract The fall armyworm, *Spodoptera frugiperda* (J. E. Smith), and the southwestern corn borer, *Diatraea grandiosella* (Dyar), can cause economic damage to maize, *Zea mays* L., grown in the southeastern United States. Maize hybrids are commercially available that have been transformed to express insecticidal crystalline proteins from *Bacillus thuringiensis* (*Bt*) Berliner. The field efficacy of seven *Bt* hybrids were tested for control of leaf-feeding fall armyworm and southwestern corn borer. All *Bt* hybrids performed better than their conventional near-isolines for control of both insects. In general, the *Bt* hybrids provided intermediate resistance to the fall armyworm and near immunity to the southwestern corn borer. Based on larval establishment and weights, the fall armyworm was more tolerant of the insecticidal proteins expressed by the *Bt* hybrids than the southwestern corn borer. There was no difference in expression of insecticidal proteins among the *Bt* hybrids. *Bt* hybrids should be advantageous for the production of maize in areas that are affected by southwestern corn borer. The moderate level of resistance in the *Bt* hybrids to fall armyworm should be further examined to determine if amplifying the expression of insecticidal proteins or integrating other control methods along with the use of current *Bt* hybrid maize is needed to protect the crop from yield reduction by this pest.

Key Words *Spodoptera frugiperda, Diatraea grandiosella, Bt, Zea mays,* corn, Cry1Ab, plant resistance

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith), can cause economic damage to maize, *Zea mays* L., grown in the southeastern United States by feeding on developing leaves in the whorl of the plant (Morrill and Greene 1973, Sparks 1986). Damage to foliage of young maize plants can cause physiological disruption and reduced grain yield (Showers et al. 1989). Fall armyworm damage can pose a threat to maize production, especially to late-season plantings in the southeastern United States (Scott et al. 1977). The southwestern corn borer, *Diatraea grandiosella* (Dyar), is native to Mexico and is an economically significant pest of maize grown in the southern United States. An increase in population densities has raised the status of the pest in the mid-South region, possibly because of the eastward migration of the pest, increasing acreages of maize grown and reduced-tillage practices. Yield loss

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occurs from larvae feeding on the developing leaves within the whorl and tunneling into the stalk on young maize and tunneling and girdling at the base of the stalk on mature maize plants (Scott and Davis 1974). Control of these insects with insecticides is usually cost prohibitive because multiple applications are necessary to achieve acceptable control. Plant resistance is an ideal control option for lepidopteran pests of maize, but only moderate improvement had been made to develop useful levels of conventional resistance for these two pests (Davis et al. 1988).

Maize has been genetically modified to express insecticidal crystalline proteins from *Bacillus thuringiensis* Berliner (*Bt*) for resistance to lepidopteran pests. Commercially available *Bt* maize hybrids express the crystalline proteins Cry1Ab (events 176, Bt11, MON810 and MON802), Cry1Ac (event DBT418), and Cry9C (event CBH351) (Koziel et al. 1993, Armstrong et al. 1995, Jansens et al. 1997). Cry1Ab and Cry1Ac proteins are from the *B. thuringiensis* subspecies *kurstaki* and Cry9C is from subspecies *tolworthi.* Maize was primarily transformed to express these Cry proteins to protect the crop from damage caused by the European corn borer, *Ostrinia nubilalis* Hübner, in the midwest United States (Armstrong et al. 1995).

In the southeastern United States, many growers plant *Bt* hybrids to protect against damage caused by the southwestern corn borer with limited published information on their effectiveness. Archer et al. (2000, 2001) discovered Cry1Ab hybrids that utilized the transformation events MON810, Bt11, and CBH351 provided excellent southwestern corn borer control for vegetative- and reproductive-stage maize while event 176 provided excellent control only in the vegetative stage. Williams et al. (1997) demonstrated a high degree of susceptibility in southwestern corn borer to MON810-event maize. *Bt*-maize confers resistance to fall armyworm damage (Lynch et al. 1999, Wiseman et al. 1999, Buntin et al. 2001, Bokonon-Ganta et al. 2003); however, the insect seems to be more tolerant of CryIA endotoxins than the southwestern corn borer (Williams et al. 1997).

For this study, the objectives were to test the field efficacy of *Bt* events MON810 and Bt11 for control of fall armyworm and southwestern corn borer leaf feeding and to quantify the total number, total weight, and mean weight of larvae harvested from test plants to better understand the effect of event MON810 expressed in five transgenic hybrids on developing larvae in maize whorls.

Materials and Methods

Transgenic maize hybrids and their closely related isolines (Table 1) were planted in Stoneville, MS, on 23 April 1999, 21 March 2000, and 15 April 2003. All of the transgenic hybrids were commercially available and non-*Bt* near isolines were obtained for each transgenic hybrid with the exception of Asgrow RX799Bt and Novartis N7639-Bt(11). The trials conducted in 1999 used Wf9 as a susceptible inbred-line check (Abel et al. 1995). Conventionally resistant checks were used in 1999 with GT-FAWCC(C5) (Widstrom et al. 1993) used for the fall armyworm study and Mp704 × Mp707 (Williams and Davis 1982, Williams and Davis 1984) used for the southwestern corn borer study. The one-row plots were 6.1 m long and 1.0 m apart. Forty-two seeds from each of the entries were planted in an individual row for each experimental unit and thinned to 30 plants. Standard maize production procedures for the area were used. Maize entries were planted in the field in a randomized complete block arrangement with four replications. In each year, two studies were planted separately, one for testing resistance to fall armyworm and the other for testing

Table 1. Commercially available maize hybrids tested at Stoneville, MS, 1999, 2000, and 2003 and their transformation events

Hybrid	Cry toxin	Transformation event	Closely related non-Bt isoline
Agrigold A6609Bt	CryIA(b)	MON810	Agrigold XA3713
Asgrow RX799Bt	CryIA(b)	MON810	
Dekalb DK626BtX	CryIA(c)	DBT418	Dekalb DK626
Dekalb DK679Bty	CryIA(b)	MON810	Dekalb DK679
Novartis N7639-Bt(11)	CryIA(b)	Bt11	
Pioneer brand 31B13	CryIA(b)	MON810	Pioneer brand 3223
Pioneer brand 33V08	CryIA(b)	MON810	Pioneer brand 3394

resistance to southwestern corn borer. All fall armyworms and southwestern corn borers used for this research were obtained from cultures maintained by the USDA, ARS, Corn Host Plant Resistance Research Unit (Mississippi State, MS) using the technique described by Davis (1989). Wild adults are collected from the field and interbred with the existing culture on an annual basis.

In 1999, the first six plants in each row of both the fall armyworm and southwestern corn borer studies were infested, using an inoculator (Mihm 1983) and autoclaved maize cob grits, at the V5-V6 (Ritchie et al. 1992) leaf stage using a split application of 15 neonatal larvae per plant and then another 15 neonatal larvae per plant one day later. Damage was rated 14 d after the initial infestation using a rating scale based on leaf feeding developed by Davis et al. (1992) where a rating of 0 to 3 is considered resistant, 4 to 5 is intermediate, and 7 to 9 is susceptible. On the same morning that the plants were rated, infested plants were harvested from the field by excising the stalks at ground level. The plants were dissected in the laboratory, and the number of larvae, total weight of larvae, and average larval weights on a per plant basis were recorded. All data were analyzed using Restricted Maximum Likelihood (REML)-ANOVA and means were separated with the LSMEANS option of PROC MIXED (Littel et al. 1996, SAS Institute 1995).

In 2000, the field evaluation of test hybrids was conducted in the same way as in 1999 with the exception that V6-V7 (Ritchie et al. 1992) leaf stage plants were infested, and the first five plants in each row were treated with either fall armyworm or southwestern corn borer. There was a 3-day and 5-day separation between the split application of southwestern corn borer and fall armyworm larvae, respectively. Infested plants were not harvested from the field after the 14 d damage ratings, and larval parameters from the test plants were not recorded. Damage ratings and statistical analysis were conducted in the same manner as in 1999.

In 2003, the field evaluation was conducted as in 1999 with the exception that V5 (Ritchie et al. 1992) stage plants were infested and there was a 1-day and 2-day separation between the split application of southwestern corn borer and fall armyworm larvae, respectively. A split application of 30 southwestern corn borers applied during the first application and another 30 southwestern corn borers applied during the second application was done in 2003. Both 7 d and 14 d damage ratings were measured from the test entries (Davis et al. 1992). At the V6 stage (Ritchie et al. 1992) of maize growth, the developing eighth leaf was harvested from two non-infested plants within each test row by grasping the leaf within the whorl and pulling it up, thus, removing it from the plant. The harvested leaves were placed in labeled paper bags and taken to the laboratory. An approximate 20-mg portion was excised from the margin of each leaf at the approximate center (between the basal end and the distal tip) of the leaf. The leaf portions were weighed, placed into a 1.5-ml microcentrifuge tube that was capped and frozen at −80°C for toxin analysis. At a later time, but no longer than 14 d from tissue harvest, the microcentrifuge tubes containing the maize tissue were removed from −80°C. The excised leaf samples were homogenized by hand within the tubes using Cry1A extraction buffer (EnviroLogix, Portland, ME) and a fitted pestle. To quantify the relative amount of δ-endotoxin present for each variety, a commercial enzyme-linked immunosorbent assay (ELISA) kit was used (EnviroLogix). Quantification of δ-endotoxin was determined photometrically (Benchmark, Bio-Rad, Hercules, CA). A standard curve was established using known quantities of Cry1A δ-endotoxin. Dilution factors, positive and negative controls, and calculations were conducted as dictated in the kit protocol and Adamczyk et al. (2001). Leaf feeding ratings and relative ppm Cry1A δ-endotoxin were analyzed using REML-ANOVA (Littel et al. 1996, SAS Institute 1995).

Results and Discussion

Fall armyworm. The ANOVA for the fall armyworm leaf feeding damage scores in 1999 showed differences among entries ($F = 22.72$; df = 7, 21; $P < 0.0001$). Transgenic maize hybrids had less fall armyworm damage than their closely-related conventional isolines (Table 2). A poor infestation of plants occurred in 1999 as evidenced by lower-than-expected damage occurring to the conventional hybrids and the susceptible check inbred line, Wf9. This may have been because of poor fall armyworm colony health or detrimental environmental factors in the field, e.g., a heavy rain after infestation. Chi-square analysis for number of larvae recovered from plants after 14 d from the Bt vs. non-Bt entries showed a difference ($\chi^2 = 24.99$, df = 11, $P = 0.0091$) with fewer larvae being found on Bt plants (n = 3) when compared to the non-Bt plants (n = 105). This was probably because of mortality of larvae feeding on the Bt plants as indicated by other studies showing an antibiosis-type mechanism of resistance for the Cry1Ab toxin to fall armyworm (Williams et al. 1997, Lynch et al. 1999).

For the conventional hybrids and check inbred lines, there were no differences for number of larvae per plant ($F = 2.80$; df = 3, 9; $P = 0.1010$); however, there were differences in total weight of larvae per plant ($F = 6.84$; df = 3, 12; $P = 0.0061$) and average larval weight ($F = 11.60$; df = 3, 9; $P = 0.0019$). Larvae recovered from GT-FAWCC(C5) had lower larval weights on a per plant basis than Pioneer brand 3223 (P3223) and Wf9 (not XA3713) and lower average larval weights than all other entries (Table 2). Among the two conventional hybrids that were tested, XA3713 had lower leaf damage scores and lower total larval weight per plant than P3223. XA3713 may have an inherent morphological or chemical resistance factor to fall armyworm leaf feeding. XA3713 did not differ from P3223 in the average weight of the larvae recovered per plant, but differed in having a lower total weight of larvae recovered per plant. This may indicate an antixenosis (nonpreference) mechanism of resistance for XA3713.

Table 2. Mean (±SEM) fall armyworm leaf feeding damage scores, total number and weight of larvae per plant and average larval weight at 14 d for transgenic and conventional maize hybrids infested with approximately 30 neonatal larvae per plant, Stoneville, MS, 1999

Entry	Bt event	Leaf feeding damage score*,**	Total wt. (mg) of larvae/plant at 14 d	Average larval wt. (mg)/entry at 14 d
A6609Bt	MON810	1.40 ± 0.33a	No larvae	No larvae
XA3713	non-Bt, A6609Bt isoline	4.75 ± 0.95b	126.75 ± 34.42ab	212.55 ± 27.29b
P31B13	MON810	1.50 ± 0.29a	Two larvae	110.28†
P3223	non-Bt, P31B13 isoline	6.25 ± 0.63c	362.25 ± 69.62c	204.64 ± 37.29b
N7639-Bt(11)	Bt11	1.50 ± 0.29a	One larvae	24.95‡
RX799Bt	MON810	1.33 ± 0.24a	No larvae	No larvae
GT-FAWCC(C5)		3.75 ± 0.49b	35.42 ± 19.10a	44.48 ± 10.31a
Wf9		7.50 ± 0.64c	272.25 ± 78.01bc	238.89 ± 22.06b

Means within a column followed by the same letter are not significantly different according to the Least Significant Difference test ($P < 0.05$). GT-FAWCC(C5) and Wf9 were used as conventional resistant and susceptible checks, respectively.

* 14 d damage scores using Davis et al. (1992) 0-9 rating scale: 0-3 = resistance; 4-6 = intermediate resistance; and 7-9 = susceptible.

** A weaker than usual infestation of fall armyworm occurred in the field, possibly because of insect colony health or detrimental environmental factors.

† Average weight of two larvae recovered from this entry.

‡ Weight from the only larvae recovered from this entry.

The ANOVA for fall armyworm leaf feeding damage scores conducted in 2000 showed differences among entries for 14 d ratings ($F = 14.29$; df = 9, 27; $P < 0.0001$) and in 2003 for 7 d and 14 d ratings ($F = 96.50$; df = 7, 24; $P < 0.0001$; $F = 116.00$; df = 7, 21; $P < 0.0001$, respectively). Fourteen-day fall armyworm damage scores in 2000 and 7 d and 14 d scores in 2003 represented intermediate resistance scores from 3.50 to 6.00 for most of the MON810 hybrids and susceptibility for DBT418 hybrid DK626BtX with average scores of 6.75 to 8.00 (Tables 3, 4). The higher damage scores given to the conventional near-isolines in 2000 and 2003 indicated that the fall armyworm establishment on the test plants was better than in 1999.

There were maize entry differences for number of larvae per plant ($F = 3.07$; df = 7, 24; $P = 0.0187$), total larval weight per plant ($F = 18.13$; df = 7, 21; $P < 0.0001$), and average larval weight ($F = 15.20$; df = 7, 21; $P < 0.0001$) for fall armyworms collected from maize whorls in 2003. There was no difference in the number of larvae established per plant between the transgenic hybrids and their conventional near-isolines, with the exception of Pioneer brand 33V08 (P33V08) having lower larval establishment when compared to its near-isoline, Pioneer brand 3394 (P3394, Table 4). Larval

Table 3. Mean (±SEM) fall armyworm leaf feeding damage scores for transgenic and conventional maize hybrids infested with 30 neonatal larvae per plant, Stoneville, MS, 2000

Entry	Bt event	2000 damage score*
A6609Bt	MON810	5.25 ± 0.48a
DK626	non-Bt, DK626BtX isoline	8.50 ± 0.29c
DK626BtX	DBT418	6.75 ± 0.63b
DK679	non-Bt, DK679Bty isoline	8.25 ± 0.25c
DK679Bty	MON810	6.00 ± 0.00ab
P31B13	MON810	5.50 ± 0.50a
P3223	non-Bt, P31B13 isoline	8.25 ± 0.25c
P33V08	MON810	5.50 ± 0.64a
P3394	non-Bt, P33V08 isoline	8.00 ± 0.41c
RX799Bt	MON810	5.00 ± 0.41a

Means followed by the same letter are not significantly different according to the Least Significant Difference test ($P < 0.05$).

* Davis et al. (1992) 0-9 rating scale: 0-3 = resistance; 4-6 = intermediate resistance; and 7-9 = susceptible.

weights were lower on all transgenic hybrids compared to larval weights on their near-isolines, indicating that the insecticidal proteins were negatively affecting the development of the larval fall armyworms. These differences varied by hybrid. For example, P33V08 had a 24-fold reduction in mean larval weights compared to the near-isoline P3394, while DK626BtX had a 2-fold reduction in mean larval weights compared to its near-isoline, DK626. Overall, the results from 2003 indicate that reduced larval development was more important than mortality in Cry1Ab control of fall armyworms at 14 d.

Southwestern corn borer. The ANOVA for the southwestern corn borer leaf feeding damage scores showed differences among entries in 1999 ($F = 115.68$; df = 6, 18; $P < 0.0001$). Transgenic maize hybrids had less southwestern corn borer damage than their conventional near-isolines (Table 5). Chi-square analysis for number of larvae recovered from plants after 14 d from the Bt vs non-Bt entries ($\chi^2 = 21.81$, df = 7, $P = 0.0027$) found a difference in larvae recovered from Bt plants (n = 0) when compared to the non-Bt plants (n = 37). The conventional hybrids were compared for larvae recovered per plant, total weight of larvae per plant, and average larval weight; however, there were no significant differences ($F = 2.19$, df = 2, 6, $P = 0.1936$; $F = 1.40$, df = 2, 7, $P = 0.3080$; $F = 0.63$, df = 2, 3.7, $P = 0.5794$, respectively).

The ANOVA for the southwestern corn borer leaf feeding damage scores showed differences among entries for 14 d ratings in 2000 ($F = 72.32$; df = 9, 30; $P < 0.0001$, Table 6) and 7 d and 14 d ratings for 2003 ($F = 68.14$; df = 7, 21; $P < 0.0001$; $F = 137.91$; df = 7, 21; $P < 0.0001$, respectively, Table 7). Transgenic maize hybrids had less southwestern corn borer damage than their conventional near-isolines in both years. There were no differences among the conventional hybrid entries for number of larvae per plant ($F = 3.15$; df = 2, 9; $P = 0.0917$). Most of the *Bt* hybrids had no

Table 4. Mean (±SEM) fall armyworm 7 d and 14 d leaf feeding damage scores, total number and weight of larvae per plant and average larval weight at 14 d for maize hybrids infested with 30 neonates per plant, and relative Cry1A (ppm) expressed in the distal tip of the developing eighth leaf for the transgenic maize hybrid entries, Stoneville, MS, 2003

Entry	Bt event	7 d leaf feeding damage score*	14 d leaf feeding score*	Number of larvae/plant at 14 d	Total wt. (mg) of larvae/plant at 14 d	Mean larval wt. (mg)	Cry1A in ppm
A6609Bt	MON810	4.00 ± 0.41a	3.50 ± 0.29a	1.42 ± 0.14bc	6.20 ± 0.62a	4.38 ± 0.19a	0.92 ± 0.13
DK626	non-Bt, DK626BtX isoline	9.00 ± 0.00c	9.00 ± 0.00c	1.36 ± 0.36bc	197.43 ± 59.95c	137.68 ± 13.51c	
DK626BtX	DBT418	7.50 ± 0.29b	8.00 ± 0.00b	1.04 ± 0.08ab	69.39 ± 4.83ab	67.26 ± 4.51b	**
P31B13	MON810	3.75 ± 0.25a	3.75 ± 0.48a	1.08 ± 0.11ab	10.77 ± 3.82a	10.58 ± 4.04a	0.70 ± 0.08
P3223	non-Bt, P31B13 isoline	9.00 ± 0.00c	8.50 ± 0.29bc	0.88 ± 0.14a	116.98 ± 11.54b	153.72 ± 45.47c	
P33V08	MON810	4.25 ± 0.25a	3.50 ± 0.58a	1.08 ± 0.11ab	6.25 ± 1.93a	5.61 ± 1.54a	0.64 ± 0.11
P3394	non-Bt, P33V08 isoline	8.75 ± 0.25c	9.00 ± 0.00c	1.83 ± 0.12c	247.72 ± 32.00c	136.37 ± 17.52c	
RX799Bt	MON810	4.25 ± 0.25a	3.75 ± 0.48a	1.04 ± 0.14ab	6.89 ± 2.00a	6.16 ± 1.16a	0.89 ± 0.20

Means followed by the same letter are not significantly different according to the Least Significant Difference test ($P < 0.05$).
* 7 d and 14 d damage scores using Davis et al. (1992) 0-9 rating scale: 0-3 = resistance; 4-6 = intermediate resistance; and 7-9 = susceptible.
** Absorbance levels below standard curve. Unable to quantify.

Table 5. Mean (±SEM) southwestern corn borer leaf feeding damage scores, total number and weight of larvae per plant and average larval weight at 14 d for transgenic and conventional maize hybrids infested with 30 neonatal larvae per plant, Stoneville, MS, 1999

Entry	Bt event	Leaf feeding damage score*
A6609Bt	MON810	1.00 ± 0.00a
RX799Bt	MON810	1.00 ± 0.00a
P31B13	MON810	1.25 ± 0.25a
N7639-Bt(11)	Bt11	1.50 ± 0.29a
Mp704 × Mp707**		4.00
P3223	non-Bt, P31B13 isoline	7.25 ± 0.48b
XA3713	non-Bt, A6609Bt, isoline	7.25 ± 0.48b
Wf9		8.00 ± 0.41b

Means followed by the same letter are not significantly different according to the Least Significant Difference test ($P < 0.05$). Wf9 is a susceptible check.

* 14 d damage scores using Davis et al. (1992) 1-9 rating scale: 1-3 = resistance; 4-6 = intermediate resistance; and 7-9 = susceptible.

** Because of low seed germination, only one replication of entry Mp704 × Mp707 could be evaluated.

Table 6. Mean (±SEM) southwestern corn borer leaf feeding damage scores for transgenic and conventional maize hybrids, Stoneville, MS, 2000

Entry	Bt event	2000 damage score*
A6609Bt	MON810	1.75 ± 0.50a
DK626	non-Bt, DK626BtX isoline	8.33 ± 0.33c
DK626BtX	DBT418	4.80 ± 0.20b
DK679	non-Bt, DK679Bty isoline	8.00 ± 0.41c
DK679Bty	MON810	1.75 ± 0.25a
P31B13	MON810	2.25 ± 0.25a
P3223	non-Bt, P31B13 isoline	8.25 ± 0.25c
P33V08	MON810	7.50 ± 0.36c
P3394	non-Bt, P33V08 isoline	1.67 ± 0.33a
RX799Bt	MON810	1.50 ± 0.50a

Means followed by the same letter are not significantly different according to the Least Significant Difference test ($P < 0.05$).

* Davis et al. (1992) 0-9 rating scale: 0-3 = resistance; 4-6 = intermediate resistance; and 7-9 = susceptible.

Table 7. Mean (±SEM) southwestern corn borer 7 d and 14 d leaf feeding damage scores, total number and weight of larvae per plant and average larval weight at 14 d for transgenic and conventional maize hybrids infested with 60 neonatal larvae per plant, and relative Cry1A (ppm) expressed in the developing eighth leaf for the transgenic maize hybrid entries, Stoneville, MS, 2003

Entry	Bt event	7 d leaf feeding damage score*	14 d leaf feeding score[a]	Total wt. (mg) of larvae/plant at 14d	Cry1A in ppm
A6609Bt	MON810	1.75 ± 0.25a	1.50 ± 0.29a		0.79 ± 0.18
DK626	non-Bt, DK626BtX isoline	8.25 ± 0.25c	9.00 ± 0.00c	39.40 ± 3.30a	
DK626BtX	DBT418	3.00 ± 0.41b	3.50 ± 0.29b	Only 1 rep with larvae	**
P31B13	MON810	1.75 ± 0.25a	2.00 ± 0.41a		0.75 ± 0.06
P3223	non-Bt, P31B13 isoline	7.50 ± 0.64c	8.50 ± 0.29c	93.27 ± 15.82b	
P33V08	MON810	2.00 ± 0.58ab	2.00 ± 0.00a		0.60 ± 0.12
P3394	non-Bt, P33V08 isoline	7.75 ± 0.25c	8.50 ± 0.29c	59.69 ± 13.51ab	
RX799Bt	MON810	2.25 ± 0.25ab	1.75 ± 0.48a		0.80 ± 0.10

Means followed by the same letter are not significantly different according to the Least Significant Difference test ($P < 0.05$).

* 7 d and 14 d damage scores using Davis et al. (1992) 0-9 rating scale: 0-3 = resistance; 4-6 = intermediate resistance; and 7-9 = susceptible.

** Absorbance levels below standard curve. Unable to quantify.

surviving larvae, except DK626BtX which had 6 surviving larvae. The conventional hybrids averaged approximately eight surviving larvae per plant after 14 d. These results demonstrate the high degree of susceptibility of southwestern corn borer larvae to Cry1Ab δ-endotoxin. Among the conventional near-isoline hybrid entries, there was a significant difference for the total larval weight per plant but no difference in average larval weight for southwestern corn borer larvae collected from maize whorls ($F = 5.00$; df = 2, 9; $P = 0.0346$; $F = 1.90$; df = 2, 9; $P = 0.2046$, respectively).

The data presented support a previous study that determined the southwestern corn borer was more susceptible to the Cry1Ab δ-endotoxin than the fall armyworm (Williams et al. 1997). Our results show that southwestern corn borer leaf feeding damage scores were generally lower than the fall armyworm scores in 2000 and 2003 (Table 3 vs Table 6 and Table 4 vs Table 7, respectively). Also, larval establishment was lower for the southwestern corn borer than for the fall armyworm (Table 4 vs Table 7). The results from 2000 and 2003 confirm previous findings that the fall armyworm and other *Spodoptera* spp. are generally more tolerant of Cry1A endotoxins than other lepidopteran pests of maize (Moar et al. 1990, Inagaki et al. 1991, Lynch et al. 1999, Strizhov et al. 1996). Nyouki et al. (1996) found that the fall armyworm is tolerant of insecticidal proteins expressed by some subspecies of *B. thuringiensis,* including *B. thuringiensis* subsp. *kurstaki,* the same subspecies that is used for the *Bt* events tested in this manuscript. There were differences between conventional hybrid lines of maize for total larval weight per plant for the fall armyworm tests of 1999 (Table 2) and 2003 (Table 4) and the southwestern corn borer test of 2003 (Table 7). This indicates that there may be inherent morphological or chemical resistance factors to fall armyworm and southwestern corn borer leaf feeding in some of the hybrid lines tested. However, there was no difference in the level of resistance when comparing the *Bt* hybrid lines, indicating that there does not seem to be an additive effect of inherent resistance on the Cry toxin resistance in the transgenic hybrid entries.

The ANOVA for the Cry1A δ-endotoxin expressed in the developing eighth leaf for both the fall armyworm ($F = 1.13$; df = 3, 13; $P = 0.3723$) and southwestern corn borer ($F = 1.95$; df = 3, 12.2; $P = 0.1741$) tests in 2003 showed no differences among MON810 event hybrids (Tables 4 and 7, respectively). Bruns and Abel (2003) and Abel and Adamczyk (2004) have shown differences in δ-endotoxin expression in maize by variable nitrogen fertilization and rates of chlorophyll content in developing leaves, respectively. Environmental and plant physiological effects on expression of Cry1A proteins should be investigated to determine if lowered protein expression is correlated with a reduction in fall armyworm control. Amplification of insecticidal protein expression or research integrating other control methods with the use of current *Bt* hybrids may be needed to protect the crop from economically important damage by the fall armyworm.

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References Cited

Abel, C. A. and J. J. Adamczyk, Jr. 2004. Relative concentration of Cry1A in maize leaves and cotton bolls with diverse chlorophyll content and corresponding larval development of fall armyworm (Lepidoptera: Noctuidae) and southwestern corn borer (Lepidoptera: Crambidae) on maize whorl leaf profiles. J. Econ. Entomol. In Press.

Abel, C. A., R. L. Wilson and J. C. Robbins. 1995. Evaluation of Peruvian maize for resistance to European corn borer (Lepidoptera: Pyralidae) leaf feeding and ovipositonal preference. J. Econ. Entomol. 88: 1044-1048.

Adamczyk, Jr. J. J., L. C. Adams and D. D. Hardee. 2001. Field efficacy and seasonal expression profiles for terminal leaves of single and double *Bacillus thuringiensis* toxin cotton genotypes. J. Econ. Entomol. 94: 1589-1593.

Archer, T. L., G. Schuster, C. Patrick, G. Cronholm, E. D. Bynum Jr. and W. P. Morrison. 2000. Whorl and stalk damage by European and southwestern corn borers to four events of *Bacillus thuringiensis* transgenic maize. Crop Prot. 19: 181-190.

Archer, T. L., G. Schuster, C. Patrick, G. Cronholm, E. D. Bynum Jr. and W. P. Morrison. 2001. Ear and shank damage by corn borers and corn earworms to four events of *Bacillus thuringiensis* transgenic maize. Crop Prot. 20: 139-144.

Armstrong, C. L., G. B. Parker, J. C. Pershing, S. M. Brown, P. R. Sanders, D. R. Duncan, T. Stone, D. A. Dean, D. L. DeBoer and J. Hart. 1995. Field evaluation of European corn borer control in progeny of 173 transgenic corn events expressing an insecticidal protein from *Bacillus thuringiensis*. Crop. Sci. 35: 550-557.

Bokonon-Ganta, A. H., J. S. Bernal, P. V. Pietrantonio and M. Setamou. 2003. Survivorship and development of fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), on conventional and transgenic maize cultivars expressing *Bacillus thuringiensis* Cry9C and Cry1A(b) endotoxins. International Journal of Pest Management. 49: 169-175.

Bruns, H. A. and C. A. Abel. 2003. Nitrogen fertility effects on *Bt* δ-endotoxin and nitrogen concentrations of maize during early growth. Agron. J. 95: 207-211.

Buntin, G. D., R. D. Lee, D. M. Wilson and R. M. McPherson. 2001. Evaluation of yieldguard transgenic resistance for control of fall armyworm and corn earworm (Lepidoptera: Noctuidae) on corn. Florida Entomol. 84: 37-42.

Davis, F. M. 1989. Rearing the southwestern corn borer and fall armyworm at Mississippi State, Pp. 27-36. *In* Toward insect resistant maize for the third world: Proceedings of the international symposium on methodologies for developing host plant resistance to maize insects. International Maize and Wheat Improvement Center (CIMMYT), El Batan, Mexico.

Davis, F. M., W. P. Williams, J. A. Mihm, B. D. Barry, J. L. Overman, B. R. Wiseman and T. J. Riley. 1988. Resistance to multiple Lepidopterous species in tropical derived corn germplasm. Miss. Agri. Forestry Exp. Stn. Tech. Bull. 157.

Davis, F. M., S. S. Ng and W. P. Williams. 1992. Visual rating scales for screening whorl-stage corn for resistance to fall armyworm. Mississippi Agric. & For. Exp. Stn. Tech. Bull. 186.

Inagaki, S., M. Miyasono, T. Ishiguro, R. Takeda and Y. Hayashi. 1991. Proteolytic processing of δ-endotoxin of *Bacillus thuringiensis* var. *kurstaki* HD-1 in insensitive insect, *Spodoptera litura:* unusual proteolysis in the presence of sodium dodecyl sulfate. J. Invertebr. Pathol. 60: 64-68.

Koziel, M. G., G. L. Beland, C. Bowman, N. B. Carozzi, R. Crenshaw, L. Crossland, J. Dawson, N. Desai, M. Hill, S. Kadwell, K. Launis, K. Lewis, D. Maddox, K. McPherson, M. R. Meghji, E. Merlin, R. Rhodes, G. W. Warren, M. Wright and S. V. Evola. 1993. Field performance of elite transgenic plants expressing an insecticidal protein derived from *Bacillus thuringiensis.* Bio/Technology 11: 194-200.

Jansens, S., A. Van Vliet, C. Dickburt, L. Buysse, C. Piens, B. Saey, A. De Wulf, A. Paez, E. Gobel and M. Peferoen. 1997. Field evaluations of transgenic corn expressing a Cry9C insecticidal protein derived from *Bacillus thuringiensis,* protected from European corn borer. Crop Sci. 37: 1616-1624.

Littell, R. C., G. A. Milliken, W. W. Stroup and R. D. Wolfinger. 1996. SAS System for Mixed Models. SAS Institute, Cary, NC.

Lynch, R. E., B. R. Wiseman, D. Plaisted and D. Warnick. 1999. Evaluation of transgenic sweet corn hybrids expressing CryIA(b) toxin for resistance to corn earworm and fall armyworm (Lepidoptera: Noctuidae). J. Econ. Entomol. 92: 246-252.

Mihm, J. A. 1983. Efficient mass rearing and infestation techniques to screen for host plant resistance to maize stem borers, *Diatraea* spp. Centro Internacional del Mejoramiento de Maiz y Trigo (CIMMYT), El Batan, Mexico.

Moar, W. J., L. Masson, R. Brousseau and J. T. Trumble. 1990. Toxicity to *Spodoptera exigua* and *Trichoplusia ni* of individual P1 protoxins and sporulated cultures of *Bacillus thuringiensis* subsp. *kurstaki* HD-1 and NRD-12. App. Environ. Microbiol. 56: 2480-2483.

Morrill, W. L. and G. L. Greene. 1973. Distribution of fall armyworm larvae. 1. Regions of field corn plants infested by larvae. Environ. Entomol. 2: 195-198.

Nyouki, F. F. R., J. R. Fuxa and A. R. Richter. 1996. Spore-toxin interactions and sublethal effects of *Bacillus thuringiensis* in *Spodoptera frugiperda* and *Pseudoplusia includens* (Lepidoptera: Noctuidae). J. Entomol. Sci. 31: 52-62.

Ritchie, W. W., J. J. Hanway and G. O. Benson. 1992. How a corn plant develops. Iowa State Univ. Science and Technology Coop. Ext. Serv. Spec. Report 48.

SAS. 1995. SAS procedure guide for personal computers, vers. 6th ed. SAS Institute, Cary, NC, USA.

Scott, G. E. and F. M. Davis. 1974. Effect of southwestern corn borer feeding on maize. Agron. J. 66: 206-212.

Scott, G. E., F. M. Davis, G. L. Beland, W. P. Williams and S. B. King. 1977. Host plant resistance is necessary for late-planted corn. Mississippi Agric. and For. Exp. Stn. Res. Rpt. 13. 4 pp.

Showers, W. B., J. F. Witkowski, C. E. Mason, D. D. Calvin, R. A. Higgins and G. P. Dively. 1989. European corn borer: development and management. North Cent. Reg. Pub. No. 327, Iowa State Univ., Ames. Pp. 1-32.

Sparks, A. N. 1986. Fall armyworm (Lepidoptera: Noctuidae): potential for area-wide management. Florida Entomol. 69: 603-614.

Strizhov, N., M. Keller, J. Mathur, Z. Koncz-Kalman, D. Bosch, E. Prudovsky, J. Schell, B. Sneh, C. Konez and A. Zilberstein. 1996. A synthetic *cryIC* gene, encoding a *Bacillus thuringiensis* delta-endotoxin, confers *Spodoptera* resistance in alfalfa and tobacco. Proc. Natl. Acad. Sci. U.S.A. 93: 15012-15017.

Williams, W. P. and F. M. Davis. 1982. Registration of Mp7O4 germplasm line of maize. Crop Sci. 22: 1269.

1984. Registration of Mp705, Mp706, and Mp707 germplasm lines of maize. Crop Sci. 24: 1217.

Williams, W. P., J. B. Sagers, J. A. Hanten, F. M. Davis and P. M. Buckley. 1997. Transgenic corn evaluated for resistance to fall armyworm and southwestern corn borer. Crop Sci. 37: 957-962.

Wiseman, B. R., R. E. Lynch, D. Paisted and D. Warnick. 1999. Evaluation of Bt transgenic sweet corn hybrids for resistance to corn earworm and fall armyworm (Lepidoptera: Noctuidae) using a meridic diet bioassay. J. Econ. Entomol. 34: 415-425.