Western Bean Cutworm, *Striacosta albicosta* (Smith) (Lepidoptera: Noctuidae), as a Potential Pest of Transgenic Cry1Ab *Bacillus thuringiensis* Corn Hybrids in South Dakota

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ABSTRACT Injuries caused by the western bean cutworm, *Striacosta albicosta* (Smith), on transgenic Cry1Ab *Bacillus thuringiensis* (*Bt*) corn hybrids were documented and quantified. The western bean cutworm is an emerging or potential pest of transgenic *Bt* corn in South Dakota. The proportion of ears infested with western bean cutworm larvae in the Cry1Ab *Bt* corn hybrids were 18–20, 38–70, and 0–34% in 2000, 2003, and 2004, respectively. The Cry1Ab *Bt* corn hybrids were almost completely free of European corn borer infestations. Untreated conventional corn hybrids were less infested with western bean cutworm larvae but more infested with European corn borer larvae. The proportion of ears infested with European corn borer larvae alone were 33, 58–80, and 8–25% in 2000, 2003, and 2004, respectively. Infestations with western bean cutworm alone were 28, 8–28, and 13–19%, respectively. Proportion of ears simultaneously infested with both western bean cutworm and European corn borer larvae were much lower than single infestations by either species alone, indicating niche overlap and competition. Simultaneous infestations by the two species on untreated conventional corn hybrids were only 8, 0–18, and 0–1% in 2000, 2003, and 2004. The corn grains harvested from injured ears were also analyzed for fumonisin and aflatoxin through quantitative enzyme-linked immunosorbent assays. More mycotoxins were found in 2003 when the levels of insect infestation in the corn ears were higher than in 2004. Results from this study underscore the need to investigate other emerging or potential arthropod pests of transgenic *Bt* corn hybrids in addition to the western bean cutworm.

KEY WORDS western bean cutworm, *Bacillus thuringiensis* corn, European corn borer, mycotoxins, South Dakota

Western bean cutworm, *Striacosta albicosta* (Smith), is a noctuid moth native to the United States and was first described in 1887 by Smith from a specimen collected from Arizona (Smith 1890, Poole 1989, Lafontaine 2004). It was first reported as a pest of edible beans (*Phaseolus vulgaris*) in 1915 in Colorado (Hoerner 1948) and corn in 1954 in Idaho (Douglass et al. 1957). The western bean cutworm is currently recognized as a pest of both corn and edible beans in Nebraska (Hagen 1962, 1963, 1976, Seymour et al. 2004). Blickenstaff and Jolly (1982) concluded that corn and edible beans are the original hosts of western bean cutworm; soybeans, teosinte, tomato, ground cherry, and black nightshade are not suitable hosts. According to Fauske (1982), the western bean cutworm is present in South Dakota but was not considered economically important. Western bean cutworm moths have been collected, using light traps, from 17 counties in South Dakota (Fauske 1982, Catangui 2002). To the best of our knowledge, the western bean cutworm has never caused economic damage to conventional corn in South Dakota before 2000.

Transgenic *Bt* corn hybrids were first commercially planted in South Dakota in 1996, and the state currently ranks first in the United States in terms of proportion of corn acres planted to *Bt* corn or *Bt* corn stacked with an herbicide resistance gene at 52% (NASS 2005). We have continuously evaluated the performances of *Bt* corn crops since 1996. In general, *Bt* corn hybrids have performed well on the field, albeit only during years when European corn borers were abundant (Catangui and Berg 2002, Catangui 2003).

During routine corn hybrid performance testing in 2000, we noticed that, although *Bt* corn hybrids were resistant to European corn borers, most of them were susceptible to ear injuries by western bean cutworm larvae (Table 2). Some of the *Bt* corn hybrids sustained up to 20% infestation rate with up to 7.45 cm^2 of the kernels destroyed per infested ear. Because of these findings, we decided to further study the potential of western bean cutworm as a pest of transgenic *Bt* corn crops in South Dakota by quantifying the injuries that they can cause on the corn ears. We

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report here results of our field studies in 2000, 2003, and 2004.

Materials and Methods

Studies were performed at the South Dakota Southeast Research Farm in Clay County in 2000, 2003, and 2004. Procedures followed in this research were similar to those of Catangui and Berg (2002) and Catangui (2003). Corn hybrids adapted to the site were obtained from various seed companies and planted using commercial equipment and cultural practices. Western bean cutworm and European corn borer moth flights were monitored using a light trap. Injuries by the western bean cutworm and European corn borer larvae to the corn plants were recorded by examining the ears and stalks at certain times during the growing season. Corn grain was harvested using a commercial combine in 2003 and 2004. Yield was not taken in 2000 because of the fact that the Cry 1 F corn hybrid was not yet approved for commercial planting in the United States. Samples of corn grain were sent to an analytical laboratory for mycotoxin analyses in 2003 and 2004.

Monitoring Moths. Nocturnal western bean cutworm and European corn borer moth activities were monitored using a light trap equipped with a 15-W black light fluorescent bulb (Philips Lighting, Somerset, NJ). The light trap was located within 1.6 km of the research plots. An insecticide-impregnated rubber strip (dichlorvos; Loveland Industries, Greeley, CO) was placed in the collection container of the trap to quickly kill all insects attracted to the light trap. The light trap operated 24 h/d from 14 May to 14 September every growing season. Western bean cutworm and European corn borer moths collected by the trap were counted daily.

Planting Summary. Corn hybrid seeds were planted using a two-row John Deere 7100 U planter (Deere & Company, Moline, IL) in 2000 and a six-row White 5700 (AGCO, Duluth, GA) planter in 2003 and 2004. Seeding rates were 66,000, 69,000, and 74,623 seeds/ha for the 2000, 2003, and 2004 growing seasons, respectively. Planting dates were 2 May 2000, 21 May 2003, and 4 May 2004. The land used in 2000 was prepared using conventional tillage in the spring from a previous crop of soybean. For the 2003–2004 seasons, the plots were on no-till land that had been on continuous corn since 2002. Fertilizer (N:P:K) was applied at the rate of 180:30:0, 170:0:0, and 289:37:0 kg/ha, respectively. Weed control in 2000 was accomplished by preplant soil incorporation of a mixture of metolachlor and flumetsulam (2.45 kg [AI]/ha; Broadstrike + Dual; Dow AgroSciences, Indianapolis, IN). In 2003, weed control was accomplished by early postemergence application of a nicosulfuron and rimsulfuron mixture (0.04 kg [AI]/ha; Steadfast; DuPont Agricultural Products, Wilmington, DE) tank-mixed with mesotrione (0.08 kg [AI]/ha; Callisto; Syngenta Crop Protection, Greensboro, NC). The pre-emergence herbicide mixture used in 2004 was composed of glyphosate (1.12 kg/ha; Roundup Original II; Monsanto Company, St. Louis, MO), mesotrione (0.17 kg [AI]/ha; Callisto; Syngenta Crop Protection), and atrazine (0.08 kg [AI]/ha; Aatrex 4L; Syngenta Crop Protection). The crop was harvested using a John Deere 3300 combine (Deere & Company) equipped with a weighing scale and a grain moisture meter.

Experimental Design, Treatments, Data Analyses. The experimental design was a randomized complete block (Gomez and Gomez 1984) with four replications per treatment. Each experimental unit was six rows wide (0.76-m spacing between rows) by 15.2 m long in 2003 and 2004. In 2000, plot size was smaller at two rows (0.76 m between rows) by 9.1 m long because of low availability of Cry 1 F *Bt* corn seeds at that time. The treatments applied on the experimental units were the different corn hybrids and seed treatments (Table 1).

Some of the corn hybrids planted in 2003 and 2004 came pretreated with systemic seed insecticides by their respective companies (Table 1). The corn hybrid seeds pretreated with 0.25 mg of clothianidin (Poncho; Gustafson, Plano, TX) per kernel were Dekalb 537 in 2003 and Dekalb DKC53–31 in 2004. Seeds pretreated with 1.25 mg clothianidin (Poncho; Gustafson) per kernel were Dekalb DKC53–32 in 2003 and Dekalb 537 in 2004. The corn hybrid seed pretreated with imidacloprid (Gaucho; Gustafson) were Dekalb DKC53–29 and Pioneer 34N42 (1.34 mg [AI]/seed) in 2003 and 2004.

Whole corn plants were examined for the presence of European corn borer and western bean cutworm larvae at certain times during the growing season. Stalk injuries caused by the European corn borer larvae were determined by splitting the stalks with a knife and measuring the larval tunnels with a ruler. Ear injuries caused by western bean cutworm and European corn borer larvae were determined by closely examining corn ears and measuring the area of kernels injured by each species. The area of kernels destroyed was quantified by measuring with a ruler the length (along the row) and width (across the row) of injured kernels per infested ear. Kernels showing any sign of feeding by the larvae were declared as destroyed kernels. If separate areas of destroyed kernels were observed in an infested ear, these areas were added together and expressed as a single measure of area of kernels destroyed per infested ear. Besides the actual presence of live larvae, unique differences in feeding pattern and frass produced allowed for identification of which larval species caused which injuries on the corn ears. Western bean cutworm larvae left larger frass pellets near the injured kernels than the European corn borer larvae. European corm borer larvae usually left silken webs on the infested ears while western bean cutworm and corn earworm larvae did not. Corn earworm larvae left powdery and less defined frass pellets than those produced by the western bean cutworm larvae. Corn earworm data are not reported here because they were not encountered in 2000 and 2004, and <2% of the ears were infested with corn earworms in 2003. The corn plants were examined for early-season larvae on 11–17, 14–18, and

Table 1. Corn hybrids evaluated against the western bean cutworm and European corn borer near Beresford, SD, from 2000 to 2004

Hybrid corn seed	Relative maturity	Treatment	Gene trademark/event/protein	Year tested	Seed company
Dekalb 537	103	Untreated	—	2003, 2004	DeKalb Genetics
Dekalb 537	103	clothianidin	—	2003, 2004	DeKalb Genetics
Dekalb DKC49–92 (*Bt*-CB)	99	*Bt* gene	YieldGard Corn Borer/MON810/Cry1Ab	2000	DeKalb Genetics
Dekalb DKC53–29 (*Bt*-RW)	103	*Bt* gene + imidacloprid	YieldGard Rootworm/MON863/Cry3Bb1	2003, 2004	DeKalb Genetics
Dekalb DKC53–32 (*Bt*-CB)	103	*Bt* gene	YieldGard Corn Borer/MON810/Cry1Ab	2004	DeKalb Genetics
Dekalb DKC53–32 (*Bt*-CB)	103	*Bt* gene + clothianidin	YieldGard Corn Borer/MON810/Cry1Ab	2003	DeKalb Genetics
Dekalb DKC53–31 (*Bt*-CB+RW)	103	*Bt* genes + clothianidin	YieldGard Corn Borer/MON810/Cry1Ab + YieldGard Rootworm/MON863/Cry3Bb1	2004	DeKalb Genetics
Garst 8773 BLT (*Bt*)	99	*Bt* gene	StarLink/CBH351/Cry9C	2000	Garst Seed
Golden Harvest 8194RR	103	Herbicide tolerance gene	Roundup Ready/MONGA21/CP4 EPSPS	2003	J.C. Robinson Seed
Golden Harvest 8350 (*Bt*-CB)	104	*Bt* gene	YieldGard Corn Borer/MON810/Cry1Ab	2003	J.C. Robinson Seed
Golden Harvest 9006 (*Bt*-CB)	112	*Bt* gene	YieldGard Corn Borer/MON810/Cry1Ab	2004	J.C. Robinson Seed
Golden Harvest 8906	110	Untreated	—	2004	J.C. Robinson Seed
Mycogen 2395	95	Untreated	—	2000	Mycogen Seeds
Mycogen 2395 (*Bt*-HX)	95	*Bt* gene	Herculex I/TC1507/Cry1F	2000	Mycogen Seeds
Pioneer 34N42 (*Bt*-HX)	111	*Bt* gene + imidacloprid	Herculex I/TC1507/Cry1F	2003, 2004	Pioneer Hi-Bred
Pioneer 34N43	110	Untreated	—	2003, 2004	Pioneer Hi-Bred
Pioneer 34N44 (*Bt*-CB)	110	*Bt* gene	YieldGard Corn Borer/MON810/Cry1Ab	2003 2004	Pioneer Hi-Bred
Pioneer 34R0 7 (*Bt*-CB)	110	*Bt* gene	YieldGard Corn Borer/MON810/Cry1Ab	2000, 2004	Pioneer Hi-Bred
Syngenta N58-D1 (*Bt*-CB)	107	*Bt* gene	YieldGard Corn Borer/*Bt*11/Cry1Ab	2003	Syngenta Seeds
Syngenta N58-F4	107	Untreated	—	2003	Syngenta Seeds
Syngenta N60B6 (*Bt*-CB)	107	*Bt* gene	YieldGard Corn Borer/*Bt*11/Cry1Ab	2004	Syngenta Seeds
Syngenta N60N2	107	Untreated	—	2004	Syngenta Seeds

9–13 August in 2000, 2003, and 2004, respectively. Evaluations for late-season larvae were accomplished on 22–29 September, 30 August to 15 September, and 11 September to 8 October in 2000, 2003, and 2004, respectively. Ten consecutive corn plants on row 2 of each of the six-row plots were dissected for stalk and ear injuries (Catangui and Berg 2002, Catangui 2003). An additional 10 consecutive plants from row 6 of each plot were examined for larvae and corn ear injuries.

Rows 3, 4, and 5 of each plot were left intact and harvested for yield and moisture content. Harvest dates were 15 October 2003 and 26 October 2004. Yields were corrected for final plant stand. Gross income was calculated as grain fresh weight × (market value − moisture dockage). Corn market value was \$8.86/quintal in 2003 and \$7.09/quintal in 2004 (South Dakota Agricultural Statistics Service 2005). Quintal (q) is a measure of weight in the metric system that is equivalent to 100 kg. Moisture dockage was \$0.20/q per percentage point above 15% grain moisture as specified by a local grain elevator.

Approximately 0.91 kg seed sample was taken from each plot during harvest, dried to 15% moisture at room temperature if needed, placed in a paper bag, and sent for mycotoxin analyses within 14 d after harvest. The Olson Biochemistry at South Dakota State University analyzed the corn seed samples for fumonisin using the Veratox Quantitative Fumonisin 5/10 Test (Neogen, Lansing MI). For aflatoxin, the Veratox Aflatoxin Test from the same company was used. Both tests were competitive direct enzyme-linked immunosorbent assays (CD-ELISA) tests in a microwell format.

Data were analyzed using either analysis of variance (ANOVA; PROC GLM; SAS Institute 1989) or the Friedman's rank test (Ipe 1987), depending on whether or not the data satisfied the assumptions of ANOVA. The Friedman's rank test is a nonparametric method for randomized complete block designs (Steel and Torrie 1980, Sokal and Rohlf 1981). A nonparametric method was used instead of the usual ANOVA when the data sets could not be normalized by common data transformation procedures such as the square root and arcsine transformations for count and percentage data, respectively (Gomez and Gomez 1984). Data normality was checked using the Shapiro-Wilk statistic ($P = 0.05$, NORMAL option, PROC UNIVARIATE; SAS Institute 1989); homogeneity of variance was tested using Levene's test ($P = 0.05$, HOVTEST option, MEANS statement, PROC GLM; SAS Institute 1989). Mean comparisons were accomplished using the Fisher protected least significant difference (LSD) test (Carmer and Swanson 1973) with ANOVA and the rank sum multiple comparison test (Ipe 1987) with the Friedman's rank test. Mean comparisons were performed only if the ANOVA or Friedman's rank test detected significant ($P < 0.05$) differences among the means.

Results

Moth flights of the western bean cutworm took place in July (Fig. 1a and b). In general, peak moth flight of the western bean cutworm occurred between the peak moth flights of the first- and second-generation bivoltine ecotype European corn borer that is

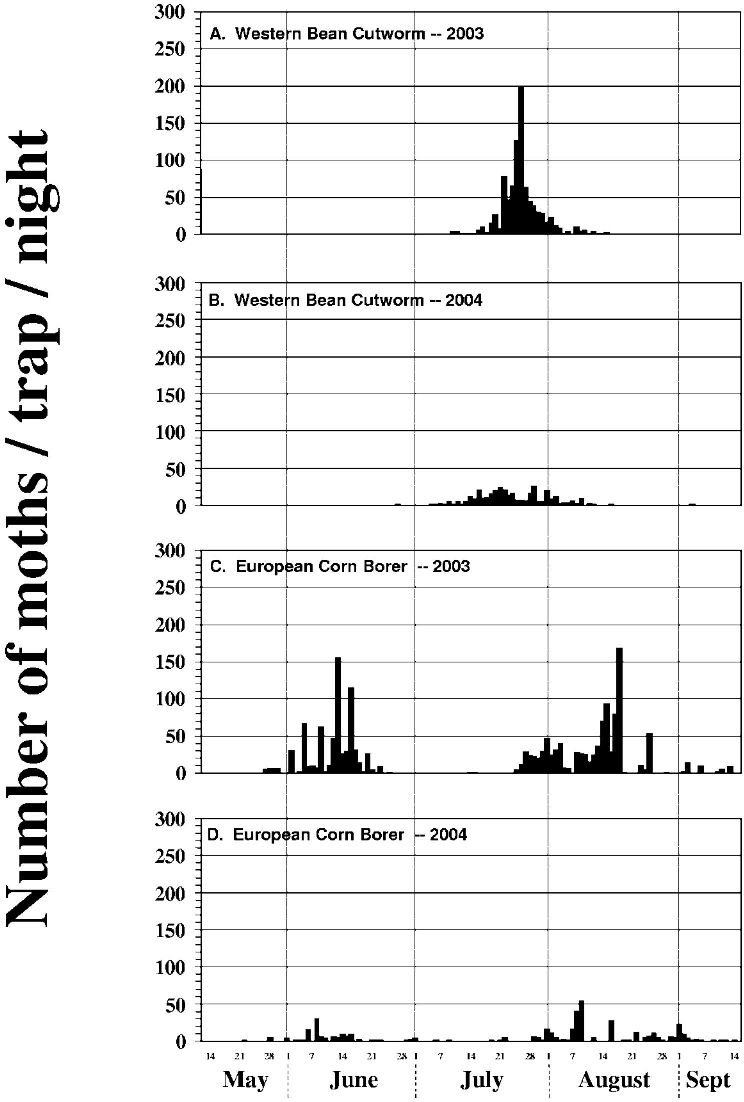

Fig. 1. (A–D) Western bean cutworm and European corn borer moth flight patterns at the South Dakota Southeast Research Farm from 2003 to 2004.

known to occur in the area (Fig. 1c and d) (Catangui and Berg 2002). Peak western bean cutworm moth flights occurred 23 and 14 d earlier than the peak second-brood European corn borer moth flights in 2003 and 2004, respectively. Moths of both species were more abundant in 2003 than in 2004. Western bean cutworm moths were not monitored in 2000. Historical and current European corn borer moth flights at the research site can be found in Berg (2005).

2000 Study. Transgenic *Bt* corn hybrids expressing the YieldGard (Dekalb C49–92 and Pioneer 34R07) and StarLink (Garst 8773 BLT) genes were all infested with western bean cutworm larvae in the ears at rates similar to that of the conventional Mycogen 2395 corn hybrid (Tables 1 and 2). However, the Mycogen 2395 *Bt* corn with the Herculex I gene was significantly less infested with western bean cutworm larvae than all the other corn hybrids ($F = 4.23$; df = 1,12; $P = 0.05$), with only 2.5% of the ears infested. The average area of kernels destroyed by western bean cutworm larvae per infested ear ranged from 1.00 to 7.45 cm^2 across all corn hybrids (Table 2). The StarLink gene was voluntarily withdrawn from the market by its manufacturer in 2000 (USEPA 2006).

Larvae of the European corn borer infested >30% of the ears in the conventional Mycogen 2395 corn hybrid (Table 2). All of the *Bt* corn hybrids had significantly lower European corn borer infestations in the ears than the conventional corn hybrid ($F = 8.81$; df = 3,12; $P = 0.0001$). Simultaneous infestations with both western bean cutworm and European corn borer larvae were found in merely 7.5% of the corn ears in the conventional corn hybrid.

Table 2. Western bean cutworm and European corn borer injuries to ears of *Bt* corn and conventional hybrids near Beresford, SD, during the 2000 season

Corn hybrid	Injury parameters (mean ± SEM)			
	Percent ears infested			Area of kernels destroyed per infested ear (cm^2)
	Western bean cutworm[a]	European corn borer[a]	Western bean cutworm and European corn borer[b]	Western bean cutworm[a]
Mycogen 2395	27.50 ± 18.87b	32.50 ± 8.54b	7.50 ± 4.79b	4.82 ± 2.87a
Mycogen 2395 (*Bt*-HXCB)	2.50 ± 2.50a	15.00 ± 11.90a	0.00 ± 0.00a	1.00 ± 1.00a
Dekalb C49–92 (*Bt*-YGCB)	17.50 ± 11.09b	0.00 ± 0.00a	0.00 ± 0.00a	4.22 ± 1.79a
Garst 8773 BLT (*Bt*-SLCB)	20.00 ± 9.13b	5.00 ± 5.00a	0.00 ± 0.00a	3.45 ± 1.72a
Pioneer 34R07 (*Bt*-YGCB)	20.00 ± 5.77b	5.00 ± 5.00a	0.00 ± 0.00a	7.45 ± 4.34a

[a] Means followed by the same letter are not significantly different ($P > 0.05$, ANOVA and Fisher's protected LSD).
[b] Means followed by the same letter are not significantly different ($P > 0.05$, Friedman's rank test and rank sum multiple comparison test).
Bt-HXCB, *Bt* corn expressing the Herculex I gene; *Bt*-YGCB, *Bt* corn expressing the YieldGard Corn Borer gene; *Bt*-SLCB, *Bt* corn expressing the StarLink gene.

No simultaneous western bean cutworm and European corn borer infestations were observed in all of the ears of the *Bt* corn hybrids, perhaps because of the fact that toxins produced through expression of all *Bt* events are known to be highly effective against European corn borer larvae (Catangui and Berg 2002). The stalks of all of the *Bt* corn hybrids were well protected by the *Bt* genes from larval infestations of first- and second-generation European corn borers (Table 3). Almost all of the *Bt* corn stalks were free of European corn borer infestations compared with 50% corn borer infestation in the conventional corn stalks.

2003 Study. All transgenic *Bt* corn hybrids expressing the YieldGard Corn Borer or Cry1Ab gene were infested with western bean cutworm larvae at 38–70% of the corn ears (Table 4). In contrast, the untreated conventional isolines within a hybrid group were significantly less infested with western bean cutworm larvae than its *Bt* corn equivalent. For example, N58-D1 (with YieldGard Corn Borer gene) had significantly higher western bean cutworm infestation in the ears than the untreated N58-F4 ($F = 6.57$; df = 1,30; $P = 0.0136$). This pattern was also observed in the Dekalb ($F = 10.12$; df = 1,30; $P = 0.0026$) and Golden Harvest ($F = 9.24$; df = 1,30; $P = 0.0038$) hybrid groups (Table 4). Pioneer 34N44 was not significantly more infested with western bean cutworm larvae than Pioneer 34N43 ($F = 0.50$; df = 1,30; $P = 0.4822$). No western bean cutworm larvae were observed in the ears of Pioneer 34N42 (expressing the Herculex I gene), making it the only *Bt* corn hybrid not infested with western bean cutworm larvae in this study.

In general, the conventional corn hybrids were more infested with European corn borer larvae than with western bean cutworm larvae despite their apparent suitability as plant hosts to each larval insect species (Table 4). The untreated conventional corn hybrids were 58–80% infested by European corn borer larvae but only 8–28% infested by western bean cutworm larvae in the ears. The area of kernels destroyed by the western bean cutworm larvae per infested ear was larger than the area destroyed by European corn borer larvae (Table 4). In the ears of the conventional hybrids, for example, the area of kernels destroyed by western bean cutworm and European corn borer larvae were 6.13 and 2.10 cm^2, respectively. Thus, on the average, western bean cutworm larvae destroyed ≈66% more area of kernels than the European corn borer larvae did per infested ear.

Within the Dekalb hybrid group, both of the transgenic *Bt* corn hybrids (Dekalb C53–32 and Dekalb C53–29) were significantly more infested with western bean cutworm larvae than the untreated isoline (Dekalb 537; Table 4). Both of these *Bt* corn hybrids were also seed treated with either imidacloprid or clothianidin (Table 1). Dekalb 537 that was seed treated with clothianidin had a western bean cutworm infestation similar to those of the *Bt* corn hybrids. *Bt* corn hybrid Golden Harvest 8350 also had significantly

Table 3. European corn borer injuries to stalks of *Bt* corn and conventional hybrids near Beresford, SD, during the 2000 season

Corn hybrid	Injury parameters (mean ± SEM)[a]			
	Percent stalks infested	Average no. of tunnels per infested stalk	Cumulative length of tunnels per infested stalk (cm)	Average no. of live larvae per infested stalk
Mycogen 2395	50.00 ± 10.80b	1.55 ± 0.18b	5.24 ± 0.95b	0.72 ± 0.44b
Mycogen 2395 (*Bt*-HXCB)	2.50 ± 2.50a	0.25 ± 0.25a	0.60 ± 0.60a	0.25 ± 0.25a
Dekalb C49–92 (*Bt*-YGCB)	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
Garst 8773 BLT (*Bt*-SLCB)	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
Pioneer 34R07 (*Bt*-YGCB)	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a

Means followed by the same letter are not significantly different ($P > 0.05$, Friedman's rank test and rank sum multiple comparison test).
[a] Because of combined first- and second-generation European corn borer larvae.
Bt-HXCB, *Bt* corn expressing the Herculex I gene; *Bt*-YGCB, *Bt* corn expressing the YieldGard Corn Borer gene; *Bt*-SLCB, *Bt* corn expressing the StarLink gene.

Table 4. Western bean cutworm and European corn borer injuries to ears of *Bt* corn and conventional hybrids near Beresford, SD, during the 2003 season

Corn hybrid	Injury parameters (mean ± SEM)				
	Percent ears infested			Area of kernels destroyed per infested ear (cm^2)	
	Western bean cutworm[a]	European corn borer[b]	Western bean cutworm and European corn borer[b]	Western bean cutworm[b]	European corn borer[b]
Syngenta N58-D1 (*Bt*-YGCB)	45.00 ± 11.90b	0.00 ± 0.00a	0.00 ± 0.00a	4.32 ± 1.05a	0.00 ± 0.00a
Syngenta N58-F4	10.00 ± 7.07a	70.00 ± 8.16b	0.00 ± 0.00a	4.58 ± 2.67a	0.70 ± 0.41a
Dekalb C53–32 (*Bt*-YGCB) + clothianidin	57.50 ± 12.50b	0.00 ± 0.00a	0.00 ± 0.00a	9.28 ± 2.13a	0.00 ± 0.00a
Dekalb C53–29 (*Bt*-YGRW) + imidacloprid	47.50 ± 18.87b	55.00 ± 15.00b	12.50 ± 9.46b	6.69 ± 2.44a	3.55 ± 1.15b
Dekalb 537	7.50 ± 2.50a	77.50 ± 2.50b	0.00 ± 0.00a	8.00 ± 4.64a	3.02 ± 0.57b
Dekalb 537 + clothianidin	30.00 ± 17.80b	60.00 ± 17.80b	0.00 ± 0.00a	5.56 ± 2.26a	3.33 ± 0.49b
Golden Harvest 8350 (*Bt*-YGCB)	70.00 ± 12.25b	0.00 ± 0.00a	0.00 ± 0.00a	6.46 ± 1.43a	0.00 ± 0.00a
Golden Harvest 8194RR	27.50 ± 13.15a	57.50 ± 4.79b	15.00 ± 11.90b	6.26 ± 2.48a	3.06 ± 0.92b
Pioneer 34N42 (*Bt*-HXCB)	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
Pioneer 34N44 (*Bt*-YGCB)	37.50 ± 8.54b	0.00 ± 0.00a	0.00 ± 0.00a	5.78 ± 0.87b	0.00 ± 0.00a
Pioneer 34N43	25.00 ± 5.00b	80.00 ± 0.00b	17.50 ± 4.79b	5.69 ± 3.47b	1.62 ± 0.73b

[a] Means (within a hybrid group) followed by the same letter are not significantly different ($P > 0.05$, ANOVA and Fisher's protected LSD).
[b] Means (within a hybrid group) followed by the same letter are not significantly different ($P > 0.05$, Friedman's rank test and rank sum multiple comparison test).
Bt-YGCB, *Bt* corn expressing the YieldGard Corn Borer gene; *Bt*-YGRW, *Bt*-corn expressing the YieldGard Rootworm gene; *Bt*-HXCB, *Bt* corn expressing the Herculex I gene.

higher western bean cutworm infestation in the ears than its non-*Bt* counterpart Golden Harvest 8194RR (Table 4). As expected, the *Bt* corn borer hybrids were completely protected from European corn borer infestation. The ears of the *Bt* rootworm hybrid (Dekalb C53–29) were unprotected from both the European corn borer and western bean cutworm larvae. Pioneer 34N42 with the Herculex I gene was free of both European corn borer and western bean cutworm infestations. Pioneer 34N44 with YieldGard Corn Borer gene was free of European corn borer but infested with western bean cutworm larvae. The untreated Pioneer 34N43 was infested with both insects. Both species simultaneously occurred only in two of the four untreated non-*Bt* corn hybrids (Golden Harvest 8194RR and Pioneer 34N43) and in the *Bt* rootworm hybrid (Dekalb C53–9). Furthermore, the proportion of ears simultaneously infested with both larvae was much less than the proportion of ears infested by either species alone (Table 4). For example, ears of the Golden Harvest 8194RR corn hybrid were 27.5% infested with western bean cutworms, 57.5% infested with European corn borers, but only 15% infested by both species simultaneously.

Table 5 shows the injuries in the cornstalks caused by second-generation European corn borer larvae, grain moisture contents, yields, and gross incomes. Corn hybrids expressing the YieldGard Corn Borer (Syngenta N58-D1, Dekalb C53–32, Golden Harvest 8350, and Pioneer 34N44) and Herculex I (Pioneer 34N42) genes were mostly free of European corn borer larvae. However, this protection from European corn borer did not necessarily translate into higher yields. Significantly higher yield than untreated conventional hybrids was recorded in only one (Dekalb) of four corn hybrid groups ($F = 2.40$; df = 16,48; $P = 0.0099$; Table 5). Within the Dekalb hybrid group, significant increases in yields and gross incomes were also recorded in the corn hybrids protected from soil insects such as Dekalb C53–29 expressing the YieldGard Rootworm gene and seed treated with imidacloprid and Dekalb 537 seed treated with clothianidin (Tables 1 and 5). The experimental site was on third-year continuous corn in 2003 and may have had infestations of soil insects such as corn rootworms and wireworms. The impact of soil insects on the performances of *Bt* corn hybrids is beyond the scope of this study.

2004 Study. The moth flights and resulting larval infestations of western bean cutworm and European corn borer were lower in 2004 than the previous growing season (Fig. 1; Tables 6 and 7). Peak western bean cutworm moth number in late July was only 26 moths per night compared with 198 moths per night in 2003. Western bean cutworm larval infestation in the corn ears was also lower at ≈34% compared with 70% in 2003 (Tables 4 and 6).

Almost all of the transgenic *Bt* corn hybrids were again infested with western bean cutworm larvae in the ears (Table 6). Three of the four corn hybrids expressing the YieldGard Corn Borer gene (Golden Harvest 9006, Dekalb C53–32, and Syngenta N60B6) were infested with western bean cutworm larvae at 6.25–21.25%. Pioneer 34N44 was not infested with western bean cutworm larvae.

Within the Golden Harvest hybrid group, the western bean cutworm infestation of the corn ears in Golden Harvest 9006 (with the YieldGard Corn Borer gene) was 21.25%. This infestation rate did not significantly differ from the untreated Golden Harvest 8906 ($F = 0.23$; df = 1,33; $P = 0.6303$; Table 5).

The Dekalb hybrid group had transgenic corn hybrids with the YieldGard Corn Borer gene alone (Dekalb C53–32), YieldGard Rootworm gene alone (De-

Table 5. European corn borer injuries to stalks of *Bt* corn and conventional hybrids near Beresford, SD, during the 2003 season

Corn hybrid	Injury parameters (mean ± SEM)[a]				Grain moisture content[c] (%)	Yield[a,d] (q/ha)	Gross income[a,e] ($/ha)
	Percent stalks infested[b]	Avg. no. tunnels per infested stalk[b]	Cum. length of tunnels per infested stalk[b] (cm)	Avg. no. live larvae per infested stalk[a]			
Syngenta N58-D1 (*Bt*-YGCB)	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	21.63 ± 0.15b	92.46 ± 4.31a	747.76 ± 34.17a
Syngenta N58-F4	77.50 ± 4.79b	2.03 ± 0.16b	9.67 ± 0.79b	1.33 ± 0.28b	19.68 ± 0.47a	83.80 ± 4.32a	697.18 ± 33.12a
Dekalb C53-32 (*Bt*-YGCB) + clothianidin	7.50 ± 4.79a	0.50 ± 0.29a	0.56 ± 0.33a	0.12 ± 0.12a	17.45 ± 0.80b	82.37 ± 2.19b	706.73 ± 17.25a
Dekalb C53-29 (*Bt*-YGRW) + imidacloprid	77.50 ± 13.15b	2.07 ± 0.23b	15.01 ± 2.72b	1.28 ± 0.26b	15.24 ± 0.56a	89.15 ± 1.33b	787.04 ± 6.28b
Dekalb 537	80.00 ± 10.80b	2.03 ± 0.11b	15.78 ± 2.06b	1.27 ± 0.36b	15.90 ± 0.64a	78.25 ± 5.78a	684.21 ± 45.53a
Dekalb 537 + clothianidin	80.00 ± 4.08b	2.07 ± 0.42b	15.36 ± 4.00b	1.36 ± 0.52b	15.57 ± 0.54a	88.92 ± 5.75b	780.98 ± 46.19b
Golden Harvest 8350 (*Bt*-YGCB)	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	18.30 ± 0.81b	76.94 3.50a	652.63 28.61a
Golden Harvest 8194RR	77.50 ± 4.79b	2.32 ± 0.30b	16.11 ± 2.39a	1.56 ± 0.34b	17.16 ± 0.21a	69.19 1.31a	596.25 10.78a
Pioneer 34N42 (*Bt*-HXCB)	2.50 ± 2.50a	0.25 ± 0.25a	0.85 ± 0.85a	0.00 ± 0.00a	22.71 ± 0.37c	84.72 3.79a	674.11 33.16a
Pioneer 34N44 (*Bt*-YGCB)	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	19.86 ± 0.69a	88.39 7.77a	733.10 62.68a
Pioneer 34N43	90.00 ± 4.08b	2.15 ± 0.26b	15.10 ± 3.26a	1.39 ± 0.31b	21.06 ± 0.31b	82.14 3.91a	669.77 29.04a

[a] Means (within a hybrid group) followed by the same letter are not significantly different ($P > 0.05$, ANOVA and Fisher's protected LSD).
[b] Means (within a hybrid group) followed by the same letter are not significantly different ($P > 0.05$, Friedman's rank test and rank sum multiple comparison test).
[c] Because of combined first- and second-generation European corn borer larvae.
[d] Adjusted to 15% grain moisture content.
[e] Grain fresh wt at harvest × (market value − moisture dock). Moisture dock = \$0.20/q per percentage point over 15%. Market value = \$8.86/q.
Bt-YGCB, *Bt* corn expressing the YieldGard Corn Borer gene; *Bt*-YGRW, *Bt* corn expressing the YieldGard Rootworm gene; *Bt*-HXCB, *Bt*-corn expressing the Herculex I gene.

kalb C53–29), and YieldGard Plus gene (Dekalb C53–21). The latter gene was a stacked gene comprising both the YieldGard Corn Borer and YieldGard Rootworm genes. Both hybrids containing the YieldGard Rootworm gene also were seed treated with clothianidin (Tables 1 and 6). Ironically, Dekalb C53–21 with the stacked YieldGard Plus gene had the highest proportion of ears infested with western bean cutworm larvae. This infestation was significantly higher than the other treatments within the Dekalb hybrid group ($F = 4.92$; df = 1,33; $P = 0.0305$). Dekalb C53–29 with the YieldGard Rootworm gene had the largest area of kernels destroyed by western bean cutworm larvae in the ears at 11.33 cm^2 (Table 6; $F = 11.10$; df = 1,33; $P = 0.0015$). No significant differences in western bean cutworm infestations were detected between corn hybrids in the Syngenta and Pioneer hybrid groups ($F < 1.46$; df = 1,33; $P > 0.2314$; Table 6).

The proportion of ears infested with European corn borer larvae was lower in 2004 than in 2003 (Tables 4 and 6). The range of infestation was 8–25% in 2004 compared with 58–80% in 2003. These differences in European corn borer larval infestations seem to reflect the lower number of moths collected from the light traps in 2003 than in 2004 (Fig. 1).

As expected, all of the *Bt* corn hybrids across hybrid groups expressing the YieldGard Corn Borer, YieldGard Plus, and Herculex I genes were completely free of European corn borer infestations in the ears (Table 6). This observation was also true in 2003 (Table 5). Varying levels of corn borer infestations in the ears were observed among the untreated conventional hybrids, insecticide-treated conventional hybrids, seed-treated conventional hybrids, and the *Bt* corn hybrid expressing the YieldGard Rootworm gene. Simultaneous occurrence in the ear of both the European corn borer and western bean cutworm larvae was observed only in the Dekalb 537 hybrid (Table 6). The 1.25% simultaneous infestation in this hybrid was again much lower than western bean cutworm alone (18.75%) or European corn borer alone (25%).

European corn borer infestations in the cornstalks were also lower in 2004 than in 2003 (Tables 5 and 7). Whereas up to 90% of the stalks of the untreated conventional corn hybrids were infested with European corn borer larvae in 2003 (Table 5), the highest infestation in the untreated conventional hybrids was only 18% in 2004 (Table 7). In general, all of the *Bt* corn hybrids expressing genes intended for European corn borer larval control (YieldGard Corn Borer, YieldGard Plus, and Herculex I) were almost completely free of corn borer infestations. Within the Dekalb hybrid group, the conventional hybrid seed-treated with clothianidin was significantly more infested with European corn borer larvae than the untreated Dekalb 537 ($F = 6.56$; df = 1,33; $P = 0.0131$; Table 7).

The differences in yields at harvest were hard to attribute to specific insect injuries during the growing season. For example, within the Golden Harvest hybrid group, it was the *Bt* corn hybrid expressing the YieldGard Corn Borer gene (Golden Harvest 9006)

Table 6. Western bean cutworm and European corn borer injuries to ears of *Bt* corn and conventional hybrids near Beresford, SD, during the 2004 season

Corn hybrid	Injury parameters (mean ± SEM)				
	Percent ears infested			Area of kernels destroyed per infested ear (cm^2)	
	Western bean cutworm[a]	European corn borer[a]	Western bean cutworm and European corn borer[b]	Western bean cutworm[b]	European corn borer[b]
Golden Harvest 9006 (*Bt*-YGCB)	21.25 ± 7.74b	0.00 ± 0.00a	0.00 ± 0.00a	5.25 ± 2.33a	0.00 ± 0.00a
Golden Harvest 8906	16.25 ± 8.98b	10.00 ± 4.08a	0.00 ± 0.00a	5.15 ± 2.65a	0.31 ± 0.24a
Dekalb C53–32 (*Bt*-YGCB)	12.50 ± 4.33a	0.00 ± 0.00a	0.00 ± 0.00a	5.67 ± 1.72a	0.00 ± 0.00a
Dekalb C53–29 (*Bt*-YGRW) + clothianidin	20.00 ± 4.56a	11.25 ± 4.27b	0.00 ± 0.00a	11.33 ± 6.11b	2.11 ± 1.12b
Dekalb C53–21 (*Bt*-YGCB+YGRW) +clothianidin	33.75 ± 18.53a	0.00 ± 0.00a	0.00 ± 0.00a	2.94 ± 1.38a	0.00 ± 0.00a
Dekalb 537	18.75 ± 11.25a	25.00 ± 6.12c	1.25 ± 1.25a	4.51 ± 2.69a	2.22 ± 1.07b
Dekalb 537 + clothianidin	11.25 ± 5.15a	12.50 ± 4.33b	0.00 ± 0.00a	2.84 ± 1.24a	2.16 ± 1.01b
Syngenta N60B6 (*Bt*-YGCB)	6.25 ± 6.25a	0.00 ± 0.00a	0.00 ± 0.00a	0.91 ± 0.91a	0.00 ± 0.00a
Syngenta N60N2	1.25 ± 1.25a	7.50 ± 4.33a	0.00 ± 0.00a	0.75 ± 0.75a	0.62 ± 0.31a
Pioneer 34N42 (*Bt*-HXCB)	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
Pioneer 34N44 (*Bt*-YGCB)	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
Pioneer 34N43	12.50 ± 6.61a	7.50 ± 4.33a	0.00 ± 0.00a	3.63 ± 1.84a	0.80 ± 0.73a

[a] Means (within a hybrid group) followed by the same letter are not significantly different ($P > 0.05$, ANOVA and Fisher's protected LSD).
[b] Means (within a hybrid group) followed by the same letter are not significantly different ($P > 0.05$, Friedman's rank test and rank sum multiple comparison test).
Bt-YGCB, *Bt* corn expressing the YieldGard Corn Borer gene; *Bt*-YGRW, *Bt* corn expressing the YieldGard Rootworm gene; *Bt*-YGCB+YGRW, *Bt* corn expressing both the YieldGard Corn Borer and YieldGard Rootworm genes; *Bt*-HXCB, *Bt* corn expressing the Herculex I gene.

that had the lowest yield (Table 5) at 120.42 q/ha ($F = 6.97$; df = 1,33; $P = 0.0107$). This yield was 5% lower than the untreated conventional hybrid (Golden Harvest 8906). During the growing season, Golden Harvest 9006 was completely free of European corn borer injuries in the cornstalks and ears, but had 21% of its ears infested with western bean cutworm larvae (Tables 6 and 7). Thus, it would seem that western bean cutworm larvae caused the yield reduction in the *Bt* corn. However, the yields of the insecticide-treated conventional hybrid were not significantly better than the untreated conventional hybrid ($F = 0.44$; df = 1,33; $P = 0.5123$), despite having significantly lower western bean cutworm and European corn borer infestations. Dekalb C53–32 (with the YieldGard Corn Borer gene) did not significantly out yield Dekalb 537 ($F = 0.09$; df = 1,33; $P = 0.7711$). Both hybrids were infested with western bean cutworm larvae, although Dekalb C53–32 was free of European corn borer infestations in the ears and stalks (Tables 6 and 7). Significant increases in yields of 8–10% were observed in the corn hybrids protected against rootworms by seed treatment alone or seed treatment in combination with the YieldGard Rootworm or YieldGard Plus genes ($F > 5.17$; df = 1,33; $P < 0.0269$). This would indicate that rootworms and perhaps other soil-inhabiting pests of corn may have been present on the field. The experimental site was on third-year continuous corn in 2004. Rootworms and other soil-inhabiting insects were not sampled in this study, and no obvious signs of root injuries such as lodging and goosenecking were observed on the plots. For some currently unknown reason, the rootworm-protected hybrids also had the highest infestations of western bean cutworm larvae in the ears (Table 6).

Mycotoxin Contents of Grain at Harvest. Figures 2–5 present the fumonisin and aflatoxin contents of the corn grain at harvest from 2003 to 2004. During the 2003 season, within the Syngenta hybrid group, there were no significant differences in grain fumonisin contents between N58-D1 with the YieldGard Corn Borer gene and untreated N58-F4 (Fig. 2). Various degrees of infestations of western bean cutworm larvae in the ears and European corn borer larvae in the ears and stalks were observed in these treatments (Tables 4 and 5). The fumonisin content of N58-D1 at harvest was 1.18 μg/g (range: 1.65–4.00 μg/g; Fig. 2). No European corn borers were observed on N58-D1 both in the ears and stalks, but 45% of the ears were infested with western bean cutworm larvae (Tables 4 and 5). The fumonisin content of untreated Syngenta N58-F4 was not significantly higher than N58-D1 despite 10% infestations of the ear with western bean cutworms and 70 and 77.5% infestations by European corn borer larvae in the ears and stalks, respectively.

Within the Dekalb hybrid group, Dekalb C53–32 with the YieldGard Corn Borer gene had the lowest average grain fumonisin content of 0.92 μg/g (Fig. 2). Dekalb C53–32 had 57.5% infestation by western bean cutworm larvae in the ears, no infestation of European corn borers in the ears, and 7.5% European corn borer infestation in the stalks (Tables 4 and 5). Dekalb C53–29 (YieldGard Rootworm; imidacloprid seed treatment) had the highest fumonisin content of 3.91 μg/g. This treatment had 47.5% infestation with western bean cutworms in the ears, 55% infestation with European corn borers in the ears, and 77.5% infestation with European corn borers in the stalks. Fumonisin contents in the untreated and clothianidin-treated Dekalb 537 were similar to Dekalb C53–29

Table 7. European corn borer injuries to stalks of *Bt* corn and conventional hybrids near Beresford, SD, during the 2004 season

Corn hybrid	Injury parameters (mean ± SEM)[a]				Grain moisture content[c] (%)	Yield[a,d] (q/ha)	Gross income[a,e] ($/ha)
	Percent stalks infested[a]	Avg. no. tunnels per infested stalk[a]	Cum. length of tunnels per infested stalk[b] (cm)	Avg. no. live larvae per infested stalk[b]			
Golden Harvest 9006 (*Bt*-YGCB)	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	22.38 ± 0.17b	120.41 ± 2.82a	732.27 ± 17.11a
Golden Harvest 8906	7.50 ± 4.79a	0.75 ± 0.48a	4.81 ± 3.38a	0.62 ± 0.38a	22.26 ± 0.24b	127.35 ± 0.65b	776.97 ± 13.93b
Dekalb C53–32 (*Bt*-YGCB)	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	18.92 ± 0.40b	111.18 ± 4.77a	730.09 ± 29.24a
Dekalb C53–29 (*Bt*-YGRW) + clothianidin	15.00 ± 6.45b	1.00 ± 0.41b	5.63 ± 2.41b	0.62 ± 0.24b	16.76 ± 0.22a	121.08 ± 2.03b	830.47 ± 12.57b
Dekalb C53–21 (*Bt*-YGCB+YGRW) + clothianidin	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	18.02 ± 0.38b	119.59 ± 0.79b	800.41 ± 9.61b
Dekalb 537	7.50 ± 4.79a	0.75 ± 0.48b	3.11 ± 2.65a	0.62 ± 0.47b	17.95 ± 0.30b	109.93 ± 5.42a	737.43 ± 40.32a
Dekalb 537 + clothianidin	22.50 ± 7.50b	0.83 ± 0.29b	5.38 ± 2.25b	0.33 ± 0.24b	17.69 ± 0.19b	125.72 ± 1.80b	846.90 ± 9.31b
Syngenta N60B6 (*Bt*-YGCB)	2.50 ± 2.50a	0.25 ± 0.25a	0.12 ± 0.12a	0.00 ± 0.00a	20.89 ± 0.19a	109.46 ± 2.91a	689.23 ± 18.07a
Syngenta N60N2	17.50 ± 7.50b	1.42 ± 0.52b	7.52 ± 4.73b	1.08 ± 0.48b	20.86 ± 0.35a	107.61 ± 2.46a	678.29 ± 19.23a
Pioneer 34N42 (*Bt*-HXCB)	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	20.81 ± 0.49a	118.63 ± 2.42a	748.41 ± 19.77a
Pioneer 34N44 (*Bt*-YGCB)	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	20.34 ± 0.36a	118.29 ± 4.67a	754.68 ± 35.16a
Pioneer 34N43	10.00 ± 7.07a	0.67 ± 0.41a	2.41 ± 1.64a	0.33 ± 0.24a	20.91 ± 0.04a	115.63 ± 1.66a	727.76 ± 9.95a

[a] Means (within a hybrid group) followed by the same letter are not significantly different ($P > 0.05$, ANOVA and Fisher's protected LSD).
[b] Means (within a hybrid group) followed by the same letter are not significantly different ($P > 0.05$, Friedman's rank test and rank sum multiple comparison test).
[c] Because of combined first- and second-generation European corn borer larvae.
[d] Adjusted to 15% grain moisture content.
[e] Grain fresh wt at harvest × (market value − moisture dock). Moisture dock = $0.20/q per percentage point over 15%. Market value = $8.86/q.
Bt-YGCB, *Bt* corn expressing the YieldGard Corn Borer gene; *Bt*-YGRW, *Bt* corn expressing the YieldGard Rootworm gene; *Bt*-YGCB+YGRW, *Bt* corn expressing both the YieldGard Corn Borer and YieldGard Rootworm genes; *Bt*-HXCB, *Bt*-corn expressing the Herculex I gene.

(Fig. 2). Figure 3 presents the aflatoxin contents of the corn grains at harvest in 2003. Most of the corn hybrids had relatively low grain aflatoxin contents except for Dekalb C53–29 and Golden Harvest 8194 RR, both of which had significant insect infestations in the ears and stalks (Tables 4 and 5). However, the other corn hybrids that were also insect-infested in the ears and stalks (Pioneer 34N43 and Syngenta N58-F4) did not have high grain aflatoxin contents.

In general, the fumonisin and aflatoxin contents of the corn grain at harvest in 2004 were lower than the previous season (Figs. 4 and 5), perhaps because of lower insect infestations (Tables 4–7). The fumonisin contents of corn grain were not significantly different within the Golden Harvest, Syngenta, and Pioneer hybrid groups (Fig. 4). Within the Dekalb hybrid group, Dekalb 537 plus clothianidin seed treatment had significantly higher fumonisin content than untreated Dekalb 537 and the hybrids containing YieldGard Corn Borer, YieldGard Rootworm, and YieldGard Plus genes. The clothianidin-treated Dekalb 537 had 15% more European corn borer infestation in the stalks than the untreated Dekalb 537.

Discussion

Efficacy of *Bt* Corn Hybrids Against the European Corn Borer. *Bt* corn hybrids that express the YieldGard Corn Borer gene and produce the Cry1Ab insecticidal protein have performed well in South Dakota since their commercial introduction in 1996. Catangui and Berg (2002) and Catangui (2003) indicated that Cry1Ab *Bt* corn hybrids were almost completely free of European corn borer larval injuries in the stalks and ears compared with conventional corn hybrids during European corn borer outbreaks. This study indicates that the Cry1Ab *Bt* corn hybrids continue to be efficacious against the European corn borer (Tables 2–7). Cry1 F *Bt* corn hybrids (expressing the Herculex I gene), recently introduced in 2003, were also efficacious against the larvae of the European corn borer.

Efficacy of *Bt* Corn Hybrids Against the Western Bean Cutworm. Western bean cutworm larvae were apparently not susceptible to the Cry1Ab and Cry9C insecticidal proteins (Tables 1, 2, 4, and 6). Thus, although Cry1Ab and Cry9C B*t* corn hybrids were almost completely free of European corn borer infestations, significant areas of the kernels in the corn ears of these *Bt* corn hybrids were destroyed by western bean cutworm larvae. In 2003, the *Bt* corn hybrids were in fact more infested with western bean cutworms than conventional corn (Table 4). Other plant physiological factors may be involved in the susceptibility of corn hybrids to western bean cutworm larvae besides the *Bt* genes for in 2003, Dekalb 537 corn hybrid seed–treated with clothianidin was significantly more infested with western bean cutworms than untreated Dekalb 537 (Table 4). The western bean cutworm larvae seemed to be susceptible to the Cry1 F protein in Herculex I *Bt* corn hybrids (Tables 2, 4, and 6).

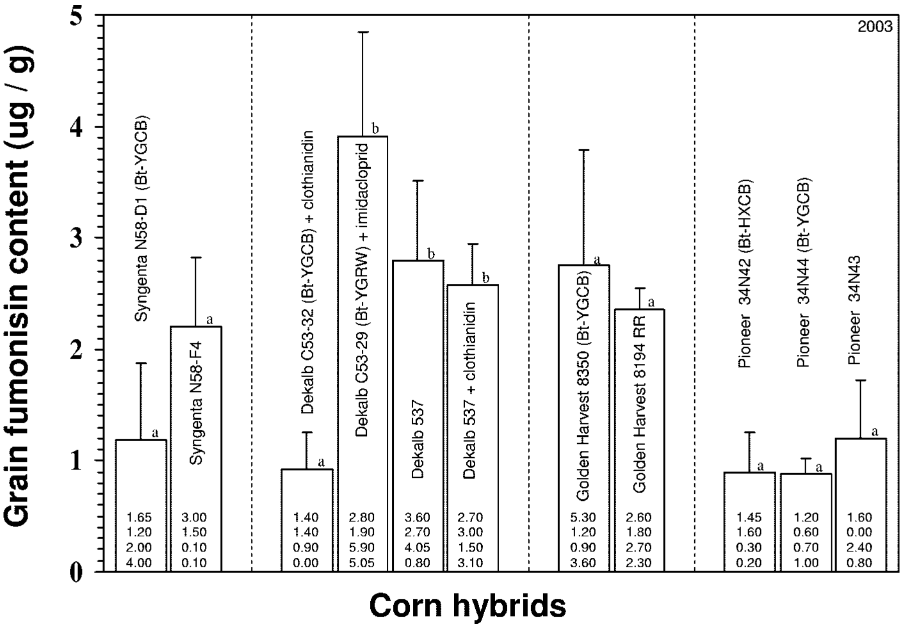

Fig. 2. Fumonisin content (mean ± SEM) of grain at harvest of *Bt* corn and non-*Bt* corn hybrids near Beresford, SD, during the 2003 season. Bars (within a hybrid group) followed by the same letter are not significantly different ($P > 0.05$, Friedman's rank test and rank sum multiple comparison test). Numbers within each bar are fumonisin levels of each replicate or experimental unit of each treatment.

The efficacy of a certain *Bt* insecticidal protein against a specific insect is related to the presence or absence of a matching receptor site in the insect midgut that can bind with the activated toxin form of the *Bt* protein (Glare and O'Callaghan 2000, Nester et al. 2002). Based on the differential efficacies of Cry1Ab,

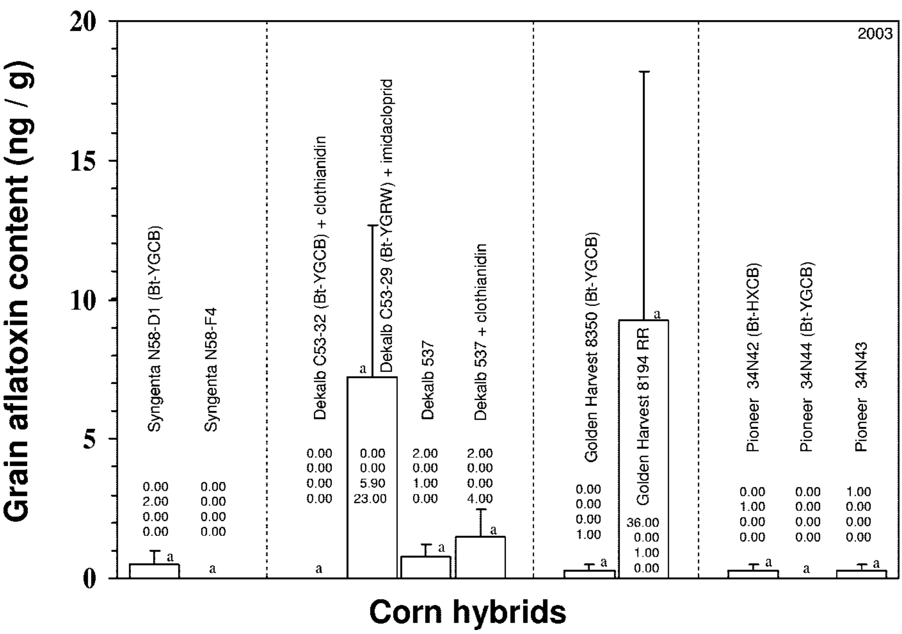

Fig. 3. Aflatoxin content (mean ± SEM) of grain at harvest of *Bt* corn and non-*Bt* corn hybrids near Beresford, SD, during the 2003 season. Bars (within a hybrid group) followed by the same letter are not significantly different ($P > 0.05$, Friedman's rank test and rank sum multiple comparison test). Numbers within each bar are fumonisin levels of each replicate or experimental unit of each treatment.

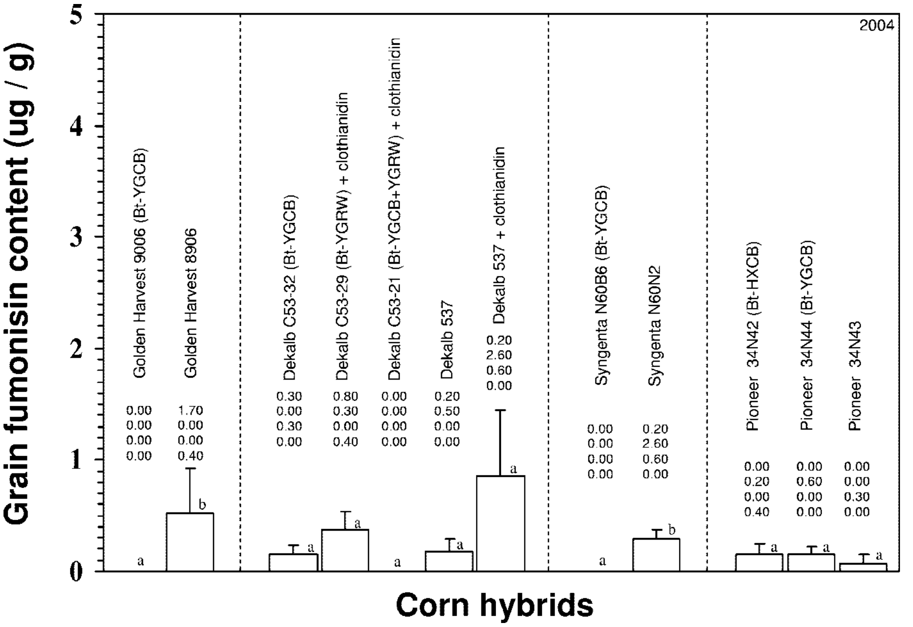

Fig. 4. Fumonisin content (mean ± SEM) of grain at harvest of *Bt* corn and non-*Bt* corn hybrids near Beresford, SD, during the 2004 season. Bars (within a hybrid group) followed by the same letter are not significantly different ($P > 0.05$, Friedman's rank test and rank sum multiple comparison test). Numbers within each bar are fumonisin levels of each replicate or experimental unit of each treatment.

Cry9C, and Cry1 F *Bt* corn hybrids against western bean cutworms, it can be surmised that the western bean cutworm larva may not have specific receptors in its midgut that could bind with Cry1Ab and Cy9C proteins, but may have the specific receptor for the Cry1 F protein. The physiology and anatomy of the western bean cutworm larva has not been studied in detail because of its status as a relatively minor pest of

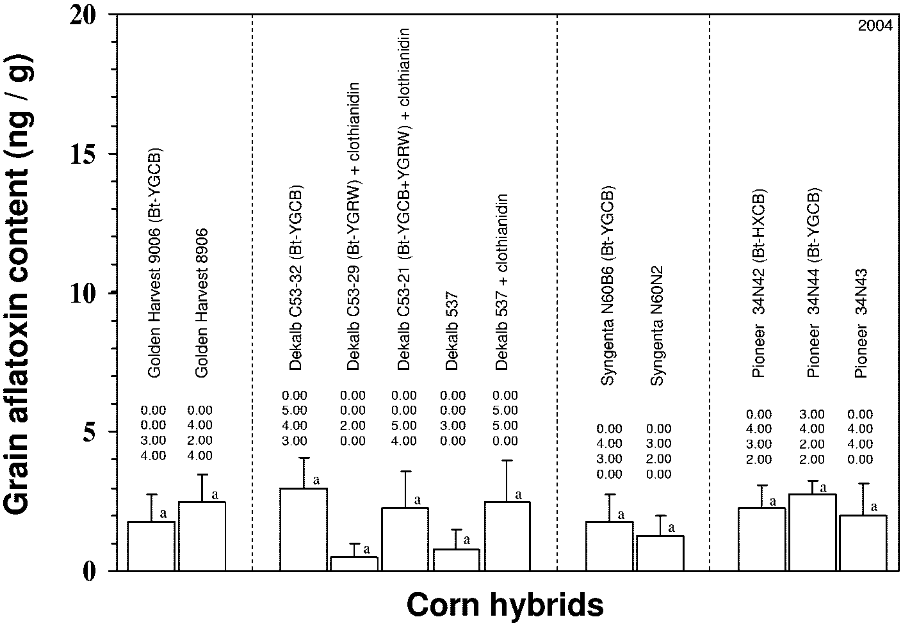

Fig. 5. Aflatoxin content (mean ± SEM) of grain at harvest of *Bt* corn and non-*Bt* corn hybrids near Beresford, SD, during the 2004 season. Bars (within a hybrid group) followed by the same letter are not significantly different ($P > 0.05$, Friedman's rank test and rank sum multiple comparison test). Numbers within each bar are fumonisin levels of each replicate or experimental unit of each treatment.

corn in the United States. Besides the western bean cutworm, there are several other lepidopteran insect pests of corn (Steffey et al. 1999), the susceptibility of which to currently deployed *Bt* corn insecticidal proteins are not known. Studies on the biotic and abiotic factors related to the life history of the western bean cutworm on corn are also wanting.

Interaction Between Western Bean Cutworm and European Corn Borer. The corn plant and the western bean cutworm are native to the United States (Nafziger and Bullock 1999, Lafontaine 2004), whereas the European corn borer is an introduced species (Pedigo 1989). We have observed the latter exploit many parts of the corn plant such as the leaves, stalks, tassel, ear shank, corn husk, ear silk, ear pith, and ear kernels, whereas the former seems to be more specialized, feeding mainly on the tassel, corn husk, ear silk, and ear kernels. In terms of ovipositional behavior of the two moth species, we observed that western bean cutworm moths laid eggs on the upper surface of the leaves close to the developing ears; second-generation European corn borer moths laid eggs on the leaf collar, lower surface of leaves, and directly on the ear husk. Peak moth flight of the western bean cutworm was about a month ahead of the European corn borer moth (Fig. 1). This may mean that western bean cutworm moths did not have to directly compete with European corn borer moths for ovipositional sites on the corn plants. Competition between the two species, if any, may therefore be mainly between the larvae as both feed on the kernels in the corn ears. Western bean cutworm and European corn borer larvae are not known to be predatory or cannibalistic (Seymour et al. 2004). Thus, it is not uncommon to see several individuals of either species coexisting in an infested corn ear for the duration of their larval stages. In Nebraska, for example, up to 20 western bean cutworm larvae had been observed feeding on a corn ear during severe infestations (Seymour et al. 2004). The detailed nature of the ecological interactions between the western bean cutworm and European corn borer is currently not known and deserve further study.

The European corn borer may be the more successful species on conventional corn. The western bean cutworm, however, is clearly the more successful species on Cry1Ab Bt-corn. On conventional corn, the European corn borer infested more ears and was more prevalent than western bean cutworms (Tables 2, 4, and 6). Our data further indicates that the proportion of ears simultaneously infested with both larvae was much less than the proportion of ears infested by either species alone. This observation may indicate competition or niche overlap (Keeton and Gould 1986). Because the western bean cutworm has never been known to cause economic injuries in corn in South Dakota before 2000, it can be surmised that the European corn borer may indeed be the more successful species between the two in cornfields planted with conventional corn.

We hypothesize that the emergence of western bean cutworm as a potential pest of corn in South Dakota may be related to the widespread planting of Cry1Ab *Bt* corn hybrids. Cry1Ab *Bt* corn hybrids favor the survival of western bean cutworms and effectively eliminate competition from European corn borers. The ecological impacts of *Bt* corn hybrids toward the numerous species of insects associated with corn production besides the target lepidopteran pest species must be studied in the future. Although considerable time and resources have been invested in resistance management to prolong the usefulness of transgenic crops against target insect pests, virtually no studies have been done on emerging or potential insect pests of transgenic crops. Transgenic crops may be viewed as an introduced or artificial disturbance of the ecosystem that may lead to a rearrangement of niches occupied by corn-associated arthropods. Continuous planting of Cry1Ab *Bt* corn hybrids over large areas, for example, may favor western bean cutworm (and other species) by effectively eliminating competition from European corn borer.

Mycotoxin Content of Grain in Relation with Insect Injuries. The association between insect injuries on the corn ears and grain mycotoxin contents is well documented (Diener et al. 1987, Dowd 2000, 2001, Clements et al. 2003, Munkvold 2003). Fumonisins and aflatoxins are naturally occurring chemicals that can harm human and animal health (Goldblatt 1969, Gelderblom et al. 1988, Wilson et al. 1990, Harrison et al. 1990, Eaton and Gropman 1994). Mycotoxins are also very stable compounds and can be concentrated in byproducts of ethanol production (Lillehoj et al. 1979, Bothast et al. 1991). Because one part of raw corn makes one-third part dry distiller's grain (Kelsall and Lyons 2003), ethanol manufacturers usually assume a three-fold magnification of mycotoxin content from raw corn to the dry distiller grain byproduct. Thus, corn used for ethanol production is routinely tested for mycotoxins to avoid harmful levels in the distiller's grain byproducts. The current levels of fumonisins on various food products considered significant are 2–4 ppm for humans and 5–100 ppm for animals (USFDA 2001). For aflatoxin, the significant levels are 0.5–20 ppb for various human food products and 20–300 ppb for animal feeds (USFDA 2000).

Results of mycotoxin analyses did not indicate a clear association between western bean cutworm larval injuries and mycotoxin contents of corn grain at harvest (Figs. 2–4; Tables 2–7). However, there were indications that the potential advantage of Cry1Ab corn *Bt* corn hybrids in protecting the ears from insect injuries and resulting grain mycotoxin contents may be nullified by the ability of western bean cutworms to infest the supposedly protected ears. Significant reduction in grain fumonisin content in the Cry1Ab *Bt* corn hybrid was observed in only one of four corn hybrid groups in 2003 (Fig. 1) and two of four hybrid groups in 2004 (Fig. 3). The aflatoxin contents of the grain at harvest were not significantly different among the conventional and *Bt* corn hybrids in both years (Figs. 2 and 4).

There were also no clear indications on which of the two insect species may be responsible for more mycotoxin production in the grain. In 2003, for example, observations in both the Syngenta and Dekalb hybrid groups indicated that injuries caused by European corn borer larvae in the ears and stalks seemed to be associated with higher grain fumonisin contents than injuries caused by western bean cutworm larvae. However, this was not the case in the Golden Harvest hybrid group. Golden Harvest 8350 had as high fumonisin content as Golden Harvest 8194 RR even though the former was completely free of European corn borer larval infestations in the stalks and ears (Fig. 2). Golden Harvest 8350 had 70% infestation with western bean cutworms and no European corn borer infestations in the ears and stalks. Golden Harvest 8194 RR had 27.5% infestation with western bean cutworms, 57.5% infestation with European corn borers in the ears, and 77.5% infestation with European corn borers in the stalks (Tables 4 and 5). Pioneer 34N42 with the Herculex I gene, which was almost completely protected from both western bean cutworm and European corn borer larvae, did not have a significantly lower fumonisin content than Pioneer 34N44, which was infested by western bean cutworm larvae, and Pioneer 34N43, which was unprotected from both insect species (Tables 4 and 5). Past studies have shown that other factors are also involved in determining the fumonisin content in corn besides insect injuries (Clements et al. 2003, Munkvold 2003). Munkvold (2003) reviewed the various infection pathways of fumonisin-producing fungi in corn and did not indicate stalk injury as a major infection route of entry. No significant differences in grain aflatoxin contents were detected in all of the hybrid groups in 2003 and 2004 (Figs. 2 and 4). A detailed discussion on aflatoxin epidemiology and the potential role of insects in aflatoxin formation on the field can be found in Diener et al. (1987) and Wiatrak et al. (2005).

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