EFFECTS OF BT TRANSFORMATION ON DISEASES OF CORN

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The availability of genetically engineered corn hybrids that are resistant to insect pests is an exciting development for corn producers and the entire corn industry. These hybrids are likely to become very popular as they continue to demonstrate dependable insect protection and yield performance. But these hybrids have other advantages as well. Because of the relationship between corn insects, particularly the European corn borer, and certain diseases, Bt hybrids have shown reduced levels of these diseases in side-by-side comparisons.

European corn borers influence the occurrence of several corn diseases, sometimes playing a major role. One way in which European corn borers affect diseases is by acting as vectors of plant pathogens. A vector is an organism (insect in this case) that transmits a pathogenic organism (fungus in this case) to its host plant. All types of stalk rot can be enhanced by European corn borer or corn rootworm damage to corn plants. Injuries by these insects provide entry wounds for the fungi, and the root and stalk damage cause stress that predisposes the plants to stalk rot development. Additionally, European corn borer larvae carry spores of *Fusarium* species from the plant surface to the interior of stalks, where stalk rot is initiated (Sobek, 1996). For these reasons, management of these insects can play a major role in corn root and stalk rot management.

Corn ear and kernel rots are affected by insect activity in a manner similar to the stalk rots. Some of the same fungi that cause the stalk rots, such as the *Fusarium* species, also infect corn kernels. Fusarium ear rot is the most common ear rot disease in the Corn Belt; it can be found to some extent in every corn field at harvest. The severity of this disease is usually low, but it can reduce yield and quality, as well as produce mycotoxins. Fungi in this group produce fumonisins, a group of toxins that can be fatal to horses and pigs, and are probable human carcinogens (Munkvold and Desjardins, 1997). Symptoms of Fusarium ear rot, caused by *F. moniliforme, F. subglutinans* and other species, are highly correlated with ear damage by European corn borer and corn earworm (*Heliothis zeae*) larvae (Christensen and Schneider, 1950; Smeltzer, 1958). Several *Fusarium* species can infect kernels without causing visible symptoms, but still affect grain quality and produce mycotoxins.

Insect activity may be the single most important factor that affects the occurrence of these diseases. European corn borer larvae carry *Fusarium* spores from the plant surface to the kernels, and provide entry wounds for the fungi. The result is an increase in disease symptoms and in symptomless infection of the kernels (Sobek, 1996). Because these fungi can produce mycotoxins, it is important to manage both symptomatic and symptomless infection. Other important ear and kernel pathogens, such as *Aspergillus* (the producer of aflatoxin), also are associated with insect damage to ears. Other groups of insects, such as thrips and Nitidulid beetles, also have been implicated as vectors and/or wounding agents for ear and kernel rots of corn.

European corn borers are probably the most important corn insects contributing to diseases. Because of the strong influence of this insect on stalk rots and ear rots, corn borer management, through its effects on diseases, can provide very tangible yield and quality benefits. We have shown that reductions in European corn borer damage, brought about by either insecticide applications or genetic engineering for resistance, can significantly reduce both stalk rots and Fusarium ear rot.

Currently available Bt hybrids produce CryIA(b), one of a group of δ-endotoxins originally produced by some strains of a bacterium, *Bacillus thuringiensis*. The expression of CryIA(b) in specific corn plant tissues is dependent on the gene promoter used in each transgenic genotype. Proprietary *cryIA(b)* transformations BT11 and MON810 (YieldGard®) utilize a CaMV 35S gene promoter that results in season-long expression of CryIA(b) in all plant tissues, whereas *cryIA(b)* transformation 176 (marketed as Maximizer® and NatureGard®) utilize a combination of two maize-derived, tissue-specific promoters : a phosphoenolpyruvate carboxylase (PEPC) promoter that results in gene expression only in green plant tissues, and a pollen-specific promoter. Kernel expression of CryIA(b) is likely to affect the extent of kernel feeding by ECB larvae and subsequently the intensity of *Fusarium* infection.

Previous Research

Although seed companies developing Bt hybrids have made disease observations for several years, there is very little previously published university research. Researchers at Cornell University have published some results showing a reduction in anthracnose stalk rot in Bt hybrids (Bergstrom et al. 1996). In this study, four Bt hybrids and their near-isogenic counterparts were compared, along with two other hybrids. Data were collected on anthracnose stalk rot, the incidence of stalk and shank tunneling, European corn borer larvae per plant, and silage yield (Table 1).

Tunneling and anthracnose stalk rot were closely correlated, and the non-Bt hybrids were much more heavily damaged by both pests. However, yields did not generally differ between the Bt hybrids and their near-isogenic lines. The highest yielding hybrid was a non-Bt hybrid.

Iowa Research, 1994-1997

Fusarium ear rot

Field experiments were conducted in 1994, 1995, and 1996 to evaluate the performance of corn hybrids genetically engineered with *Bacillus thuringiensis* genes encoding for production of CryIA(b) (Munkvold et al. 1997). In each experiment, transgenic hybrids were compared with near-isogenic corn hybrids lacking *cryIA(b)* genes (Table 2). Near-isogenic hybrids were supplied by cooperating seed companies, and were either identical with (except for the *cryIA(b)* genes) or very closely related to their corresponding transgenic hybrids. In 1994, insecticidal treatments were included to limit ECB activity to specific plant growth stages.

Table 1. Anthracnose stalk rot, European corn borer damage and silage yield for 10 hybrids grown in Poplar Ridge, NY, in 1996. Data are from Bergstrom et al. (1996).

Hybrid	Anthracnose stalk rot (% of plants)	Stalk and shank tunneling (% of plants)	ECB larvae per plant	Silage yield (t/a)
Mon810 (Bt)	0.00 a	0.00 a	0.00 a	18.75 a
B73/Mo17	11.25 bcd	43.75 bcd	0.51 bc	16.63 b
Mycogen X6821NG (Bt)	2.50 ab	2.50 a	0.09 ab	16.51 b
Mycogen X5790	12.50 bcd	35.00 bc	0.55 c	15.84 b
NK X4734 CBR (Bt)	6.25 abc	1.25 a	0.00 a	16.57 b
NK N4640	22.50 d	47.50 cd	0.68 c	16.25 b
Ciba Maximizer 101	3.75 ab	7.50 a	0.20 abc	15.80 b
Ciba 4372	12.50 bcd	26.25 b	0.44 bc	17.02 b
Cornell 281	12.50 bcd	56.25 d	0.40 abc	14.06 c
Pioneer 3525	15.00 cd	40.00 bcd	0.36 abc	19.72 a

Table 2. Maize hybrids included in field experiments examining the effects of CryIA(b) expression by maize hybrids on *Fusarium* infection of kernels.

Experiment	Hybrid	*cryIA(b)* transformation	CryIA(b) expression in kernels
1994	Monsanto[a] 802	MON810	yes
	B73/Mo17	none	no
1995	Monsanto[a] 802	MON810	yes
	Monsanto[a] 810	MON810	yes
	B73/Mo17	none	no
1996	Monsanto 810	MON810	yes
	B73/Mo17	none	no
	Ciba[e] MAX454	176	no
	Ciba 4494	none	no
	Mycogen[c] NG7059BT	176	no
	Mycogen[c] 7050cb[d]	none	no
	Northrup King[b] X6534CBR	BT11	yes
	Northrup King[b] N6800	none	no

[a] Monsanto Corp., St. Louis, MO
[b] Northrup King Co., Golden Valley, MN
[c] Mycogen Corp., San Diego, CA
[d] Hybrid with naturally-occurring resistance to European corn borer.
[e] Ciba Seeds, Greensboro, NC

In 1994, an experiment was planted on 14 May at the Iowa State University (ISU) Woodruff Farm in Story County, IA. There were two corn hybrids (Monsanto Corp., St. Louis, MO) and seven treatments involving insecticide applications and/or manual ECB infestation, with six replicate blocks. Insecticide treatments were designed either to limit ECB activity to specific corn growth stages or to mimic standard ECB control practices. Manual infestations were intended to mimic first-generation (growth stage V10-V12) and second-generation (growth stage R1) ECB infestation. Manual infestations were conducted by using a volumetric dispenser to drop approximately 50 neonatal ECB larvae into the whorl (stage V10-V12) or ear-leaf axil (stage R1) of each plant. A second experiment was planted at the ISU Woodruff Farm on 22 May 1995, with three corn hybrids (Monsanto Corp., St. Louis, MO) and four treatments involving ECB infestation and/or insecticide treatment. Both hybrids did not receive all four treatments. In 1996 an experiment was planted on 13 and 14 May at the ISU Johnson Farm in Story Co., IA; the experiment included eight hybrids from several seed companies, no insecticide treatments, and two ECB infestation treatments.

In each experiment, ten arbitrarily selected ears per plot were visually evaluated in the field for Fusarium ear rot and insect damage to kernels. Data were recorded as the number of kernels displaying symptoms on each ear. Ear rot incidence was calculated as the percentage of plants per plot with symptoms of Fusarium ear rot. Ear rot severity was calculated as the mean number of kernels with symptoms per ear.

Bt hybrids that express CryIA(b) in kernels consistently had lower Fusarium ear rot incidence (% of plants) and severity (number of kernels) than near-isogenic non-Bt hybrids (Figs. 1-3). Bt hybrids that do not express CryIA(b) in kernels sometimes had lower ear rot incidence and severity, but sometimes did not. There were significant correlations among ear rot occurrence, symptomless infection, and corn borer damage (Table 3).

Table 3. Linear correlation coefficients among incidence and severity of Fusarium ear rot symptoms, symptomless *Fusarium* infection, and incidence and severity of European corn borer damage (ECB) to maize ears in field experiments conducted near Ames, IA, in 1994, 1995, and 1996.

Year		Ear rot severity	Symptomless infection	ECB incidence	ECB severity
1994	Ear rot incidence	0.769**[a]	0.369**	0.882**	0.842**
	Ear rot severity		0.302**	0.759**	0.799**
	Symptomless infection			0.398**	0.391**
	ECB incidence				0.967**
1995	Ear rot incidence	0.822**	0.391*	0.534**	0.522**
	Ear rot severity		0.303*	0.438**	0.468**
	Symptomless infection			0.361*	0.345*
	ECB incidence				0.957**
1996	Ear rot incidence	0.754**	0.270*	0.662**	0.639**
	Ear rot severity		0.297*	0.549**	0.657**
	Symptomless infection			0.255*	0.289*
	ECB incidence				0.841**

a * = correlation significant at $P \leq 0.05$; ** = correlation significant at $P \leq 0.01$.

Stalk Rot and Gray Leaf Spot
Stalk rot and gray leaf spot were evaluated in 1997 in large plots located in a commercial field near Centerville, IA. There were ten hybrids including four pairs of Bt and near-isogenic lines. Novartis hybrids did not have near-isogenic lines included in the experiment. Natural European corn borer and disease infestations were assessed; no manual infestation or inoculation was performed. Gray leaf spot was assessed by rating the percentage of the ear leaf area that was diseased at the late dough stage on ten plants per plot. After maturity, five stalks per plot were split and the total length of corn borer tunneling and stalk rot were measured. The experiment was repeated in another commercial field near Dallas Center, IA, but gray leaf spot assessments were not made. Only the results from Centerville are shown here.

Gray leaf spot differed greatly among hybrids but was not affected by Bt transformation (Fig. 4). Stalk rot severity was significantly lower in Bt hybrids than in non-Bt hybrids, regardless of the type of Bt transformation (Fig. 5). Stalk rot severity and Bt tunneling were closely correlated.

Stalk Rot and Leaf Disease Development in Relation to European Corn Borer

Corn stalk rots develop in at least three different ways (in order of importance): 1. Infection through roots; 2. Infection through stalk injuries; and 3. Infection through leaf sheaths. Only the second pathway for stalk rot development is related to European corn borer activity. Therefore it is expected that in many fields there will be little difference in stalk rot severity between Bt hybrids and their near-isogenic counterparts. When environmental conditions are very favorable for stalk rot development, the fungi enter primarily through the roots. Under these conditions Bt corn may provide some benefit for stalk rot reduction. Because Bt hybrids have less corn borer injury, they suffer less physiological stress; therefore they may be less susceptible to stalk rot. Under more moderate stalk rot severity, stalk injury may be the more important pathway for stalk rot infection. In these fields we consistently see large reductions in stalk rot with Bt hybrids.

Leaf disease susceptibility also can be affected by plant stress and we might expect that diseases such as gray leaf spot will be reduced in Bt hybrids. So far, we have not seen any evidence of this, although seed company personnel have made observations of reduced leaf disease in Bt hybrids. It is possible that this observation is due to slightly later maturity in Bt hybrids compared to near-isogenic non-Bt hybrids. Plants damaged by corn borers will mature (die) prematurely, and as they begin to die, they become more susceptible to leaf diseases.

Conclusions

1. Bt hybrids that express Bt proteins in kernels consistently have reduced Fusarium ear rot incidence and severity.
2. Bt hybrids have reduced stalk rot incidence and severity under some conditions.
3. We did not detect any effects of Bt transformation on leaf diseases.

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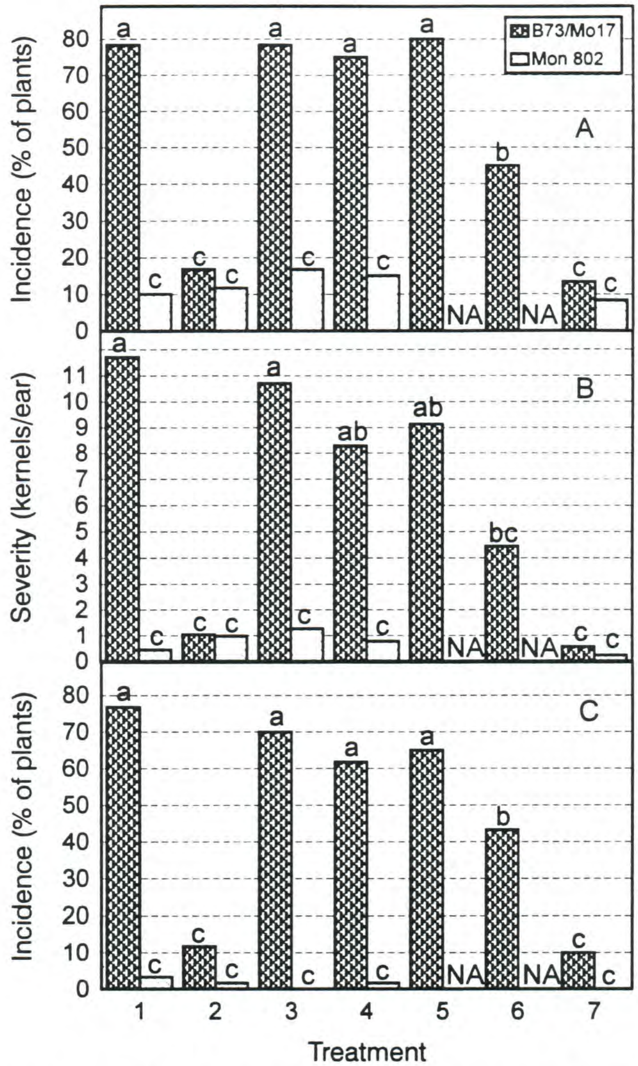

Fig. 1. A, Incidence and B, severity of Fusarium ear rot; and C, incidence of European corn borer (ECB) damage to ears in standard (B73/Mo17) and trans-genic maize hybrids (Mon 802) in the field in 1994. Treatment 1, plants were manually infested with 50 neonatal ECB larvae at stages V10 to V12 and at stage R1; treatment 2, an insecticide, Pounce 1.5G (active ingredient permethrin 1.5%), was applied weekly (0.56 kg/ha) from stage VT to R4 and plants were manually infested with ECB larvae at stages V10 to V12 only; treatment 3, Pounce 1.5G was applied weekly from stage VE to V15 and plants were manually infested at stage R1 only; treatment 4, no insecticide or manual infestation; treatment 5, plants were manually infested at stages V10 to V12 and at stage R1 and *Bacillus thuringiensis kurstaki* (Dipel 10G, 0.32% a.i.) was applied at 14.6 kg/ha 5 days after infestation; treatment 6, plants were manually infested at stages V10 to V12 and at stage R1, and Pounce 1.5G was applied 5 days after infestation; and treatment 7, Pounce 1.5G was ap-plied weekly from stage VE to R4.

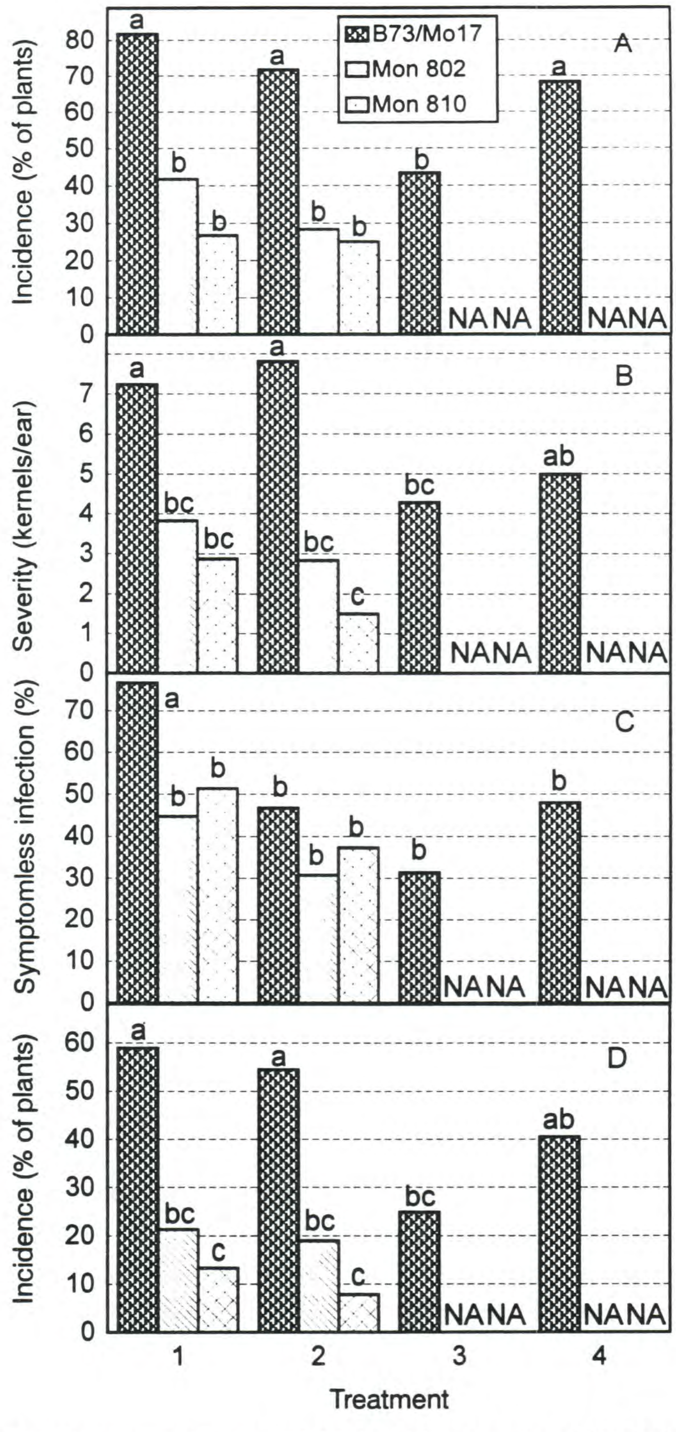

Fig. 2. Results of field experiment 1995A with standard (B73/Mo17) and transgenic (Mon 802 and Mon 810) maize hybrids. A, Incidence and B, severity of Fusarium ear rot; C, symptomless Fusarium infection of kernels; and D, incidence of European corn borer (ECB) damage to ears. Treatment 1, plants were manually infested with 50 neonatal ECB larvae at stages V10 to V12 and at stage R1; treatment 2, no manual infestation or insecticide; treatment 3, Pounce 1.5G (active ingredient permethrin 1.5%) was applied on 11 August; and treatment 4, Dipel 10G (*Bacillus thuringiensis kurstaki*, 0.32% a.i.) was applied on 11 August.

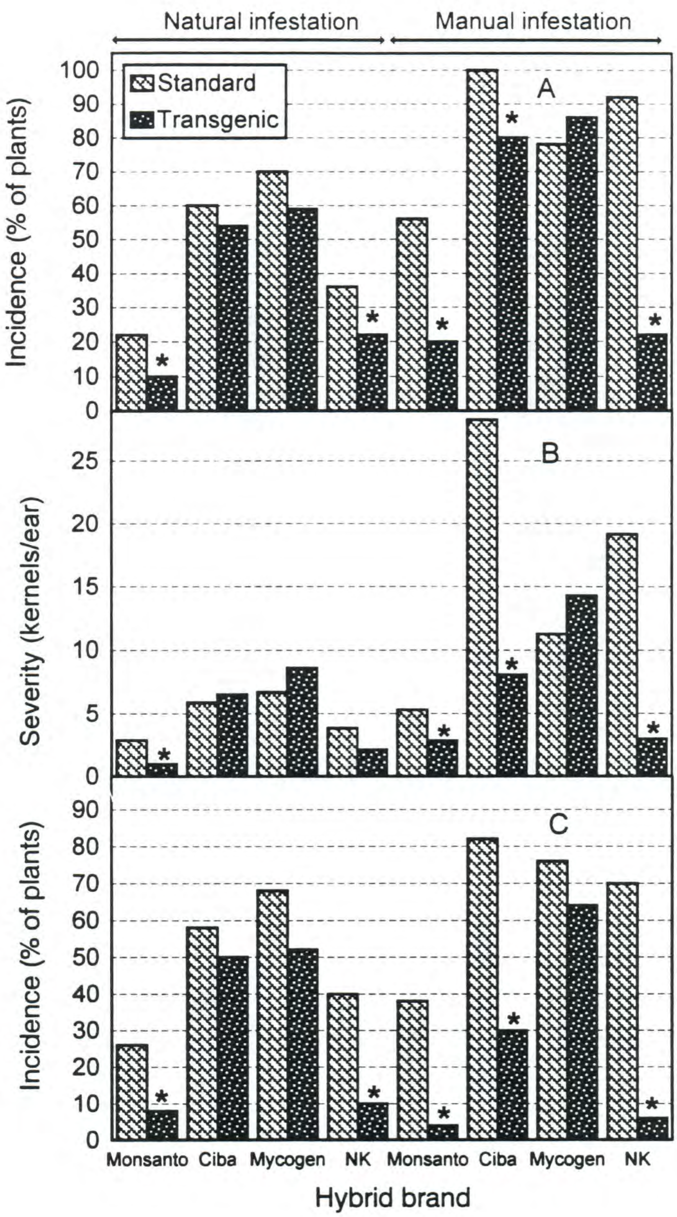

Fig. 3. Results of field experiment in 1996 with standard and transgenic maize hybrids. A, Incidence and B, severity of Fusarium ear rot; and C, incidence of European corn borer (ECB) damage to ears. Transgenic hybrids are paired with their near-isogenic counterparts. Manually infested plants were infested with 50 neonatal ECB larvae at stages V10 to V12 and at stage R1. * indicates a significant difference between standard and transgenic

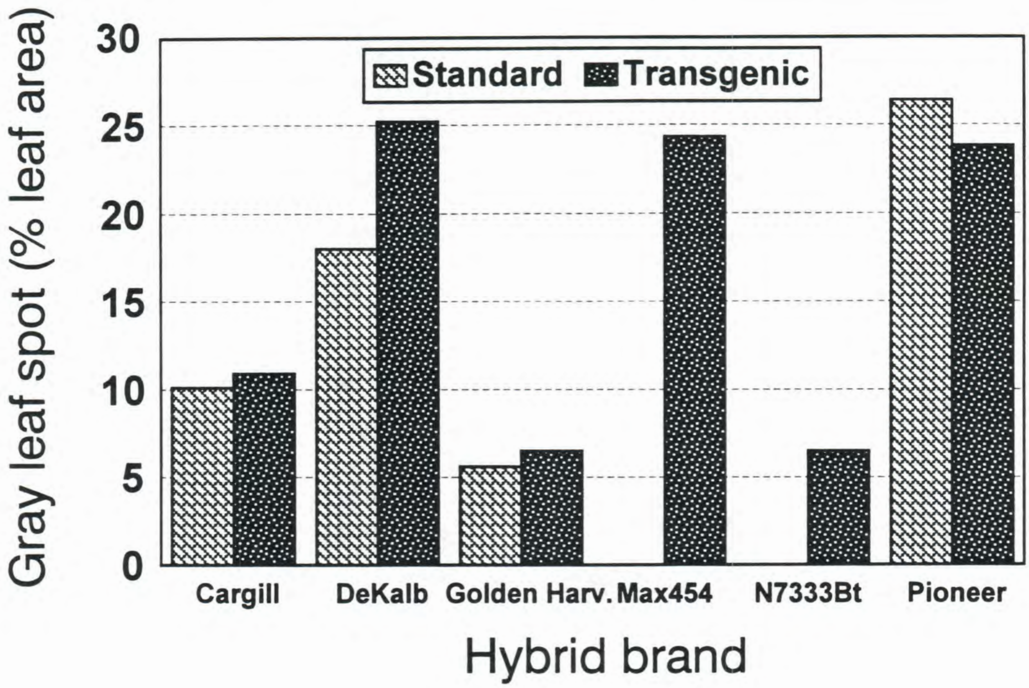

Figure 4. Gray leaf spot severity in transgenic and nontransgenic hybrids grown near Centerville, IA, in 1997. Hybrids are Cargill 7997 and 8021Bt, DeKalb DK 566 and DK566Bt, Golden Harvest H2530 and H2530Bt, Pioneer 3489 and 34R06, and Novartis Max454 and N7333Bt. Differences between paired Bt and non-Bt hybrids were not significant.

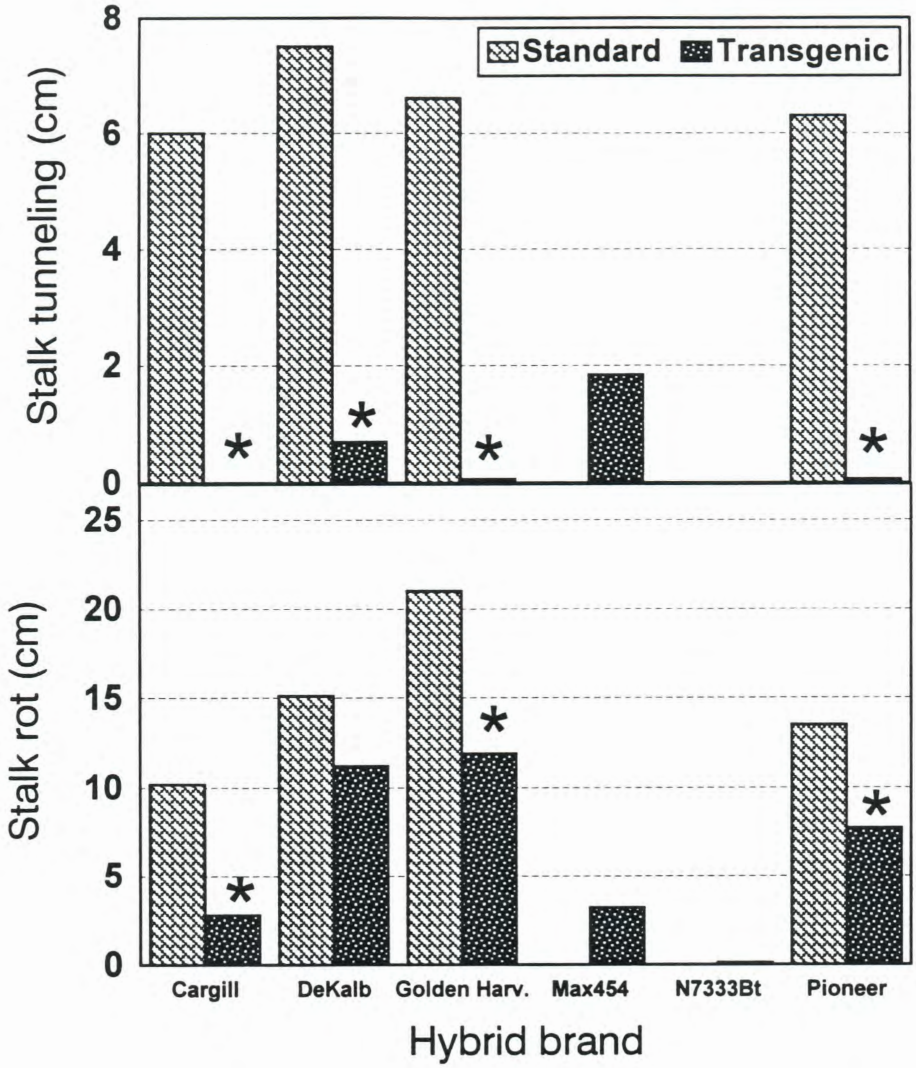

Figure 5. European corn borer tunneling and stalk rot severity in transgenic and nontransgenic hybrids grown near Centerville, IA, in 1997. Hybrids are Cargill 7997 and 8021Bt, DeKalb DK 566 and DK566Bt, Golden Harvest H2530 and H2530Bt, Pioneer 3489 and 34R06, and Novartis Max454 and N7333Bt. * indicates transgenic hybrid was significantly different from near isogenic line.