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Development, yield, grain moisture and nitrogen uptake of Bt corn hybrids and their conventional near-isolines

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Abstract

There are concerns over the economic benefits of corn (*Zea mays* L.) hybrids with the Bt trait transferred from *Bacillus thuringiensis*. A field experiment including three to seven pairs of commercial hybrids and their transgenic Bt near-isolines were grown side-by-side for three consecutive years in Ottawa, Canada (45°17′N, 75°45′W; 93 m above sea level) to determine (i) which hybrid had the highest yielding potential, (ii) if there was a differential response of Bt and non-Bt hybrids to N application, and (iii) under natural infestation of European corn borer (ECB), whether there was a yield advantage of Bt over non-Bt hybrids to justify their cost. We found that some of the Bt hybrids took 2–3 additional days to reach silking and maturity, and produced a similar or up to 12% lower grain yields with 3–5% higher grain moisture at maturity, in comparison with their non-Bt counterpart. Although N application increased grain yield and N uptake in 2 of the 3 years, there was no N-by-hybrid interaction on yield or other agronomic traits. Most Bt hybrids had similar to or lower total N content in grain with higher N in stover than their respective non-Bt near-isolines. Under extreme weather conditions (e.g. cool air temperature at planting and severe drought during the development), some of the hybrids (both Bt and non-Bt) required up to 400 additional crop heat units (CHU) to reach physiological maturity than indicated by the supplying companies. Our data suggest that within the same maturity group, it was the superior hybrids (non-Bt trait) that led to the greatest N accumulation, and the highest grain yield. Under the conditions tested, there was no yield advantage of Bt hybrids in comparison with their conventional counterparts when stalk lodging and breakage of the non-Bt counterpart by ECB was low to moderate.

Keywords: Corn or maize; Grain yield; Nitrogen response; Bt hybrids; Transgenic isolines; Agronomic benefit

1. Introduction

European corn borer [ECB, *Ostrinia nubilalis* (Hübner)] is a very destructive insect pest of corn (*Zea mays* L.) in Europe and North America and can cause severe economic losses to producers by reducing grain

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yield and quality (Lynch, 1980; Heinrich, 1988; Tenuta et al., 1999). Since its first introduction into North America in 1910 from a central European broom factory (Tenuta et al., 1999), agronomists, entomologists and plant breeders have devoted tremendous efforts to combat this pest (Lewis et al., 2001). It is estimated that an average of 1 ECB cavity per stalk across an entire field can reduce yield as much as 5% by the 1st generation and 2.5% by the 2nd generation (Bode and Calvin, 1990; Mason et al., 1996; Tenuta et al., 1999).

Managing ECB can be achieved through resistant hybrids, crop rotation, adjusting planting dates, scouting and the use of insecticides above economic thresholds. Once the borer larvae have moved inside the plant, insecticides and biological measures offer virtually no protection or control (Mason et al., 1996; Velasco et al., 2002). Growing resistant cultivars has been the most efficient and economic approach for controlling this pest (Velasco et al., 2002) because of its easiness for field operation. Corn hybrids transformed with a gene from the bacterium *Bacillus thuringiensis* (Bt) to express the insecticidal lepidopteran-active crystalline protein (Cry1Ab) endotoxin has proven to give effective control of ECB (Koziel et al., 1993).

Since their commercial introduction in 1996, the effectiveness and potential impact of Bt hybrids on soil ecology and environments have been extensively studied. In a study conducted in US Corn Belt, Pilcher and Rice (2001) reported that while there was no significant difference in egg density between Bt and isoline non-Bt hybrids, early planting resulted in the significant increase of ECB population during the first generation whereas 40–65% of the eggs were laid in the late planting for the second generation. Their results suggest that it is difficult to recommend planting date adjustments as a practical management tool for ECB and Bt hybrids. There are, however, limited publications to show the agronomic benefits of Bt hybrids under low or moderate (<2 cavities per plant) ECB natural infestation. In general, Bt hybrids with the MON810 or Bt11 event are very effective in controlling ECB. Clements et al. (2003) reported that Bt hybrids could reduce fumonisin (a toxin from *Gibberella zea*) concentration in grain during seasons when ECB is abundant. The benefits of Bt corn are to provide season-long protection against ECB and reduced use of insecticides. One field study by Graeber et al. (1999) showed that under low ECB infestation, Bt hybrids had similar yield and other agronomic performance to their near-isoline non-Bt hybrids.

Widespread adoption of the Bt corn technology may result in the development of pest resistance, effects on non-target and soil micro-organisms, and perhaps a reduction in the rate of residue decomposition (Dinel et al., 2003), thereby affecting soil carbon and nitrogen transformations. Saxena et al. (1999) showed that endotoxin to control ECB remains active in soil for some time when it binds to clays and humic acids. Dinel et al. (2003) reported that the ratio of saturated and unsaturated lipids in soils cropped to Bt hybrid was different from non-Bt corn. There are also other environmental concerns of potential risks of transgenic hybrids such as effects on biodiversity and presence of genetically modified materials on other products (Garcia et al., 1998; Scriber, 2001; Conner et al., 2003).

Many questions concerning the benefits of Bt corn and whether these benefits are worth the extra cost for seed remain to be answered. Like other cultivars, Bt hybrids should bring yield or quality advantage with equivalent yield to their conventional non-transgenic counterpart in order to keep their viability. In a 2-year study conducted in Wisconsin, Lauer and Wedberg (1999) reported that yield of Bt hybrids was 4–8% greater than standard hybrids when inoculated with ECB, but was 8% less than standard hybrids when they were treated with an insecticide. Traore et al. (2000) compared Bt and non-Bt hybrids in terms of temperature and drought stress resistance and reported that there was 9% yield advantage of Bt over non-Bt hybrids when ECB pressure was high. In the absence or under low ECB infestation, Bt hybrids performed similarly to non-Bt conventional counterparts (Filion and Meloche, 2003; Graeber et al., 1999). They also demonstrated that incorporation of the Bt gene into corn hybrids provided a high level of protection against ECB, but had little if any agronomic advantage.

In the Northern Corn Belt of USA and Canada, grain moisture concentration at harvest is an important consideration (Dwyer et al., 1994; Ma and Dwyer, 2001). It has been reported that Bt hybrids tended to have higher percent grain moisture than their non-Bt counterparts (Lauer and Wedberg, 1999). When deciding which hybrids to be grown in a specific field, producers have to consider historical density of ECB, crop rotation, and the potential benefit of growing Bt corn (Rice and Pilcher, 1998; Martin and Hyde, 2001).

Corn response to fertilizer N application rates has been widely studied with different hybrids, cropping sequence, row spacing and population densities and planting dates. There have been no comparative studies on N uptake and accumulation of Bt hybrids and their near-isolines. In a row spacing experiment, Bt hybrids appeared to take up more N than non-Bt hybrids under 0 and low N application regimes (Ma et al., 2003). Similarly, Singer et al. (2000) found that ECB damage was greater in manure (organic fertilizer containing nitrogen and other plant nutrients) applied plots and Bt corn with manure treatment yielded 19% more in an outbreak year 1997. There is a need to determine if Bt hybrids require different fertilizer N application rates.

It is hypothesized that Bt hybrids would have greater yield advantage and different response to fertilizer N than their non-Bt near-isolines. The overall objective of this study was by growing three to seven pairs of Bt and their respective non-Bt near-isoline counterpart side-by-side for three consecutive growing seasons to assess under natural infestation of ECB, if the Bt corn hybrids had yield advantage over their non-Bt near-isolines to justify their cost. Specifically, we determined (i) which hybrid had the highest yield potential with the largest N uptake, (ii) if there were differences in CHU requirement and grain moisture at harvest between Bt and non-Bt isoline hybrids, and (iii) whether there was a differential response of Bt and non-Bt hybrids to N application.

2. Materials and methods

2.1. Hybrid pairs

Three pairs in 2000, six pairs in 2001 and seven pairs in 2002, of conventional hybrids and their corresponding, transgenic Bt near-isolines, were used for this study. The same three pairs of Bt and non-Bt hybrids (total of six hybrids) were repeated across the 3 years, and six pairs were common for the recent 2 years (Table 1). Hybrid pairs tested were Pioneer Brand "Pioneer 3905"/"Pioneer 39F06 Bt", "Pioneer 3893"/"Pioneer 38W36 Bt" from Pioneer Hi-Bred, Dupont Co., DeKalb Brand "DK385B"/"DK389

Table 1
The recommended crop heat units (CHU) for different corn hybrids by the supplying companies, and actual requirement of CHU to physiological maturity in 2001 and 2002 growing seasons in Ottawa, Canada

Hybrids	Suppliers	Recommended CHU	CHU required to physiological maturity (0 ML)[a]	
			2001	2002
Pioneer 3905	Pioneer/Dupont Co.	2650	2945	2978
Pioneer 39F06 Bt	Pioneer/Dupont Co.	2650	2898	2978
Pioneer 3893	Pioneer/Dupont Co.	2700	2942	3014
Pioneer 38W38 Bt	Pioneer/Dupont Co.	2775	2995	3026
DK385B	DeKalb/Monsanto	2750	2984	3010
DKC389 BTY	DeKalb/Monsanto	2775	2994	>3057
DK355	DeKalb/Monsanto	2650	2982	3026
DKC36-71 Bt	DeKalb/Monsanto	2650	2994	3041
DK427	DeKalb/Monsanto	2850	NT[b]	>3057
DKC42-22 Bt	DeKalb/Monsanto	2850	NT	>3057
N2555	Syngenta seeds	2675	2982	3033
N2555Bt	Syngenta seeds	2675	2978	3041
N15-B4	Syngenta seeds	2600	2976	3014
N17-C5 Bt	Syngenta seeds	2600	2972	3014

[a] Some of the hybrids failed to reach physiological maturity (0 milk line) at the first fall killing frost; killing frost occurred on 9 October 2001 and 14 October 2002.

[b] Not tested in the corresponding year.

BTY", "DK355"/"DKC36-71 Bt", "DK427"/"DKC42-22 Bt" from Dekalb Seeds, Monsanto Co., and Syngenta Brand "N2555"/"N2555 Bt" and "N15-B4"/"N17-C5 Bt" from Syngenta Seed Technology. These hybrids are listed requiring 2600–2850 crop heat units (CHU; Brown and Bootsma, 1993) to reach physiological maturity and are suitable for eastern Ontario conditions. Whole plant tissues of most transgenic hybrids expressed Cry1Ab protein and originated from the MON810 Bt, while the N2555 Bt and N17-C5 Bt from Syngenta, contained the Bt11. MON810 is the truncated form of Cry1Ab, and Bt11 consists of the Cry1Ab plus PAT (phosphinothricin *N*-acetyltransferase) genes which confer glufosinate ammonium resistance. According to the seed companies, the transgenic cultivars were isoline conversions of the corresponding conventional commercial hybrids with >98% genetic similarity.

2.2. Experimental procedures

The field experiment was conducted on a Dalhousie clay loam, classified as Eutrocryept Cryept Inceptisol in the USDA system and the Gleyed Melanic Brunisol in the Canadian system in Ottawa, Ontario (45°17′N, 75°45′W; 93 m above sea level). In 2000, the experiment was planted in a site previously cropped to spring wheat (*Triticum aestivum* L.). To increase the potential ECB natural infestation, the 2001 and 2002 experiment was planted in a field (different sections each year) previously cropped to corn. Prior to fertilizer application, soil samples were taken at 0–30 cm depth and analyzed for organic matter (OM), total N, soil test phosphorus (P) and potassium (K), and soil pH. The soil contained 10.1% OM, 33% sand, 45% silt and 22% clay with a pH value of 6.1 in water. In each year, the hybrid and N rates were arranged in a split plot design with four replications. Nitrogen rate (0, 60, 120, 180 kg N ha^{-1}) was the main plot, and the hybrid pair was the subplot. The subplot was a 9.5 m long with eight rows of corn spaced 0.76 cm apart. Planting was done on 16 May in 2000, and 17 May in 2001 and 2002.

Plots were broadcast with P and K fertilizers according to soil test recommendations during the land preparation. Around the V6 to V7 stages (Ritchie and Hanway, 1993), fertilizer N as urea-ammonium nitrate (27.5% N) was injected as double bands in furrows at approximately 5 cm away from the corn row according to the planned rates (0, 60, 120, 180 kg N ha^{-1}). Several herbicides were applied for weed control: metolachlor, cyanazine and atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) in 2000; Prime Extra (Atrazine and Dual) in 2001; Primetra II Magnum in 2002. Additional mechanical weeding was used to control escaped weeds in all years.

Dates to 50% silking (R1) and physiological maturity (zero milk line or black layer; R6) were recorded based on observations taken on a minimum of three replications three times per week. Shortly after physiological maturity, or killing frost in case hybrids that had not reached maturity, percent milk line and black layer if appropriate, were recorded with progression of milk line rated from 100 to 0% at maturity (Afuakwa and Crookston, 1984; Ma and Dwyer, 2001). Harvest index (HI) for each plot was determined on five plants taken from a designated area randomly selected and marked shortly after emergence. All the sample areas were properly bordered. The plants were separated into stover and ears, which were dried at 70 °C to obtain constant weight. Cobs were put back to the stover after shelling. After dry weight was recorded, all samples went through a coarse grinding, and then a subsample was taken and ground to pass a 1 mm screen. Total N concentration of each sample was determined by the micro-kjelhahl method. Harvest index was calculated as the ratio of grain dry weight to total aboveground biomass (stover + grains). Total N accumulation in grain and stover was calculated as the product of grain or stover dry matter (based on the HI and grain yield) by the component N concentration.

In 2000 and 2001, some of the pairs used for the study were found to have an intermediate level of ECB damage in an adjacent field (Ma et al., 2003). Observations on ECB damage were therefore, recorded in 2002. Stalk lodging, number of cavities per stalk and tunnel length were measured on selected pairs of Bt and non-Bt hybrids with the 0 and 120 kg N ha^{-1} treatments. In each plot stalk lodging was assessed on 30 plants from the middle rows with scale of 0 = no lodging, and 100 = completely lodged. The same 30 plants per plot were used for the estimation of ECB infestation. The number of cavities was counted between the ear-node and at the node

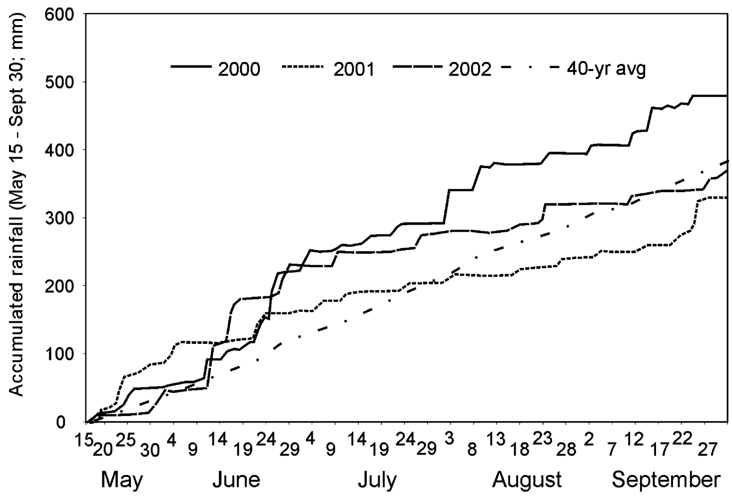

Fig. 1. Accumulated total precipitation (mm) from 15 May to 30 September in 2000, 2001, and 2002 and the 40-year average at the experimental site.

aboveground level on each plant, the stem was then split from the center and the tunnel length was measured.

At final harvest, grain yield was determined by combine harvesting the middle two rows (13.7 m^2) and corrected to a 155 $g\,kg^{-1}$ water basis. Grain moisture concentration was recorded at the time of yield determination.

All data were subjected to analyses of variance each year according to the appropriate experimental design (SAS Institute, 1996). Treatment means were separated according to the *F*-protected least squares difference test ($P \leq 0.05$).

3. Results

3.1. Weather pattern and accumulation of crop heat units

Year 2000 was wet throughout the growing season with a normally distributed rain. A prolonged period

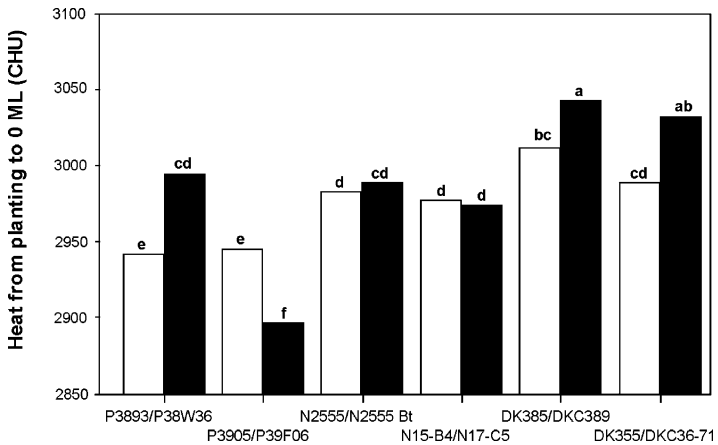

Fig. 2. Comparison of crop heat units (CHU) requirement from planting to physiological maturity of Bt (solid bar) and their respective conventional non-Bt (empty bar) near-isolines in 2001. Bars with different letters are significantly different according to the *F*-protected $LSD_{0.05}$ test.

of drought occurred in June, July and August of 2001, and the total rain during the growing season was only 56% of the average rainfall of 40 years. In 2002, periods of drought were encountered during flowering and early grain filling (Fig. 1). Some of the late maturity hybrids did not reach physiological maturity (R6) although there was sufficient CHU accumulated to meet their CHU requirements as suggested by the supplying companies (Table 1). From planting to the first fall frost, accumulated CHUs were 2613 in 2000, 3004 in 2001, and 3057 in 2002. There was insufficient CHU for the full season hybrids to reach physiological maturity in 2000, but the total heat units in 2001 and 2002 were more than enough to reach physiological maturity. In 2002, we also noted that black layer appeared after killing frost, a phenomena common in the cool region (Ma and Dwyer, 2001).

3.2. Phenology

In 2000, no Bt hybrids reached physiological maturity before the killing frost while their corresponding non-Bt hybrids matured or almost reached maturity (Fig. 3A). Dates from planting to silking

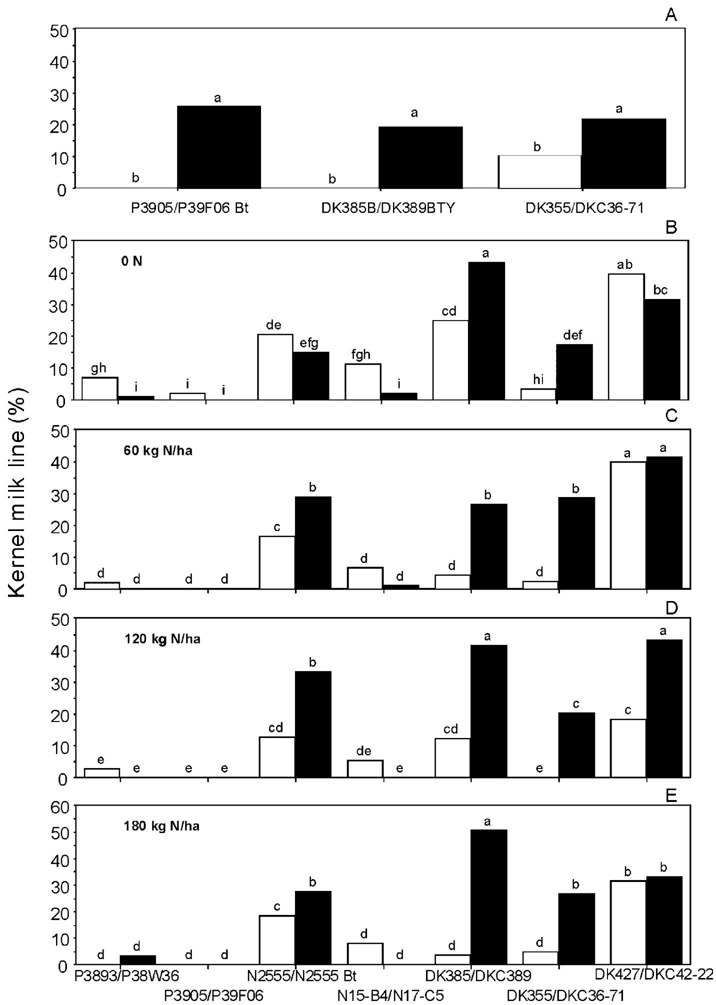

Fig. 3. Kernel milk line stages at shortly after fall killing frost as affected by Bt (solid bar) and their respective conventional non-Bt (empty bar) near-isolines in 2000 (A) and with different N rates in 2002 (B–E). Bars with different letters are significantly different according to the *F*-protected $LSD_{0.05}$ test.

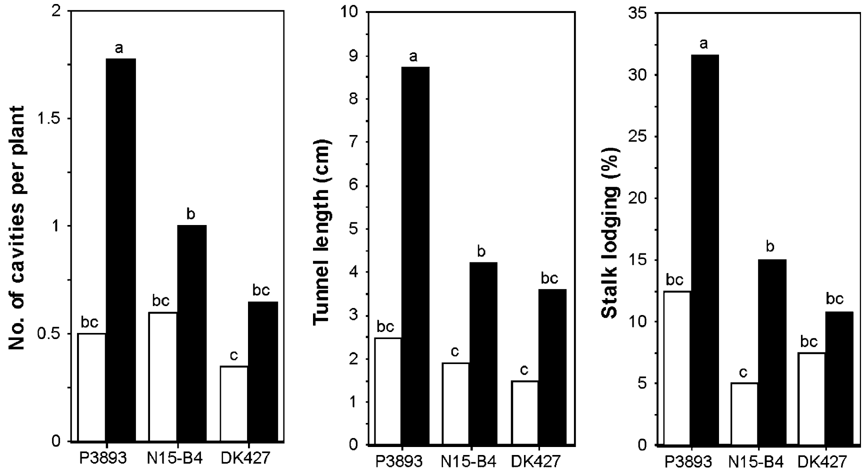

Fig. 4. European corn borer (ECB) cavities, tunnelling and stalk lodging in non-Bt hybrids under 0 (empty bar) and 120 kg N ha^{-1} (solid bar) rates in 2002. Bars with different letters are significantly different according to the F-protected LSD$_{0.05}$ test.

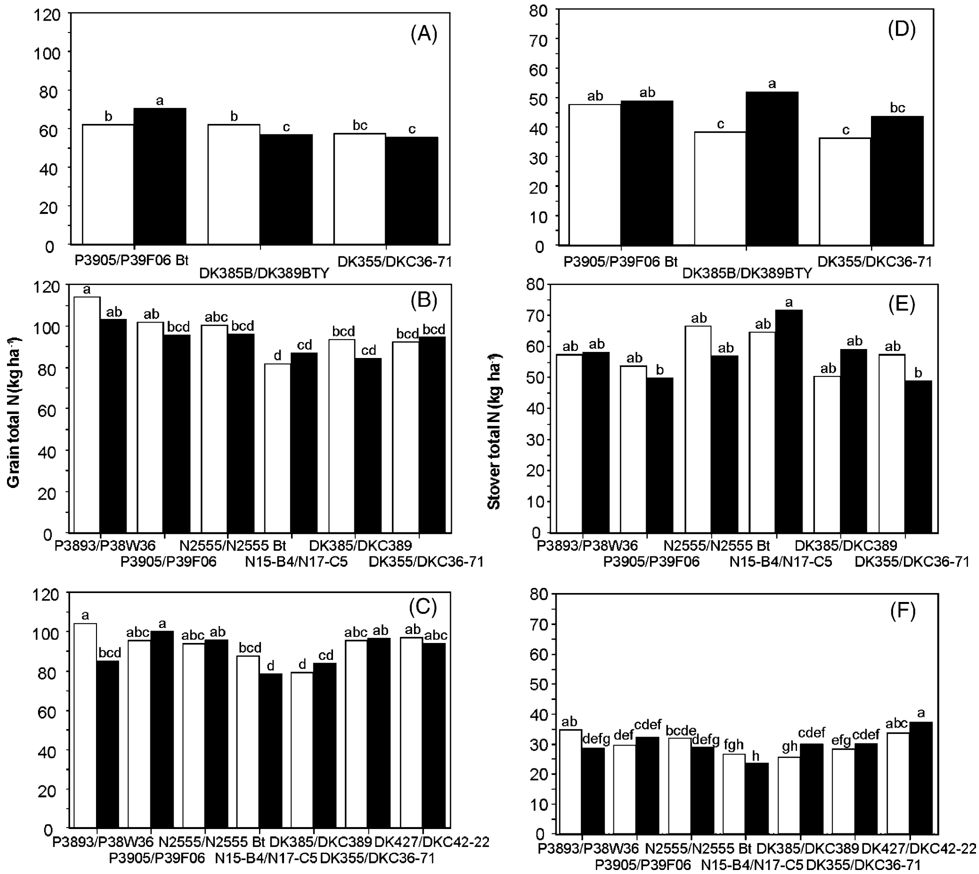

Fig. 5. Grain and stover total nitrogen contents of corn as affected by Bt hybrids (solid bar) in pairs with their conventional non-Bt (empty bar) near-isolines in 2000 (A, D), 2001 (B, E), and 2002 (C, F). Bars with different letters are significantly different according to the F-protected LSD$_{0.05}$ test.

Table 2
Analysis of variance: probabilities and degree of freedom (d.f.) for the N application and hybrid pairs main effects and all possible interactions on grain yield and other agronomic traits in 2000, 2001 and 2002

Source	d.f.	Grain yield (kg ha^{-1})	Grain moisture (g kg^{-1})	Harvest index (HI) (%)	Grain total N (kg ha^{-1})	Stover total N (kg ha^{-1})	Thermal requirement (CHU)	
							Silking	Maturity
2000								
Nitrogen (N)	3	NS[a]	NS	0.02	NS	0.03	NS	NS
Error A	9							
Hybrid pair (G)	2	0.01	0.01	0.07	0.04	NS	0.02	NS
Bt treat (B)	1	NS	0.06	0.02	NS	NS	0.03	0.04
G × B	2	0.01	NS	0.01	0.08	NS	0.001	NS
N × G	6	NS	NS	NS	NS	NS	NS	NS
N × B	3	NS	NS	NS	NS	NS	NS	NS
N × G × B	6	NS	NS	NS	NS	NS	NS	NS
Error B	18							
2001								
Nitrogen (N)	3	NS	NS	NS	0.06	0.001	NS	NS
Error A	9							
Hybrid pair (G)	5	0.001	0.001	0.001	0.001	0.10	0.001	0.001
Bt treat (B)	1	0.06	0.05	NS	NS	NS	NS	0.001
G × B	5	NS	0.001	0.06	NS	NS	0.001	0.001
N × G	15	NS	NS	NS	NS	NS	NS	NS
N × B	3	NS	NS	NS	NS	NS	NS	NS
N × G × B	15	NS	NS	NS	NS	NS	NS	NS
Error B	130							
2002								
Nitrogen (N)	3	0.001	0.001	0.01	0.001	0.001	0.001	NS
Error A	9							
Hybrid pair (G)	6	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Bt treat (B)	1	0.07	0.001	0.04	NS	NS	0.001	0.001
G × B	6	NS	0.001	NS	0.01	0.001	0.001	0.001
N × G	18	NS	NS	NS	NS	NS	NS	NS
N × B	3	NS	NS	NS	NS	NS	0.01	0.03
N × G × B	18	NS	NS	NS	NS	NS	NS	NS
Error B	156							

[a] Not significant at the probability of $P \leq 0.10$.

between Bt and non-Bt hybrids were similar (data not shown). Under the periods of drought and hot conditions in both 2001 and 2002, the heat required to reach maturity for all the hybrids tested was greater than what the companies have listed (Ontario Corn Research Committee, 2003). In 2001, some of the Bt hybrids took 2–3 additional days or 30–100 more CHU to reach maturity than the non-Bt hybrid, the only exception was Pioneer 3905 and Pioneer 39F06 (Fig. 2).

In 2002, cool temperatures in May and early June were followed by extended periods of drought and hot weather from July to September. Silking was delayed by about a week for most hybrids. As a result, several hybrids did not reach maturity by the first fall killing frost (Fig. 3B–E). Shortly after the killing frost, percent kernel milk line was significantly higher for Bt than for non-Bt hybrids of several pairs. In general, it appeared that although the calculated CHU from planting to early fall frost was more than the hybrid maturity requirement listed by the supplying companies, the thermal regime was inadequate for several hybrids, especially for Bt hybrids (Table 1).

3.3. Stalk lodging and tunneling by corn borer

Three pairs of Bt and their non-Bt near-isolines were investigated in 2002 season. Our data showed that in all cases, all the Bt corn stalks were clean without any notable cavities or tunnel. For the conventional hybrids, there was an N-by-hybrid interaction on the number of cavities, tunnel length and percent lodging. In general, Pioneer 3893 showed the most damage by ECB with 0.5–1.7 cavities per stalk, especially with high N rates (Fig. 4), tunnel length ranged from 1.5 to 9.5 cm. Accordingly, stalk breakage ranged from 5 to 15% for Syngenta N15-B4 to 14–33% for Pioneer 3893. Among the three non-Bt hybrids, Pioneer 3893 was the most susceptible variety while N15-B4 and NK427 were similar in response to ECB (Fig. 4). The greater damage to corn by ECB under high N treatments than unfertilized control confirms an earlier report that second generation ECB damage is severer in fertilized than control treatments.

3.4. Response to nitrogen

Total plant N uptake differed significantly among the hybrid pairs and fertilizer N rates (Table 2). However, there was no N-by-hybrid interaction on either total N uptake or accumulation of N in the grain in all years. Grain total N between Bt hybrids and their corresponding non-Bt isolines were not statistically different in most cases (Fig. 5). As Bt corn generally yielded slightly less than non-Bt hybrids in this study, they also had 6–9% lower total grain N (Fig. 5), with the exception of the N17-C5 versus N15-B4 which was opposite in 2001. Some of the full season hybrids, e.g. DK427 and DKC42-22, though produced the greatest yield in 2002 (Fig. 6C), had the lowest N concentration in the grain and thus the lower total

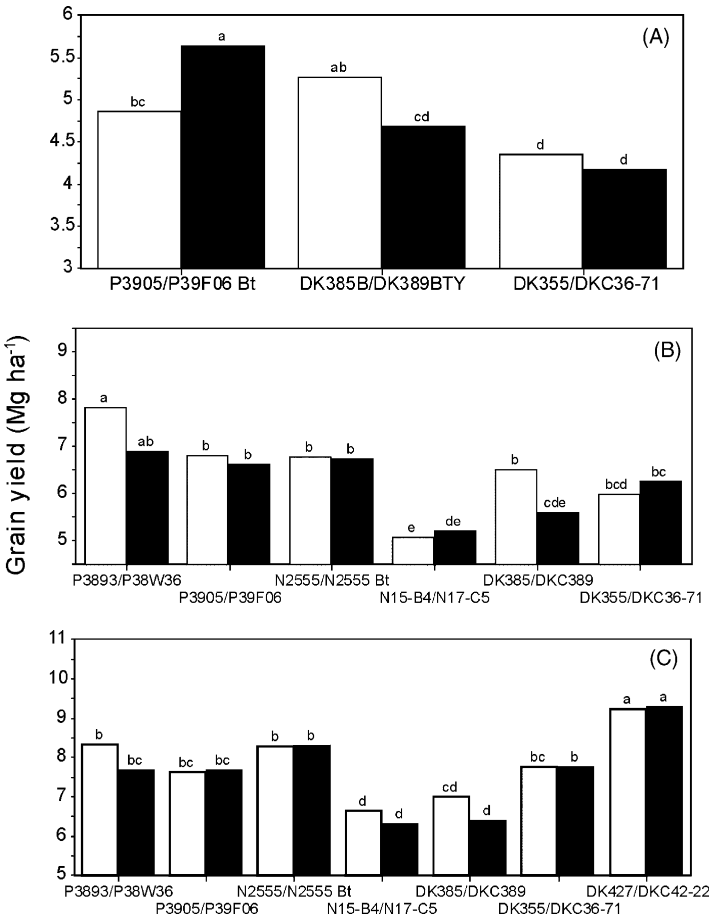

Fig. 6. Grain yield of corn as affected by Bt hybrids (solid bar) in pairs with their conventional non-Bt (empty bar) near-isolines in 2000 (A), 2001 (B), and 2002 (C). Bars with different letters are significantly different according to the F-protected $LSD_{0.05}$ test.

grain N (crude protein) than early to mid maturity hybrids. The data showed that a larger amount of N in Bt hybrids ended up in the stover (Fig. 5). Some exceptions also existed, e.g., total stover N for N2555Bt was 17% lower than N2555; and 15% lower for DNC42-22 (Bt) than for DK427 in 2001.

3.5. Grain yield and moisture

Grain yields varied among hybrid pairs and N rates from year to year. In all cases, there was no N-by-hybrid interaction on yield or any other measured variables (Table 2). In general, full season maturity hybrids yielded slightly or significantly greater than early ones. However, within a pair, there was no significant difference in grain yields between Bt and non-Bt hybrids. Year 2000 had an overall low grain yield due to wet and cool growing season and a shortfall of CHU (Fig. 6A). The Bt hybrid Pioneer 39F06 produced the highest yield, and surpassed its near-isoline, Pioneer 3905, by 13%. While Bt hybrids in all other pairs had lower yields than their conventional near-isoline hybrids. Due to the substantial shortage of CHU in 2000, even the highest yield of Pioneer 39F06 was far below the average yield (7–8 Mg ha^{-1}) in a normal year. In 2001, grain yields among hybrid comparisons were significantly different ($P < 0.01$), but there was no difference between the Bt and non-Bt pairs (Fig. 6B). The non-Bt hybrid Pioneer 3893 produced the highest yield, which was 12% more than its Bt counterpart Pioneer 38W36 (Fig. 6B). For most pairs, the 2002 yield was similar to 2001

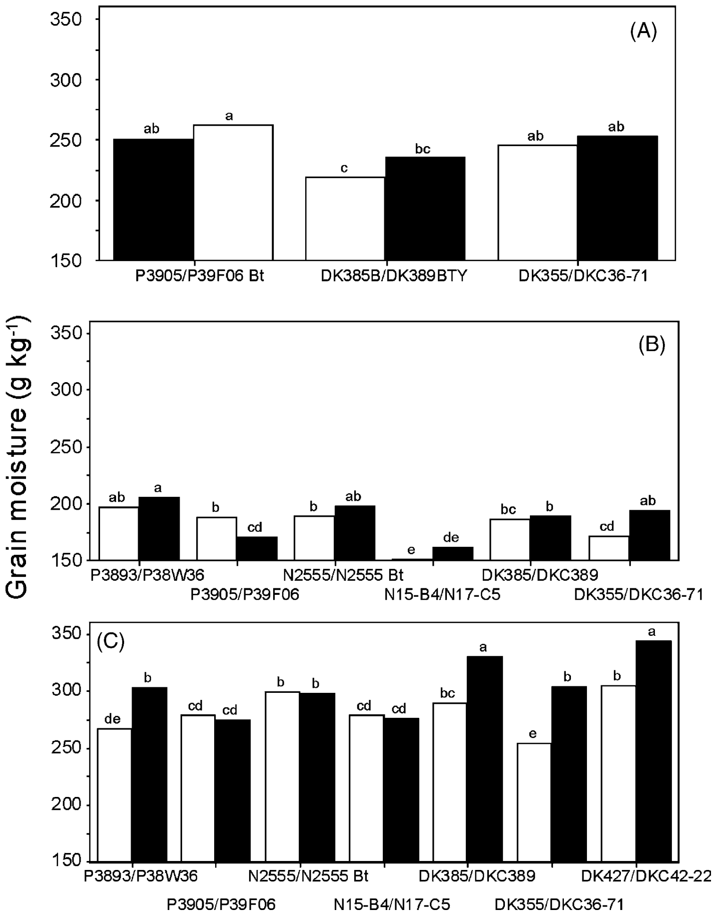

Fig. 7. Grain moisture of corn as affected by Bt hybrids (solid bar) in pairs with their conventional non-Bt (empty bar) near-isolines in 2000 (A), 2001 (B), and 2002 (C). Bars with different letters are significantly different according to the F-protected $LSD_{0.05}$ test.

although Pioneer 3893 out-yielded its Bt near-isoline by 8% (Fig. 6C). The Dekalb hybrid pair, DKC42-22 and DK427 (2850 CHU) tested in 2002, had the highest yields. Across the years of 2001 and 2002, none of the six Bt hybrids of the six common pairs produced significantly greater yield than their conventional counterparts.

There was no significant difference in grain moisture in year 2000 as almost all of the hybrids were premature with substantially greater moisture after fall killing frost (Fig. 7A). In 2001, Bt hybrids usually had slightly or significantly higher grain moisture than non-Bt hybrids in five of the six pairs compared (Fig. 7B). Grain moisture for Bt hybrids was 5–20 g kg^{-1} higher than their non-Bt isoline pairs. In 2002, grain moisture at harvest was much higher than in 2001. However, the trend between Bt and conventional near-isoline hybrids was about the same: some Bt hybrids (e.g. Pioneer 38W36, DKC36-71) having 5% higher grain moisture than their conventional non-Bt paired hybrids (Fig. 7C). The plants and grains of the Bt hybrids appeared to lose moisture slower than non-Bt hybrids perhaps due to lack of stalk injury by ECB. This could be a serious limitation in the northern short to mid-growing season Corn Belt.

4. Discussion

Over the three growing seasons of this field study, the same three pairs of Bt and non-Bt hybrids (total of six hybrids) were tested each year, and six pairs (12 hybrids) were common in 2001 and 2002. In all cases, there was no any indication that the Bt hybrids produced greater yield and/or took up more N or partitioned more N into the grain than their non-Bt near isolines. It was also noted that the higher yielding full season hybrids e.g. DK427 and DKC42-22 had a lower N concentration in grains. This suggests that the same amount of N was diluted to a relatively greater grain yield and that they did not have the ability to take up higher amount of N corresponding to their biomass. Based on the plant N uptake and grain yield data, it can be concluded that there is no difference between Bt and non-Bt near-isoline hybrids in their response to fertilizer N application. Total grain N or crude protein content in corn is an important quality trait for food and feed. Further investigation is needed to explore genotypic differences in grain protein content, as indicated earlier (Ma and Dwyer, 2001).

Hybrids with the Bt trait appeared to be greener at harvest. The later maturing and higher moisture concentration at maturity in Bt hybrids might have been caused by their stay-green characteristic, which maintains leaves and stalk green at physiological maturity (Subedi and Ma, 2005). Similarly, it should be noted that under the extreme weather conditions (e.g. cool temperature at planting and severe drought during the development), some of the hybrids tested took up to 400 additional CHU to reach physiological maturity, compared to their maturity given by the supplying companies (Table 1). Whether this has occurred due to the fact that the hybrid maturity was inaccurately assessed by the supplying companies or the unusual rainfall and temperature regimes encountered during the study years compared with the long term climate pattern. A study on the actual CHU requirement by corn hybrids in a changing climate is under way (Ma and Stewart, 2004).

Transgenic Bt corn hybrids have increasingly been used by the producers because they stand up to harvest time regardless of ECB infestation level. Undoubtedly, under heavy infestation, Bt hybrids provide an additional high level of protection against ECB (Graeber et al., 1999; Filion and Meloche, 2003; Lauer and Wedberg, 1999; Martin and Hyde, 2001). The question is whether using Bt corn provides additional advantages on yield or nitrogen response under low to moderate pest infestation conditions. Our study showed that under the conditions tested, Bt hybrids had no higher yield potentials and thus added no yield benefits over their near-isolines. It is speculated that current conventional hybrids have been bred to have high yield potentials under low to moderate infestation of ECB because of their great tolerance to ECB damage. On the other hand, in Bt hybrids, Bt gene or related cluster genes and promoters may have acted as a negative impact on dry matter and/or grain yield when ECB infestation pressure is low and/or corn crop encounters drought or other environmental stresses. The protection of corn crop from ECB damage was not realized through increased dry matter production. Another potential negative effect associated with the Bt hybrids is greater grain moisture at harvest, which may increase the cost for drying (Dwyer et al., 1994). In general, loss of whole plant and grain moisture in a Bt crop during the grain filling period is slower than non-Bt hybrid as showed in our study, which was probably due to the fact that unlike non-Bt hybrids, there was no cavity caused by ECB in Bt hybrids. A 2–5% high grain moisture would represent a significant drying cost. Plant breeders should improve grain yield in future selection of new Bt and other transgenic hybrids. When choosing hybrids for the following growing season, producers must consider the potential risk of ECB and possible yield reductions in each field before paying the higher seed premium of Bt corn. In general, Bt hybrid seeds have a \$25–30 premium ha^{-1}, and a 4% yield increase over the conventional non-Bt hybrids would level off the seed cost. Planned adoption of Bt and non-Bt hybrid use in a region would also help keep the value of the Bt hybrid technology and avoid or alleviate the threat of pest resistance. Therefore, unless it is certain that ECB infestation would be above the economic threshold, use of the current Bt hybrids may not be cost/benefit justified, and widespread use of Bt hybrids in lower ECB problem areas not only increases the cost of production, but may also lead to negative impact on non-target organisms in the environment (Garcia et al., 1998; Scriber, 2001; Conner et al., 2003).

5. Conclusions

Within the same maturity group, it was the superior genotype (not Bt trait) that led to the greatest N uptake, and the highest grain yield. The study addressed three key questions i.e., that (i) under the conditions tested and with natural ECB infestation, there was no yield advantage of Bt hybrids in comparison with their conventional counterparts when stalk breakage of the conventional hybrids by ECB was low to moderate (<2 cavities/plant), (ii) Bt hybrids have a similar response to fertilizer N application rates compared to non-Bt near-isoline hybrids, and (iii) under low to moderate ECB infestation conditions, Bt hybrids do not justify their premium on seed cost. Therefore, it is very important for the growers to consider the level of pest infestation and economic threshold before deciding to use Bt hybrids. Of course, one can argue that Bt hybrids have the benefit of protection for potential ECB damage and enhancing harvesting, which may not outweigh the potentially greater costs of seed and grain-drying. Our study does suggest that for the next generation of Bt or other transgenic hybrids against ECB or other pests, combination of transgenic traits with superiority in yield, N use and agronomic performance will be desired to justify the higher technology fee. Where there is no severe infestation of ECB, corn producers may not benefit from the use of current Bt hybrids.

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