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Field-Grown Bt and non-Bt Corn: Yield, Chemical Composition, and Decomposability

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ABSTRACT

Bt (*Bacillus thuringiensis*) corn (*Zea mays* L.) accounted for 74.5% of the corn acreage in eastern Canada in 2009. Reports that Bt corn has greater yield and lignin concentrations than unmodified corn have raised questions about its effect on the soil ecosystem. Our objectives were to evaluate the biomass of field-grown Bt and non-Bt corn, the chemical composition of different corn components that remain as residues in the field after harvest, and the effect of the Bt modification on residue decomposition. Nine Bt corn hybrids and their near isolines were field-grown in 2008 and 2009. Grain and stover yields were measured and leaves, stems, and roots were collected and analyzed for lignin, C, and N concentrations. Stem sections from a Bt/non-Bt corn pair were buried in the field and sampled periodically during 1 yr. No difference in yield or lignin concentrations due to the Bt gene was noted; however, N concentration in Bt stems was significantly greater than in non-Bt stems in 1 yr of the 2-yr study. Leaves had less lignin and a lower C/N ratio than stems and roots in both years. In buried field litterbags, the decline in C/N ratio and mass loss suggests that Bt stems were decomposing more rapidly than non-Bt stems. We conclude that the Bt gene does not affect the agronomic performance or the chemical composition of corn in fields without herbivory, and that Bt corn residue may be more susceptible to decomposition than non-Bt corn residue.

Genetically modified Bt corn is planted extensively in North America. In 2009, 1.3 million hectares in eastern Canada (Ontario and Quebec) were planted with corn and 74.5% of hybrids were Bt corn (Agriculture and Agri-Food Canada, 2009; Canadian Corn Pest Coalition, 2010). The Bt corn hybrids are popular with farmers in this region because the genes from the *Bacillus thuringiensis* gram-positive bacterium produces crystal-like proteins (Cry proteins) for protection against European corn borer (ECB, *Ostrinia nubilalis* H.) and corn rootworm (*Diabrotica* spp.).

Concerns have been raised about other effects of Bt corn on the soil ecosystem. Cry proteins can persist in soil and could affect populations of nontarget soil organisms, but reports generally indicate few direct effects (Tapp and Stotzky, 1998; Stotzky, 2000; Hopkins and Gregorich, 2003; O'Callaghan et al., 2004; Clark et al., 2005; Icoz and Stotzky, 2008).

Indirect effects of Bt corn on the soil ecosystem could arise because Bt corn exhibits greater biomass accumulation (Motavalli et al., 2004a) due to less herbivory compared with non-Bt (NBt) hybrids, which could have implications for residue management. Grain yield is an important measure of agronomic performance when assessing Bt and NBt corn hybrids. In some studies, Bt hybrids produced up to 11% more grain (Dillehay et al., 2004; Mungai et al., 2005; Subedi and Ma, 2007) whereas other studies reported either no difference or more grain production from NBt hybrids (Folmer et al., 2002; Dignac et al., 2005). Variations in grain yield were related to hybrid type differences, growing conditions, and herbivory stress; these factors may also affect the biomass accumulated in nongrain components. Greater stover and aboveground biomass with Bt hybrids was reported in fields where NBt hybrids were infested with ECB (Folmer et al., 2002; Jung and Sheaffer, 2004; Motavalli et al., 2004b; Fang et al., 2007) whereas Folmer et al. (2002) and Mungai et al. (2005) reported no differences although ECB infestations were observed in their experiments. A review of six studies showed that silage yield and aboveground biomass from Bt hybrids ranged from 17% lower to 47% higher than NBt hybrids (Yanni et al., 2010). Greater stover production from Bt corn would directly affect the amount of residue C that goes into soil following harvest, with consequences for soil organic matter, microbial community dynamics, soil C storage, and residue management (Hadas et al., 2004; Icoz and Stotzky, 2008).

In addition to greater residue input in Bt corn agroecosystems, the chemical composition of the Bt corn residue may differ from NBt corn residue and hence alter the decomposition rate. Differences in chemical composition, primarily the lignin concentration, have been reported between Bt and NBt isolines, but it is not clear why (Saxena and Stotzky, 2001; Poerschmann et al., 2005). Jung and Sheaffer (2004) noted that the insertion of the Bt gene into the corn genome is random and, unless by chance it was inserted in the lignin biosynthetic pathway, this gene should not interfere with lignin production. However, Saxena and Stotzky (2001) reported significantly greater lignin concentrations in 10 Bt hybrids with different insertion events

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Abbreviations: Bt, *Bacillus thuringiensis*; ECB, European corn borer; NBt, non-*Bacillus thuringiensis*.

Table 1. Mean annual temperature, total precipitation, and crop heat units during the 2008 (May–September) and 2009 (May–October) growing seasons at Saint-Anne-de-Bellevue, QC, Canada (Environment Canada, 2010a).

Climatic conditions	May		June		July		Aug.		Sept.		Oct.
	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2009
Mean temperature, °C	11.9	12.2	19.3	17.4	20.7	19.5	19.2	20.1	16.2	14.5	6.8
	(13.2)†		(18.1)		(21.0)		(19.8)		(14.6)		(8.1)
Total precipitation, mm	66.4	79.0	50.8	68.8	69.6	128	54.6	81.6	38.8	42.8	107
	(71.4)		(88.6)		(93.6)		(104.2)		(96.0)		(77.2)
Acquired crop heat units‡	127	192	735	659	841	786	761	808	372	544	145

† Values in parentheses are normals for 1971–2000 in Saint-Anne-de-Bellevue (Environment Canada, 2010b).
‡ Acquired crop heat units calculated from emergence day until harvesting day.

(MON 810 and Bt11 gene transformations), which had from 33 to 97% more lignin than isogenic NBt corn grown in the field and in growth chambers. Other reports have shown either greater lignin concentrations in Bt corn (Masoero et al., 1999; Poerschmann et al., 2005) or no difference between Bt and NBt corn isolines (Rossi et al., 2003; Jung and Sheaffer, 2004; Mungai et al., 2005). The discrepancy among these studies is likely due to different methods of lignin analysis as well as differences in crop maturity stages and the plant part analyzed. Another factor that was reported to be different between Bt and NBt tissue is the C/N ratio, which was reported to be lower in Bt than NBt tissue (Flores et al., 2005). The presence of the Cry proteins in Bt corn, which are produced at levels of 0.44 to 111 ng mg^{-1} in leaf tissue (Yanni et al., 2010), is an extra N source. As alteration of lignin and N concentrations could affect residue degradation, the residence time of residue C may be different in Bt and NBt corn agroecosystems.

The abovementioned differences in residue quality and quantity are hypothesized to slow decomposition of residue from Bt corn and pose a challenge to producers with respect to residue management. There is some evidence to support this hypothesis, as some reports from agricultural communities indicate that Bt corn residues are tough and more difficult to manage than NBt corn residues (Lehman et al., 2010), requiring improved tillage machinery (Lyseng, 2010) to deal with the hard residue. Flores et al. (2005) also reported 20 to 39% less CO_2 evolution from soils amended with Bt corn residue compared with soils with NBt residue from a laboratory incubation study. Slower decomposition means longer residence time of residue C in the field and a better chance for C stabilization in the soil. However, experimental studies don't necessarily support this hypothesis; research has mostly indicated that Bt corn residue degrades at the same rate as NBt residue (Hopkins and Gregorich, 2003; Zwahlen et al., 2007; Lehman et al., 2008a; Lehman et al., 2008b; Tarkalson et al., 2008; Lehman et al., 2010) even when the Bt residue had significantly greater lignin content (Mungai et al., 2005; Fang et al., 2007). With the exception of Lehman et al. (2010), the decomposition studies described above tested the decomposition of ground or chopped corn residue that was not subjected to herbivory injury. The ECB-infested NBt stem internodes used by Lehman et al. (2010) showed similar decomposition rates as Bt-protected stems buried in a clay loam soil over an 8-mo period.

This study was predicated on the hypothesis that Bt corn produces more grain and stover biomass under field conditions, but we did not expect differences in lignin or C and N contents between Bt and NBt near isolines. The first objective of this study was to evaluate the biomass (harvested grain plus stover residue) and chemical composition of nine field-grown Bt corn hybrids and their near isolines (NBt corn hybrids) grown in eastern Canada. The second objective was to determine if the chemical composition of Bt and non-Bt corn differed and would therefore alter the residue decomposition rates. Decomposition of Bt and NBt corn residues was tested with intact corn stems in a 1-yr buried litterbag experiment; based on previous litterbag experiments, the hypothesis was that Bt and NBt stems would have similar decomposition rates in the field.

MATERIALS AND METHODS

Location, Experimental Design, Soil and Corn Hybrid Characteristics, Harvesting

The experiment was performed in two growing seasons during 2008 and 2009 in a field located at the Emile A. Lods Agronomy Research Centre of McGill University in Sainte-Anne-de-Bellevue, Quebec, Canada (45°24' N, 73°56' W). The field was planted with continuous corn before this experiment and was prepared by moldboard plowing to 17 cm in the fall, followed by spring cultivation with a tandem disk (10 cm) and seed-bed preparation using a triple-K cultivator (10 cm). The soil was a Chicot sandy loam soil (Gray Brown Luvisol; fine-loamy, mixed, frigid Typic Hapludalf) having 661 g kg^{-1} sand, 159 g kg^{-1} clay, a pH of 5.97, 14.1 g organic C kg^{-1}, and 1.6 g total N kg^{-1}. Soil fertility was moderate, based on soil test values of 112 mg K kg^{-1} and 90.9 mg P kg^{-1} (Mehlich-III extractable). Monthly mean temperature, total precipitation, and crop heat units during the 2008 and 2009 growing seasons (May–October) are given in Table 1 (Environment Canada, 2010a).

The experiment was designed as a complete factorial with Bt gene modification (Bt and NBt) and hybrid type (9 hybrid pairs) as the main factors, with three replicates of each factorial combination for a total of 54 plots. The treatments were distributed in a complete randomized design since the field was homogeneous in slope and other physical characteristics. The nine Bt hybrids and nine NBt near isolines selected for this study are described in Table 2. The field, 60 by 19 m, was divided into 54 plots, each 5 by 3 m, and each plot contained four rows with 75-cm row spacing. Preseeding fertilizers, 40 kg N ha^{-1} and 20 kg P_2O_5 ha^{-1} of calcium ammonium nitrate and monoammonium phosphate fertilizer mix (23–12–0), were banded on 13 May 2008 and 30 Apr. 2009. Plots were hand seeded on 14 May 2008 and 5 May 2009 at a rate of 30 seeds per row for a plant density of 80,000 plants ha^{-1}. Postemergence herbicides were applied on 28 May 2008 and 26 May 2009 to control weeds, namely 1.1 L ha^{-1} Dimethenamid (*S*-2-chloro-*N*-(2,4-dimethyl-3-thienyl)-*N*-(2-methoxy-1-methylethyl)-acetamide), 1.25 L ha^{-1} Dicamba

Table 2. Characteristics of 18 hybrids used in the field experiment (Canadian Seed Trade Association, 2006; Maizex Seeds, 2008; Syngenta Seeds Canada, 2008).

Hybrid	Trait†	Crop heat units	Stalk strength‡	Root strength‡	Plant height§	Bt protein/genetic modification event	Company
N23-F7	CB/LL	2700	2	5	M	Cry1Ab/Bt11	Syngenta
N23-F9	–	2700	2	5	S–M	–	Syngenta
N25N	GT/CB/LL	2750	3	2	T	Cry1Ab/Bt11	Syngenta
N25N-GT	GT	2750	3	2	T	–	Syngenta
N29-A2	CB/LL	2850	4	2	M–T	Cry1Ab/Bt11	Syngenta
N29-G7	LL	2850	4	2	S	–	Syngenta
N34-F1	CB/LL	2950	2	3	M–T	Cry1Ab/Bt11	Syngenta
N34F/GT	GT	2950	2	3	M–T	–	Syngenta
N45-A6	CB/LL	3100	3	3	M–T	Cry1Ab/Bt11	Syngenta
N45A-LL	LL	3100	3	3	M–T	–	Syngenta
MZ2263	Bt	2600	1	2	M–S	Cry1Ab/MON810	Maizex
MZ226	–	2550	2	2	M–S	–	Maizex
MZ3877	Bt RR	2775	1	2	S	Cry1Ab/MON810, NK603	Maizex
MZ27–00RR	RR	2725	2	2	S–M	NK603	Maizex
MZ3888	Bt	2900	1	1	T–M	Cry1Ab/MON810	Maizex
MZ310	–	2850	Not available		M–T	–	Maizex
MZ5444	Bt	3350	1	3	T	Cry1Ab/MON810	Maizex
MZ540	–	3300	2	3	T	–	Maizex
				Hybrids for decomposition experiment			
38W22	HX1¶	2750	6	4	M–T	Cry1F/TC1507	Pioneer
38W21	–	2700	6	4	M–T	–	Pioneer

† Bt, *Bacillus thuringiensis*; CB, Corn borer protection; GT, glyphosate tolerant; LL gluphosinate herbicide resistant; RR, Roundup Ready.

‡ Strength 1–9, 1 = best.

§ Plant height observed in the field: T, tall; M, medium; S, short.

¶ HX1, Herculex 1 insect protection technology by Pioneer Hi-Bred International and Dow AgroSciences LLC.

(3,6-dichloro-2-methoxybenzoic acid), and 2.5 L ha^{-1} Atrazine (6-chloro-N^2-ethyl-N^4-isopropyl-1,3,5-triazine-2,4-diamine). A side-band application of calcium ammonium nitrate and potassium chloride mix (22–0–12) was done on 12 June 2008 and 17 June 2009 at a rate of 140 kg N ha^{-1} and 76 kg K_2O ha^{-1}. In 2008, some seeds and seedlings in three plots were removed by crows (*Corvus* spp.), which necessitated reseeding of these plots on 13 June. Also in 2008, much of the grain was consumed by crows, and only some ears at the centers of each plot were undamaged. The ears from the center of the plots were carefully chosen, selecting the least damaged ears so that yield measurement was a good representation of average yield. In 2009, the field was covered with netting (2 by 2 cm mesh) to protect young seedlings, and was removed when the plants were at the 5- to 6-leaf stage, and ear netting (0.7 by 0.7 cm mesh) was also placed on 10 corn ears per plot at the blister (R2) development stage.

Harvesting was done 126 d after seeding in 2008 when most hybrids had reached the black layer stage (Ritchie et al., 1986); the hybrids N45-A6, N45A-LL, MZ5444, and MZ540 were at the mid-dent (R5) stage of development. In 2009, harvesting was done 139 d after seeding when all hybrids had reached physiological maturity. In both years, 10 plants that were undamaged or minimally affected by crows were hand harvested from the two middle rows in each plot; the ears (without husks) were separated from the stalks and the weight of each recorded. Two stalks, designated for moisture determination, were chopped and weighed. The remaining eight stalks were then separated into leaves (including ear husks) and stems (including the first node above the soil surface), and the ears into grain and cob. Stems from 2009 were cut at the ear and the lower portion was used for analysis; the upper part of the stem is less lignified than the lower (older) part (Smith, 1977), so this choice was made to allow comparison between whole stem lignin (2008) and lower stem lignin concentration. Stalk and grain yields (kg ha^{-1}) were calculated based on the dry weights from 10 plants and a planting density of 120 plants per 15 m^2 plots. Roots from two plants per plot were collected to a depth of 25 to 30 cm within a week of the aboveground harvesting and thoroughly cleaned to remove adhering soil particles. All collected material was dried at 60°C for 48 h and ground using a Wiley mill to pass through a 1-mm mesh sieve.

Plant Tissue Analysis

Ground leaves, stems, and roots were analyzed for fiber content (hemicellulose, cellulose, lignin), carbon, and nitrogen. Fiber analysis, based on the Goering and Van Soest (1970) gravimetric method, was done using an Ankom Fiber Analyzer (Ankom Technology, Fairport, NY) to measure Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), and Acid Detergent Lignin (ADL) followed by ashing. Hemicellulose was estimated as the difference between NDF and ADF, cellulose as the difference between ADF and ADL, and lignin estimated from ADL after sample ashing. Carbon and nitrogen were analyzed on a Carlo-Erba CN Flash EA Analyzer (Milan, Italy).

Field Decomposition Experiment

Decomposition of field-grown corn stems was tested using one Bt/NBt hybrid pair grown at the Central Experimental Farm of the Eastern Cereal and Oilseed Research Centre (AAFC) in 2008. Ten Bt corn stems (Pioneer 38W22) and ten NBt corn stems (Pioneer 38W21) were acquired for this experiment (Table 2). While there were no visible cavities in the Bt corn

Table 3. Means of grain and stover yield (Mg ha^{-1}) of 18 Bt (*Bacillus thuringiensis*) and NBt (non-*Bacillus thuringiensis*) corn hybrids field-grown in 2008 and 2009. Values are the means of Bt and NBt near-isoline pairs ± standard error (*n* = 3). Yield is reported on dry basis.

Hybrid pair	Bt/NBt	2008 Grain yield	2008 Stover yield	2009 Grain yield	2009 Stover yield
		Mg ha^{-1}			
N23-F7	Bt	12.5 ± 1.7 ab†	9.3 ± 1.7	16.5 ± 0.4 ab	8.6 ± 0.9 a
N23-F9	NBt	11.1 ± 0.7 ab	10.0 ± 1.6	16.6 ± 1.2 ab	9.7 ± 1.1 a
N25N- GT/CB/LL	Bt	13.7 ± 1.4 ab	9.6 ± 1.8	15.1 ± 1.0 a	8.7 ± 0.6 a
N25N-GT	NBt	11.2 ± 0.4 ab	7.9 ± 1.2	15.3 ± 0.5 a	8.0 ± 0.9 a
N29-A2	Bt	11.8 ± 0.9 ab	9.2 ± 1.1	19.4 ± 0.6 ab	10.6 ± 1.1 ab
N29-G7	NBt	11.8 ± 1.7 ab	9.1 ± 1.1	16.3 ± 1.2 ab	9.1 ± 1.1 ab
N34-F1	Bt	13.9 ± 1.3 ab	10.7 ± 1.9	18.4 ± 1.5 ab	9.6 ± 0.7 ab
N34F-GT	NBt	11.6 ± 1.3 ab	8.1 ± 0.6	19.3 ± 0.8 ab	11.5 ± 1.4 ab
N45-A6	Bt	13.0 ± 2.1 ab	9.0 ± 1.2	19.0 ± 2.3 ab	10.3 ± 1.5 ab
N45A-LL	NBt	10.6 ± 1.4 ab	7.9 ± 0.8	15.9 ± 1.3 ab	9.5 ± 1.0 ab
MZ2263	Bt	7.4 ± 1.4 a	11.1 ± 1.2	16.7 ± 1.0 ab	9.7 ± 0.8 a
MZ226	NBt	8.7 ± 1.7 a	10.2 ± 2.8	16.2 ± 0.6 ab	8.7 ± 0.6 a
MZ3877	Bt	12.3 ± 2.5 ab	10.0 ± 1.2	17.9 ± 1.0 ab	9.1 ± 0.7 a
MZ27–00RR	NBt	11.4 ± 1.7 ab	10.5 ± 1.9	15.8 ± 1.3 ab	8.1 ± 0.7 a
MZ3888	Bt	10.4 ± 3.0 ab	11.7 ± 1.1	19.6 ± 1.6 b	12.4 ± 2.7 ab
MZ310	NBt	11.4 ± 0.4 ab	8.3 ± 1.3	20.5 ± 1.7 b	10.0 ± 0.3 ab
MZ5444	Bt	14.3 ± 1.4 b	14.8 ± 2.4	19.8 ± 2.5 ab	15.4 ± 2.0 b
MZ540	NBt	14.6 ± 0.6 b	11.5 ± 0.6	16.6 ± 2.8 ab	12.0 ± 1.9 b
		Significance probability level (*P*)			
Hybrid		0.0196	ns‡	ns	0.0026
Bt Gene modification (GM)		ns	ns	ns	ns
Hybrid × GM		ns	ns	ns	ns

† Values within a column followed by different letters are significantly different at α = 5% calculated by Tukey's test.

‡ ns, not significant.

stems, up to five holes with tunneling length of up to 15 cm by ECB were noted on the NBt stems. The experimental design included one factor (Bt gene modification) with six replicates and seven sampling dates. Eighty-four litterbags, 10 by 10 cm with a 1-mm^2 mesh size, were constructed from nylon–polyester mesh according to the description in Trofymow and CIDET Working Group (1998). Each set of stems (Bt and NBt) were pooled and dry stem sections, 5 cm long and 1.7–2 cm in diameter, were cut from the internode sections below the ear of the 10 plants, weighed, and placed in the litterbags. The bags were buried in the field at a depth of 5 cm in May 2009 and six bags from each treatment were collected monthly for the first 6 mo, and one set of bags kept over the winter and collected 1 yr after they were buried. Litterbags were buried in no-till plots with low residue input in a long-term corn agroecosystem on the Macdonald Research Farm in Saint-Anne-de-Bellevue, Quebec, Canada (45°30' N, 73°35' W). Soil at this site was a sandy loam, frigid Typic Endoaquent (Dystric Gleysol) of the Saint-Amable and Courval series with 815 g kg^{-1} sand, 96 g kg^{-1} clay, and 19.9 g organic C kg^{-1}. Further details of this long-term experiment were described by Burgess et al. (1996), Dam et al. (2005) and Halpern et al. (2010). Litterbags were buried in the field at 5-cm depth between six corn rows with about 1-m spacing between bags. The field was planted with corn shortly after the bags were buried. The bags were washed within hours of collection and dried at 50°C, after which they were weighed and mass loss calculated as the difference in weight at time = 0 and sampling time. Extra care was taken while cleaning the bags collected after 5 mo to avoid loss of broken pieces. The physical state of the stems was good enough to allow complete removal of any adhering soil particles so that there was no need to correct the mass for inorganic material by burning the samples.

Statistical Analysis

Analysis of variance (ANOVA), using the Proc GLM procedures on SAS software (SAS Institute, 2009), was done to test the effects of hybrid, Bt gene modification (GM), and their interaction on yield, fiber content, and C and N contents of field-grown corn. Because the hybrids had different crop heat requirements (CHU), the difference between CHU requirement and actual CHU acquired in each year was calculated and used as a covariable in the model. Log transformations were used to normalize the data where needed. Mean separation of significant treatment effects was done using least square means with the Tukey adjustment. An ANOVA was used to test the effect of Bt gene modification on the chemical composition of corn plant components (leaves, stems, roots) after pooling the data among hybrids, and to test the effect of Bt gene modification on the mass loss of stems at each sampling time. The NLIN procedure (SAS Institute, 2009) was used to fit the mass loss data into a single exponential decomposition model (Olson, 1963).

RESULTS

Yield of Field-Grown Bt and NBt Corn Hybrids

No ECB infestation was observed in 2008 and 2009, but damage by birds affected the average grain yield in 2008. There was no difference in grain yield (presented on a dry matter basis) or stover yield among near isolines, however the hybrid type had a significant effect on grain yield in 2008 ($P = 0.0196$) and on stover yield in 2009 ($P = 0.0026$) (Table 3). In general, the Bt hybrids yielded

Table 4. Means of lignin concentration (g kg^{-1}), C/N ratio, and N concentration (g kg^{-1}) of 9 Bt and 9 NBt field-grown corn hybrids in 2008 and 2009. Means of Bt (*Bacillus thuringiensis*) and NBt (non-*Bacillus thuringiensis*) for each corn component are given. Values are the means ± standard error (*n* = 27).

Bt gene modification (GM)	2008			2009†		
	Leaves	Stems	Roots	Leaves	Stems	Roots
			Lignin (g kg^{-1})			
Bt	37.7 ± 2.17	67.8 ± 3.41	90.4 ± 4.10	25.8 ± 1.27	71.9 ± 3.06	72.5 ± 3.12
NBt	40.0 ± 2.20	71.3 ± 2.91	91.9 ± 2.85	27.0 ± 0.91	74.7 ± 2.19	71.6 ± 2.75
GM	ns‡	ns	ns	ns	ns	ns
Hybrid	<0.0001	ns	0.0005	ns	0.0005	<0.0001
Hybrid × GM	<0.0001	ns	ns	ns	ns	ns
			C/N ratio§			
Bt	27.4 ± 1.36	72.6 ± 7.20	66.9 ± 5.18	31.8 ± 1.08	60.8 ± 3.29	55.0 ± 2.42
NBt	29.2 ± 1.25	67.8 ± 5.52	60.8 ± 3.64	35.5 ± 1.38	84.2 ± 3.96	36.7 ± 1.14
GM	ns	ns	ns	ns	<0.0001	<0.0001
			N (g kg^{-1})§			
Bt	17.2 ± 0.70	8.26 ± 0.83	8.41 ± 0.63	14.4 ± 0.40	8.09 ± 0.40	8.88 ± 0.46
NBt	16.1 ± 0.71	8.20 ± 0.71	8.61 ± 0.47	13.4 ± 0.55	5.78 ± 0.25	12.8 ± 0.34
GM	ns	ns	ns	ns	<0.0001	<0.0001

† Log transformation done for 2009 root lignin, 2009 leaf N and C/N, and 2009 root C/N.
‡ ns, not significant.
§ Hybrid and Hybrid × GM were not significantly different for N and C/N ratio for any component in any year.

about 1 Mg ha^{-1} more grain than the NBt hybrids in both years, but the difference was not statistically significant. Grain moisture content (data not shown) was similar between Bt and NBt hybrids in both years, on average 40% in 2008 and 30% in 2009. Lower grain moisture content in 2009 was likely due to the longer growing season and greater CHU accumulation.

Lignin, Carbon, and Nitrogen Concentrations of Field-Grown Bt and NBt Corn Hybrids

The Bt gene did not affect the lignin concentration of corn components in 2008 or 2009, but hybrid type strongly affected the lignin concentration in leaves and roots (2008) and in stems and roots (2009) (Table 4). The hemicellulose and cellulose in corn tissues were not affected by Bt gene modification or hybrid type (data not shown). In 2008 and 2009 respectively, hemicellulose content was between 22 to 32% and 26 to 35% in leaves, 17 to 28% and 15 to 25% in stems, and 24 to 31% and 26 to 35% in roots. Cellulose content in 2008 and 2009 respectively, was 24 to 39% and 28 to 40% in leaves, 26 to 50% and 27 to 54% in stems, and 35 to 48% and 34 to 48% in roots. Pooling the data among hybrid types showed that the lignin concentration of leaves was consistently smaller than stem and root lignin in both years whereas root lignin concentration was significantly ($P < 0.05$) greater than stem lignin in 2008 only (Table 5).

Hybrid type did not affect the C and N concentrations, or the C/N ratio, of corn components in any year and there was also no interaction between Bt gene modification and hybrid type. However, the Bt gene had a significant ($P < 0.05$) effect on the N concentration of stems and roots in 2009 (Table 4). The N concentration was 40% greater in Bt stems than NBt stems and 44% smaller in Bt roots than NBt roots in 2009. Comparing corn components (Table 5) shows that leaves had significantly ($P < 0.05$) lower C/N ratios than stems and roots in both years. The C/N ratio of stems and roots was inconsistent, with a significantly ($P < 0.05$) lower C/N ratio in roots than stems in 2009 only (Table 5).

Decomposition of Field-Grown Bt and NBt Corn Stems

Stem materials used in the litterbag study had comparable chemical composition. The Bt stems contained 105 g kg^{-1} lignin and a C/N ratio of 217, whereas the NBt stems had 103 g kg^{-1} lignin and a C/N ratio of 219. After 1 yr in the field, the Bt stems lost 56% of their mass compared with 43% for NBt stems (Fig. 1) and had a significantly ($P < 0.05$) lower C/N ratio than NBt stems (Table 6). The NBt stems had greater mass loss than Bt stems in month five ($P = 0.0105$) but total mass loss after 1 yr was not different between Bt and NBt stems. Decomposition rate constants were $k = 0.0871 \pm 0.0054$ for the Bt stems and $k = 0.0912 \pm 0.0054$ for the NBt stems.

DISCUSSION

The experiments that were designed to test differences between Bt and NBt hybrids in yield and chemical composition were performed in 2 yr with a relatively large number of hybrids so that variability in hybrid types could be accounted for. That the corn was grown in the same field during the two seasons, however, limits generalizing the results over space. On the other hand, the climatic conditions and soil conditions (pH, soil temperature, and tillage management) are similar to those in the southeastern Quebec and eastern Ontario regions and can be used to reasonably

Table 5. Lignin concentration (g kg^{-1}) and C/N ratio in stems, leaves, and roots of corn hybrids grown in the field in 2008 and 2009. Values are the means ± standard error (*n* = 54).

Component	2008		2009	
	Lignin	C/N	Lignin	C/N
	g kg^{-1}		g kg^{-1}	
Leaves	38.8 ± 1.68 a†	28.3 ± 1.01 a	26.4 ± 0.85 a	33.6 ± 0.99 a
Stems	69.6 ± 2.44 b	70.2 ± 4.93 b	73.3 ± 2.06 b	72.5 ± 3.31 b
Roots	91.2 ± 2.71 c	63.9 ± 3.47 b	72.1 ± 2.25 b	45.9 ± 2.00 c
		Significance probability level (*P*)		
Component	<0.0001	<0.0001	<0.0001	<0.0001

† Values within a column followed by different letters are significantly different at α = 5% calculated by Tukey's test.

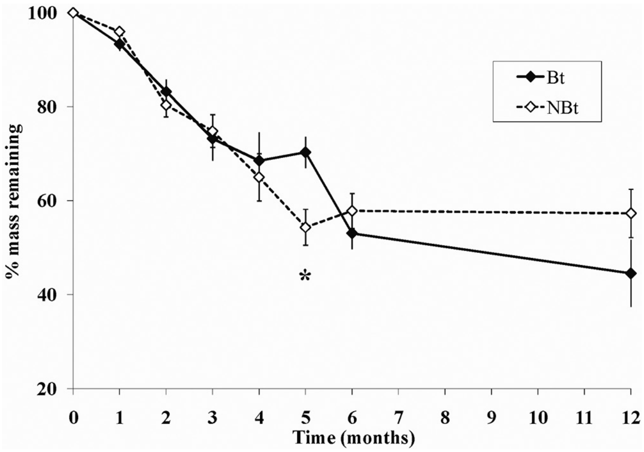

Fig. 1. Stem mass remaining of Pioneer 38W22 (Bt, *Bacillus thuringiensis*) and Pioneer 38W21 (NBt, non-*Bacillus thuringiensis*) after 1 yr in the field. Error bars are the standard error (n = 6). Significantly different values at α = 5% are marked with an asterisk.

Table 6. Carbon to N ratio, and C and N concentration (g kg^{-1}) of Pioneer stems Bt (*Bacillus thuringiensis*) 38W22-Bt and NBt (non-*Bacillus thuringiensis*) 38W21 at time 0 and 1 yr in the field. Statistical significance ($P \leq 0.05$ at – = 5%) for effect of Bt gene modification at time = 1 yr is indicated by asterisk. Values are the means ± standard error.

Time	Treatment	C	N	C/N ratio
		——— g kg^{-1} ———		
Time = 0 (n = 2)	Bt	461 ± 0.20	2.1 ± 0.04	217 ± 4.06
	NBt	459 ± 0.22	2.1 ± 0.01	219 ± 1.35
Time = 1 yr (n = 6)	Bt	479 ± 3.7	10.5 ± 1.1*	47.5 ± 4.9*
	NBt	487 ± 2.3	6.73 ± 0.8	77.5 ± 8.7

estimate effects in corn agroecosystems in those regions. The Luvisolic soil order to which the soil belongs covers about 8.8% of Canada's land area, with large areas in the central to northern interior plains and smaller areas in the regions south of the permafrost (Mckeague and Stonehouse, 2010). The hybrids selected were chosen because they are suitable to the growing conditions of the region of interest with the two hybrid pairs (N45-A6/N45A-LL and MZ5444/MZ540) having CHUs at the upper limit of the CHU requirements for southeastern Quebec and eastern Ontario (CEROM, 2009; OMAFRA, 2010).

Since no ECB stress was observed in either study year, we did not expect an effect of the Bt gene on yield. Grain yield differed among some hybrids, likely due to genotypic factors and growing conditions. Grain yield was greater in 2009 than 2008, but the longer growing season in 2009 did not result in greater stover biomass accumulation during that year. The longer growing season and 270 mm more precipitation contributed to greater grain yield in 2009 than in 2008. Even with the careful selection of least damaged ears in 2008, crow damage reduced grain yield in both Bt and NBt hybrids, so should not have affected the analysis regarding the tested variables. Results from 18 hybrids indicate that there is no yield advantage with Bt corn in sites or years without ECB infestation. However, the Bt hybrids did produce 1 Mg ha^{-1} (data not shown) more grain with no herbivore stress, so more pronounced differences between Bt and NBt hybrids are expected with higher ECB infestation levels. Due to similarity in the stover yield between Bt and NBt hybrids we conclude that there would be no difference in the quantity of corn residue returned to the soil after grain harvest with these hybrids. The results are not surprising because the incorporation of the Bt gene into corn should not have affected its potential grain production. Had there been some ECB infestation injuries in the NBt hybrids, grain yield would have been affected by the damage as a result of the stem tunneling damage, which would disrupt nutrient translocation, and as a direct result of larval feeding on the grain. Similarly, we would expect smaller stover yield from NBt hybrids with some ECB infestation due to the tunneling damage of the feeding larvae. Our findings are consistent with other studies of field-grown Bt and NBt hybrids. Mungai et al. (2005) reported similar stem, leaf, and aboveground biomass from Bt and NBt field-grown hybrids in Missouri, USA, although grain yield was greater in Bt than NBt hybrids in one of two study years, which had greater ECB infestation. In South Dakota and Minnesota, USA, respectively, Lehman et al. (2010) and Jung and Sheaffer (2004) reported no difference in aboveground biomass between Bt and NBt hybrids grown in field sites where ECB damage was observed. Subedi and Ma (2007) found that stalk dry matter and total dry plant weight were similar for Bt and NBt hybrids in Ontario, Canada, which is consistent with our results; however, they reported greater dry matter in kernels and leaves in the Bt hybrids for 2 yr when moderate ECB damage occurred (i.e., stems were infested by corn borers with three or more holes but no stalk lodging was observed).

Fiber content was not affected by the Bt gene and this is consistent with previous studies by Rossi et al. (2003), Mungai et al. (2005), Jung and Sheaffer (2004), Lehman et al. (2008a, 2008b, 2010), and Tarkalson et al. (2008). However our results do not agree with Saxena and Stotzky (2001), who reported up to 97% greater lignin content in Bt corn stem sections, between the third and fourth nodes, than in NBt corn stems. The corn stem sections taken in this study (all nodes in 2008, first to seventh nodes in 2009) differed from those selected in the Saxena and Stotzky (2001) study, but other work suggests that such large differences in lignin content between Bt and NBt corn stems is unusual. Lehman et al. (2010) reported similar lignin contents between Bt and NBt corn stems, based on stem sections (second to third internode) from three Bt hybrids producing the Cry1Ab protein and their near isolines. The ADL method used in this study and by Lehman et al. (2010) was different from the acetyl bromide method used by Saxena and Stotzky (2001) but, as Jung and Sheaffer (2004) reported, those two methods are well correlated and should not give disparate results. It is possible that the fiber analysis method used in this study was not sensitive enough to measure extra lignin deposition in the Bt stems and leaves, however such small depositions would not be expected to have major effects on ecosystems under Bt corn production.

The greater N concentration in the Bt below-ear stems caused a 28% reduction in the C/N ratio compared with the same NBt stem section, which agrees with the findings of Flores et al. (2005) for stems of a Bt11 hybrid. Comparing the below-ear sections (first to seventh node) to whole stems (first to 14th node) in this study revealed that the N concentration was similar in both sections of the NBt stems (5.5 to 5.8 g kg^{-1}), which is also similar to that of the Bt whole stems (5.5 g kg^{-1}), and only the Bt below-ear stems had greater N concentrations (8.09 g kg^{-1}). Based on this result and the observation that the greater N concentration in the Bt stems is not a result of differences in biomass or yield, it could possibly be related to the production of the Cry1Ab protein, which would require greater N uptake and increased protein synthesis in stems, specifically in the older tissue. The increased N concentration in the stems was accompanied by a decrease in N concentration in the roots of the Bt hybrids, suggesting an increased translocation from the roots to the aboveground plant parts. Though not statistically significant, there was numerically greater N concentration in leaves of Bt hybrids, compared with NBt hybrids in 2008 and 2009, which could also possibly be related to the Cry1Ab protein production.

The similarity in mass loss from Bt and NBt stems after 1 yr agrees with the results of Lehman et al. (2010) and was expected since previous studies have indicated this pattern even for residue with different fiber contents (Mungai et al., 2005; Fang et al., 2007). From the data presented by Tarkalson et al. (2008) and Lehman et al. (2010), we estimated that stalk and stem mass loss from litterbags accounted for 58% of the initial weight (after 11 mo) and 70% of the initial weight (after 8 mo), respectively. In contrast, mass loss from litterbags in this study accounted for 56% or less of the initial weight after 12 mo, indicating a lower decomposition rate, which was likely due to cooler weather conditions at our experimental site in eastern Canada. Assuming that stems constitute about 50% of the stover weights presented in Table 3, the annual residue input from corn stems would be about 5 Mg ha^{-1} yr^{-1}; at about 46% C, this constitutes an addition of 2.3 Mg C ha^{-1} yr^{-1} and half of the residue C is expected to remain in the soil after 1 yr in our region of eastern Canada. It should be noted that comparison of the field decomposition rates between Bt and NBt was from one hybrid pair and should be used carefully as a possible outcome but not as a basis for generalization.

Although mass loss results suggest no difference in decomposition of Bt and NBt stems, the C/N ratio of stems recovered after 1 yr indicates that the Bt stems were decomposing more rapidly. Organic residues lose C and concentrate N as they decompose and are incorporated in the soil organic matter, which has a C/N ratio of 10 (Havlin et al., 2005). These results indicate that decomposition of Bt and NBt stems is comparable or even slightly faster for Bt stems due to the smaller C/N ratio, contrary to some reports that Bt residues are tougher and slower to decompose than unmodified corn residues.

We have shown that the Bt gene does not affect the agronomic performance or general chemical composition of corn, which appear to be controlled by genotypic and phenotypic characteristics of the hybrids. However, this study was conducted at a site without ECB infestation and it is expected that herbivory would lead to greater grain yield and biomass production with Bt than NBt hybrids. Corn stems are expected to retain about 50% of their mass after being left in an Ontario or Quebec corn field for 1 yr, which in addition to root input, constitute an annual input of about 1.1 Mg C ha^{-1} yr^{-1} of residue C being returned to the soil. Bolinder et al. (1999) reported an average of 19.6% of corn residue C is retained as soil organic matter from shoots and roots; at this rate 0.2 Mg C ha^{-1}, or 26×10^4 Mg C from corn land (1.3 million ha) in eastern Canada, would be retained in soil organic matter, contributing to the estimated potential of 0.08 Pg C yr^{-1} sequestered from crops due to residue management (Lal and Bruce, 1999). The tendency of the Bt residue to decompose at a faster rate than non-Bt residue after 6 to 12 mo in the field is not clearly explained by the initial chemical composition of the residue and has to be confirmed by longer-term studies using more hybrid pairs and monitoring of the chemical changes throughout the decomposition period.

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