Plant Soil (2011) 341:165–177
DOI 10.1007/s11104-010-0632-7

REGULAR ARTICLE

European corn borer injury effects on lignin, carbon and nitrogen in corn tissues

Sandra F. Yanni · Joann K. Whalen · Bao-Luo Ma · Yves Gelinas

Received: 14 August 2010 / Accepted: 26 October 2010 / Published online: 6 November 2010
© Springer Science+Business Media B.V. 2010

Abstract Plant herbivores often stimulate lignin deposition in injured plant tissue, but it is not known whether corn (*Zea mays* L.) reacts to European corn borer (ECB, *Ostrinia nubilalis* Hubner) injury in this manner. Bt (*Bacillus thuringiensis*) genetic modification is also reported to affect lignin in corn. This study evaluated the effects of ECB injury and the Bt gene on the chemical composition and decomposition of corn tissues. Eight near isolines (Bt and NBt) were grown in pots and half were infested with ECB. The experiment was repeated in 2 years. ECB injury increased the lignin concentration in corn leaves in one of 2 years and lowered the C:N ratio in injured stems. Lignin concentration in leaves was greater in Bt than NBt corn in 1 year and Bt stems had greater N concentration than NBt stems in 1 year of the 2 year study. ECB injury affected the composition of lignin-derived phenols, however ECB infested and non-infested stems lost the same amount of mass after 5 months in buried field litterbags. In conclusion ECB injury and the Bt gene had subtle effects on the chemical composition of corn tissue, which did not alter the short-term decomposition of corn residues.

Keywords Bacillus thuringiensis (Bt) corn · C:N ratio · European corn borer · Lignin · Lignin-derived phenols · Litterbag decomposition

Responsible Editor: Juha Mikola.

S. F. Yanni · J. K. Whalen (✉)
Department of Natural Resource Sciences,
Macdonald Campus, McGill University,
21,111 Lakeshore Road,
Ste-Anne-de-Bellevue, Quebec, Canada H9X 3V9
e-mail: Joann.whalen@mcgill.ca

B.-L. Ma
Agriculture and Agri-Food Canada, Central Experimental Farm, Eastern Cereal and Oilseed Research Centre,
Ottawa, Ontario, Canada K1A 0C6

Y. Gelinas
Department of Chemistry and Biochemistry,
Concordia University,
7141 Sherbrooke St. W.,
Montreal, Quebec, Canada H4B 1R6

Introduction

European corn borer (ECB) (*Ostrinia nubilalis*, Hubner) is a tunneling insect that bores into corn (*Zea mays* L.) stems causing physical damage, disruption of nutrient and water flow (Martin et al. 2004), stalk lodging and grain damage leading to yield loss. An estimated 5.5% yield loss from first generation larvae and 2.8% yield loss from second generation larvae occurs when plants are infested with one larva per stalk (Bode et al. 1990), and greater yield losses are expected with higher ECB infestation levels. Traditional ECB control measures start with planting resistant hybrids such as those that produce the defensive toxin DIMBOA (2,4-dihydroxy-7-methoxy-1, 4-benzoxazin-3-one) (Ostrander and Coors 1997) and hybrids with increased stalk strength (Martin et al. 2004), field scouting early in

the season and applying insecticidal sprays such as formulations of *B. thuringiensis* and permethrin, which could provide 80% and 67% control of first generation and second generation ECB, respectively (Clark et al. 2000). Transgenic Bt corn was introduced commercially in 1996 and has gradually replaced the traditional control measures because of its high efficacy in controlling first and second generation larvae, leading to greater yield in years when ECB infestation levels exceed economic thresholds (Dillehay et al. 2004).

Wounding by insects is a stress factor to which plants respond by several direct (e.g. production of primary and secondary metabolites that affect the herbivores) and indirect (e.g. attracting predators of herbivores) defensive measures (Kessler and Baldwin 2002). One of the first responses to wounding is the production of enzymes for phenylpropanoid metabolism (Douglas 1996; Hahlbrock and Scheel, 1989). Among the end products of this pathway is lignin, which serves to reinforce the cell wall (Baron and Zambryski 1995; Dixon and Paiva 1995; Ecker and Davis 1987). Stress-induced lignin is deposited in the secondary cell wall following insect injury in plants such as tobacco (Lagrimini 1991), woody angiosperms (Hawkins and Boudet 1996), and *Arabidopsis* (Cheong et al. 2002; Delessert et al. 2004; Howe and Schaller 2008). Induced lignin deposition as a response to pathogen fungal attack is also well documented for many plant taxa (Nicholson and Hammerschmidt 1992; Stange et al. 2001; Vance et al. 1980; Walter et al. 1990; Zhang et al. 2007). Fungal invasion often begins after tissues are wounded by herbivorous insects and can lead to increased deposition of lignin or lignin-like compounds. For instance, Bergstrom and Nicholson (1999) reported that anthracnose infections, caused by *Colletotrichum graminicola*, find easy access through the ECB injury corn sites and ECB larva also act as vectors for the fungus and Lyons et al. (1993) reported that lignin formation was the final of three steps involving phenylpropanoid synthesis following corn infection with *Helminthosporium maydis*. Pascholati et al. (2008) reported increased levels of phenylalanine ammonia-lyase (PAL), the first enzyme in phenylpropanoid biosynthesis pathway, in corn mesocotyls that were wounded by rubbing with Al_2O_3 but did not assess lignin production. Tiwari et al. (2009) reported no effect of ECB infestation level, with up to six larvae per plant, on acid detergent fiber of whole corn plants at the half kernel milkline stage. Therefore, injury can indirectly lead to lignin deposition through fungal infections but whether herbivore injury acts as a direct stimulus to stress-induced lignin or lignin-like deposition in corn tissues is not yet well known.

Lignin is a structural and defensive plant compound of consequence for the global carbon cycle. At present, the global CO_2 flux from heterotrophic respiration is estimated at 55 Pg C y^{-1} (Reay and Pidwirny 2010) compared to an estimated net primary production of 60 Pg C y^{-1} (Janzen 2005). The soil C input and output are therefore in equilibrium as long as the litter quantity and quality, the main biotic factors that control litter decomposition, remain steady. A change in litter quality, such as higher lignin content, would slow litter decomposition, leading to longer residence time and the eventual conversion of plant C compounds into stable soil organic C. This is expected because lignin is the most resistant plant component to attack by microbes due to its complex molecular composition and the fact that it is bound to other compounds, such as hemicellulose, in plant cell walls (Campbell and Sederoff 1996; Hammel 1997; Hopkins et al. 2001; Jeffries 1994; Rasse et al. 2006). While insect wounding could potentially increase the lignin content of corn tissues, it has been suggested that genetic modification also affects corn lignin content. In some laboratory and field studies, Bt corn had greater lignin content than non-Bt (NBt) corn (Poerschmann et al. 2005; Saxena and Stotzky 2001) and decomposed more slowly when residues were mixed with soil (Castaldini et al. 2005; Dinel et al. 2003; Flores et al. 2005).

Another litter quality attribute that affects decomposition is the C:N ratio (Cadisch and Giller 1997). Decomposition rate constants are usually negatively correlated with initial lignin content, lignin:N ratio and the C:N ratio (Cadisch and Giller 1997; Fogel and Cromack 1977; Johnson et al. 2007; Melillo et al. 1982; Vanlauwe et al. 1997). Damage to leaves and vascular tissue by herbivory affects translocation of photosynthates and nutrients within the plant (Martin et al. 2004; Mason et al. 1996) and consequently the C:N ratio of plant organs. Upon herbivore attack, plants may store sugars and photoassimilates in stems or roots or may increase nutrient uptake and rate of photosynthesis as a means of coping with injury (Howe and Schaller 2008; Kessler and Baldwin 2002), all of which can affect the C:N ratio of plant tissues.

The litterbag method is a common way to study decomposition of plant material in the field. Lehman et al. (2008), Tarkalson et al. (2008) and Zwahlen et al. (2007) reported no differences in decomposition rates of litter from Bt and NBt corn hybrids using this technique. Lehman et al. (2010) also reported no differences in decomposition between ECB-injured corn stalks and cry1Ab-protected corn stalks after about 1 year in the field. Another way of assessing decomposition is through the lignin degradation parameters. The acid to aldehyde ratios of vanillyls (Ad/Al_v) and syringyls (Ad/Al_s) are often used to assess the degree of lignin decomposition (Goñi and Montgomery 2000; Hedges et al. 1988; Hedges and Ertel 1982; Loh et al. 2008; Otto and Simpson 2006; Poerschmann et al. 2005; Poerschmann et al. 2008). Poerschmann et al. (2005), using thermochemolysis and CuO oxidation techniques, reported that Bt corn stems were more susceptible to degradation than NBt stems. No comparison of lignin degradation between ECB injured and non-injured corn has been found in the literature.

The objectives of this study were 1) to determine if infestation with ECB affects the lignin, carbon and nitrogen in stems and leaves of NBt corn hybrids, as a result of injury, and 2) to compare the decomposition rate of stems that were injured by ECB to those that were not injured. Since the plants were not grown aseptically, there was a possibility of fungal infection through the injury site however, no distinction was made between direct ECB infestation effects and indirect fungal infection effects, so the measured lignin deposition response represented the combined direct and indirect effects. A secondary objective was to compare Bt and NBt corn stems and leaves in terms of lignin, carbon and nitrogen content. The ECB (*O. nubilalis*) selected for this study enters and creates tunnels within corn stems, potentially affecting the chemical composition of stems. As stem injury could affect metabolic processes and nutrient transport within the plant, we also considered the effect of ECB injury on the chemical composition of leaf tissue.

Materials and methods

Greenhouse experiment

A pot experiment was carried out over two growing seasons in 2008 and 2009 to evaluate the effect of ECB injury and genetic modification on lignin, carbon and nitrogen content of corn tissues (stems and leaves). This involved a factorial experiment with two levels of ECB injury (ECB and No-ECB) and two genetic modifications (Bt and NBt). Corn hybrids selected for this study included four Bt hybrids (MZ3888, MZ5444, N45-A6, N33D2-MF2) and their non-Bt (NBt) near-isolines (MZ310, MZ540, N45A-LL, N33H6-MF1). A completely randomized design was used to select and prepare four replicate pots for each hybrid by ECB treatment, for a total of 64 pots.

Pots were placed on a greenhouse sundeck that was protected from the sides and open at the top, so they received the same amount of sunlight and precipitation as field-grown corn. Six kilograms of soil were added to each pot with the addition of 900 ml of perlite in 2008; perlite was not used in 2009 since the soil was naturally well-drained, even when coarsely sieved and hand-packed into pots for the greenhouse study. The soil was a Chicot sandy loam with 661 g kg^{-1} sand, 159 gkg^{-1} clay, 14.1 g organic Ckg^{-1}, 1.6 g N kg^{-1}, and pH of 6.0, collected from the Emile A. Lods Agronomy Research Centre of McGill University in Ste-Anne-de-Bellevue, Quebec, Canada (45°24'N, 73°56'W). Urea/KCl (29.2-0-22.3) and monocalcium phosphate (0-46-0) fertilizers were added at a rate of 1.6 g and 0.3 g per pot, respectively, prior to seeding. Five corn seeds were planted in each pot and thinned to one plant per pot at the 2–3 leaf stage. Hoagland solution was added every 2–3 weeks, starting 2 months after seeding, and provided a total of 460 mgN per pot, 117 mgK per pot, and 61 mg P per pot over the growing period.

Pots designated for the ECB treatment were manually infested with ECB eggs, which were purchased from French Agricultural Research Inc. (Lamberton, MN, USA) and incubated at 25°C until they reached the 'blackhead' stage, at which time they were placed on the plants. Two infestation events were performed—at the first infestation, three egg masses (each mass consisting of about 50 eggs) were placed in the leaf whorl. In the second infestation, two egg masses were placed in the leaf axil of the 3rd leaf from the top plus two egg masses in the leaf axil of a leaf close to the ear. In 2008, eggs of the first infestation did not hatch due to an intense rainfall event (15.8 mm) that occurred one day after placement of the egg masses. Except for the first infestation in 2008, all pots were transferred inside

the greenhouse for one week at the time of infestation. In 2009, the plants were seeded inside the greenhouse and kept for 3 weeks.

Corn was harvested after 128 days of emergence in 2008 and 126 days in 2009. Because the plants were initially kept in the greenhouse in 2009, they all accumulated more crop heat units and reached physiological maturity (black layer) whereas in 2008 the late-maturing hybrid pair MZ5444/MZ540 were at the mid-dent R5 growth stage when harvested. At harvest, the plants were separated into leaves, stems (without the tassel), and roots. The roots were carefully washed to remove adhering soil. The number of ECB holes and tunnel lengths in the stems were counted and measured. In 2009, only the stem section between nodes one (above soil level) and nine was collected; this choice was made because the top portion of the stems is composed of relatively new (not highly lignified) tissue. This permits an unbiased comparison of chemical composition in the whole stem (year 2008) and the older lower stem (year 2009). Plant tissues were dried at 50°C for 48 h and ground using a Wiley mill to pass through a 1 mm mesh sieve prior to analysis.

To estimate lignin content, Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), and Acid Detergent Lignin (ADL) followed by ashing was measured according to the Goering and Van Soest (1970) gravimetric method using an Ankom Fiber Analyzer (Ankom Technology, Fairport, NY). Carbon and nitrogen contents were measured with a CN Flash EA Analyzer (Carlo-Erba, Milan, Italy).

Decomposition of ECB injured stems under field conditions

Litterbags were prepared and placed in a long-term corn agroecosystem to compare the decomposition rates of stems from the ECB and no-ECB treatments. The experimental design was a completely randomized design with corn stems selected at random from the following treatments of the greenhouse experiment: ECB and no-ECB infestation of two Bt hybrids (MZ3888, MZ5444) and two NBt hybrids (MZ310, and MZ540) during the 2008 season. For each factorial treatment (2 ECB treatments × 4 corn hybrids), five replicate stem sections prepared for destructive sampling at five sampling dates, making a total of 200 litterbags. Stems were cut into sections, 5 cm long and with variable diameter, depending on whether they had come from the bottom (older) or the top (younger) part of the stem. For this reason, the stems were categorized according to a range of diameters as follows: thin =3–5 mm, medium =6–9 mm, and thick =10–13 mm. Weighed stem pieces were placed in mesh nylon/polyester bags, 10 cm × 10 cm with a 1 mm^2 mesh size, which were constructed based on a modified design of The Canadian Intersite Decomposition Experiments (Trofymow and CIDET Working Group 1998). The bags were buried at 5 cm depth in a field located at the Macdonald Campus Farm in Ste Anne de Bellevue, Quebec, Canada (45°25'N, 73°56'W) where corn has been grown under no-till cultivation for more than 15 years. The corn hybrid grown in 2009 in the field was Mycogen (hybrid 2J463) grain corn and the bags were distributed between the corn rows, over 5 rows with about 1 m spacing between bags. Litterbags were buried in May 2009, five litterbags were collected for measurement every month for 5 months, and the last sampling date was in October 2009.

Litterbags were cleaned with distilled water within hours of sampling and dried at 50°C for 48 h. For the first 3 months, the collected stems were still intact and easy to clean whereas stems from the last two sampling dates were more degraded and had to be cleaned over a 0.5 mm mesh sieve to insure that all the pieces were retrieved. Mass loss was calculated as the difference in dry weight between time 0 and each sampling date. There were no adhering soil particles left on the stems so ashing to correct the weight for non-organic material was not required.

Lignin molecular characterization

Lignin molecular characterization by the alkaline CuO oxidation method was used to access the state of decomposition of selected stems. Analysis was conducted on un-decomposed stems (time 0) and decomposed stems collected after 5 months in the field from the MZ540 hybrid treatments, NBt with no ECB infestation, (n=2 replicates) and NBt infested with ECB (n=2 replicates). Stems were ground with an Udy cyclone mill fitted with a 1 mm mesh sieve. The method, which is adapted from Goñi and Montgomery (2000), is briefly described: ground plant tissue containing about 1.5–2 mg organic

carbon was weighed into Teflon vessels with 250 mg cupric oxide, 25 mg ferrous ammonium sulfate and 7.5 ml of 2 N NaOH, and purged with N_2 before capping and loading into a Speedwave MWS-2 microwave (Berghof/America, Florida, USA) for 150°C for 1.5 h to allow the oxidation reaction to occur. The supernatant was then transferred to clean tubes, 0.1 ml (580 $\mu g ml^{-1}$ concentration) of an internal standard (ethyl vanillin) was added and the mixture was centrifuged to collect the clear supernatant. The precipitate was re-dissolved in 1 N NaOH, centrifuged again and the supernatant added to the first solution. The collected supernatant was acidified with HCl and the organic phase separated from the aqueous phase by vigorous shaking with ethyl acetate. Because the presence of water affects derivatization, the organic layer was subjected to a series of drying and re-dissolving in ethyl acetate to ensure that the solution was water-free. After the final drying step, the residue was re-dissolved in 250 μl pyridine and 250 μl BSTFA and derivatized by heating at 80°C for 1 h. The samples were analyzed on an Agilent 6890 N gas chromatograph fitted with a Gerstel temperature-programmable injector and a flame ionization detector (GC-FID) to quantify the lignin-derived phenols. The stationary phase of the column (30 m× 0.25 mm×0.25 μm thickness) was made of 5% phenyl 95% dimethylpolysiloxane and helium was used as the carrier gas at a constant flow rate of 1.5 $ml min^{-1}$. The initial oven temperature was set at 100°C and maintained for 10 min., followed by a ramp of $20°C min^{-1}$ to a final temperature of 320°C that was held for 10 min. A series of standard solutions were prepared from eight compounds that are of interest and analyzed with a gas chromatograph-mass spectrometer (GC-MS) having the same column as the GC-FID for identification of the phenol peaks. A blank and a series of standards for each of the identified phenols were analyzed with the samples to develop calibration curves for concentration calculations.

Statistical analysis

The effect of hybrid, genetic modification (Bt, NBt), ECB injury and the interaction of Bt and ECB on lignin content, N concentration and C:N ratio in stem and leaf tissue was evaluated by analysis of variance using the GLM procedures of SAS software (SAS Institute Inc. 2009) after ensuring that the residuals complied with the presumption of normality. Log transformation was used in some cases to normalize the data. Orthogonal contrast analysis was performed to test the effect of ECB infestation by comparison of infested versus non-infested corn tissue, to test the effect of ECB injury by comparing injured versus non-injured corn tissue, and to test the effect of the Bt gene through comparison of non-infested Bt and NBt corn tissue. Least square means with the Tukey adjustment for multiple comparisons were then calculated and reported for significance at the 95% confidence level. The GLM procedure (SAS Institute Inc. 2009) was also used to test the effects of Bt and ECB on decomposition rate (weight loss of stems) by date using stem thickness as a co-variable. Monthly decomposition rate constants were calculated by fitting the data into the single exponential model (Jenny et al. 2006; Olson 1963; Wider and Lang 1982) using the NLIN procedure on SAS software (SAS Institute Inc. 2009). No statistical analysis was conducted on the CuO oxidation results, which was performed without replication.

Results

Effect of ECB injury and the Bt gene on chemical composition of corn

Plants with ECB infestation were those treated with ECB eggs, whereas ECB injured plants were those that showed damage due to ECB feeding. In the following tables, we compare the responses of corn plants that were (1) infested with ECB and (2) injured by ECB using pre-planned orthogonal contrasts. There were no noticeable fungal infections on the injury sites or inside the ECB tunnels when the stems were cut so fungal effects on lignin deposition were assumed to be minimal.

Variation in biomass accumulation of pot-grown corn plants was mainly related to hybrid type (Table 1). As expected, insect injury occurred only in the NBt plants that were infested with ECB. Although the first ECB infestation event in 2008 was not successful, the overall ECB stem injury due to one successful ECB infestation in 2008 was more severe than two ECB infestations in 2009, leading to less biomass accumulation in NBt injured than NBt non-injured stems in 2008 (P=0.0374) (Table 1). When compared to the general population, the ECB

Table 1 Biomass (g plant^{-1}) of corn leaves and stems from 8 hybrids as affected by ECB injury and the Bt gene, and mean tunnel length (cm) in ECB-infested stems in 2008 and 2009. The hybrids were paired near-isolines that were not genetically modified (NBt) or contained the Bt gene. Values are the mean± standard error (n=4)

Hybrid (isoline)	Leaves		Stems			
	2008 biomass	2009 biomass	2008 biomass	2009 biomass	2008 Average tunnel length (cm)	2009 Average tunnel length (cm)
ECB infestation						
MZ 310 (NBt)	13.8±1.1	30.3±1.8	11.6±1.4	28.4±4.2	12.6	9.5
MZ 3888 (Bt)	17.0±2.0	28.3±1.1	12.7±0.6	21.0±1.2	0	0
MZ 540 (NBt)	23.1±1.9	40.3±0.5	20.6±3.5	41.9±5.3	10.3	6.5
MZ 5444 (Bt)	20.4±0.6	36.1±1.2	17.9±1.7	30.1±3.0	0	0
N45ALL (NBt)	17.5±1.4	33.9±1.0	16.0±0.2	38.8±4.4	13.9	6.5
N45A6 (Bt)	17.6±1.4	33.3±0.7	15.9±1.7	28.5±1.1	0	0
N33H6MF1 (NBt)	24.1±2.2	39.6±0.9	22.4±2.3	51.5±13.4	30.5	3
N33D2MF2 (Bt)	21.4±0.5	34.5±0.9	24.4±1.6	34.2±5.1	0	0
No ECB infestation						
MZ 310 (NBt)	16.7±0.3	27.7±0.7	15.1±0.9	19.4±0.7	0	0
MZ 3888 (Bt)	17.9±0.8	25.9±1.4	15.7±1.6	20.7±4.2	0	0
MZ 540 (NBt)	21.0±0.8	38.8±2.3	21.1±1.4	57.8±15.0	0	0
MZ 5444 (Bt)	23.4±1.0	33.9±1.2	22.6±1.1	33.3±5.6	0	0
N45ALL (NBt)	19.5±0.6	33.3±1.3	21.1±1.4	47.8±1.7	0	0
N45A6 (Bt)	21.5±1.0	32.0±0.8	20.2±1.0	25.7±1.0	0	0
N33H6MF1 (NBt)	21.8±2.9	39.6±1.3	19.5±0.3	38.7±7.4	0	0
N33D2MF2 (Bt)	22.1±1.6	36.2±1.1	19.1±1.0	36.2±6.5	0	0
Treatment effects (Probability level)[3]						
Hybrid	P<0.0001	P<0.0001	P<0.0001	P<0.0001	NS	NS
ECB injury	NS	NS	P=0.0374	NS	P=0.0002	P=0.0010
Bt gene	NS	P<0.0001	NS	P<0.0001	P=0.0002	P=0.0010
ECB x Bt	NS	NS	NS	NS	NS	NS
Contrast analysis (Probability level)						
ECB injury (injured vs. uninjured plants)[1]	NS	NS	NS	P=0.0475	P<0.0001	P<0.0001
Bt gene (uninjured plants)[2]	NS	NS	NS	NS	n.d.	n.d.

[1] The effect of ECB injury on biomass and tunnel length was compared for NBt corn (n=16) plants infested with ECB egg masses and all other corn plants (n=48)

[2] The effect of Bt gene on biomass was determined for non-infested NBt corn (n=16) and non-infested Bt corn (n=16) plants

[3] *NS* not significant (P>0.05), *n.d.* not determined

injured plants had similar (2008) or greater biomass accumulation in stems (2009) than uninjured plants, as revealed by contrast analysis (Table 1). Genetic modification had no effect on leaves or stems in 2008 but the Bt gene negatively affected (P<0.0001) leaf and stem biomass in 2009. This effect is attributed to the greater biomass accumulation in NBt plants that were susceptible to ECB injury, as orthogonal contrast analysis showed no difference in leaf and stem biomass between non-infested Bt and NBt plants during the study.

The goal of this study was to determine how ECB injury and the Bt gene would affect the chemical composition of corn tissues, so we pooled data from the eight corn hybrids within each ECB treatment, which represent a sub-population of corn hybrids grown

in southwestern Quebec, Canada. As expected, some of the variation in lignin, nitrogen and C:N ratio was attributable to hybrids, as indicated in Tables 2 and 3.

Leaf lignin content was greater in ECB-injured NBt plants in 2008 (P=0.0074) but not in 2009 (Table 2). Leaf lignin content was affected by the Bt gene differently in the two seasons. In 2008, NBt leaves had more lignin than Bt leaves (P=0.0012) however there was no effect of the Bt gene on lignin content in non-infested plants as indicated by contrast analysis (Table 2). In 2009, the Bt leaves had more lignin than the NBt leaves and this was confirmed by the contrast analysis (P<0.0001). In 2009, there was a strong interaction between Bt and ECB (P<0.0001); the greatest lignin content (37.8 gkg^{-1}) was in the non-infested Bt leaves and the smallest lignin content (28.7 gkg^{-1}) was in the non-infested NBt leaves. There was no effect of ECB infestation or ECB injury on lignin content in the stems in both years. Table 2 also shows that lignin content in the stems was not affected by the Bt gene in both years. It was expected that the lignin concentration in the 2009 stems (older section below the ear) would be greater than that of the whole stem analyzed in 2008 however, this was not the case; the effect of ECB and Bt on lignin concentration was not affected by the age of the selected stem sections.

The nitrogen concentration and C:N ratio in leaves (2009) and stems (2008, 2009) were significantly affected by ECB injury whereas the Bt gene had some significant (P<0.05) effects on the nitrogen concentration and C:N ratio in corn tissue during this study (Table 3). No ECB effect was observed on leaf N content and C:N ratio in 2008 but contrast analysis showed that leaves of injured plants (n=16) had less N and greater C:N ratio compared to non-injured plants (n=48), which was not the case in 2009 (Table 3). There was a Bt × ECB interaction effect on N content of leaves, which was inconsistent over both years. There was consistently greater N concentration in the ECB injured stems compared to the non-injured stems, which resulted in a significantly smaller C:N ratio in these treatments (P<0.01, Table 3).

Table 2 Lignin content (gkg^{-1}) of corn leaves and stems as affected by ECB injury and the Bt gene in 2008 and 2009. Data were pooled among four Bt and NBt near-isolines (hybrids). Values are the mean±standard error (n=16)

	Leaves 2008	Leaves 2009	Stems 2008	Stems 2009
Lignin gkg^{-1}				
NBt ECB	39.3±1.8	$33.2\pm0.8_a$*	67.7±3.0	56.7±3.0
Bt ECB	33.6±1.6	$33.5\pm0.8_a$	63.2±2.5	58.6±2.5
NBt no-ECB	34.4±1.5	$28.7\pm0.8_b$	72.2±3.1	55.3±3.2
Bt no-ECB	31.6±0.9	$37.8\pm1.3_c$	72.4±2.5	63.2±2.7
Treatment effects (Probability level)[4]				
Hybrid	P<0.0001	NS	NS	P=0.0251
ECB injury	P=0.0074	NS	NS	NS
Bt gene	P=0.0012	P<0.0001	NS	NS
ECB x Bt	NS	P<0.0001	NS	NS
Contrast Analysis (Probability level)				
ECB injury (infested plants)[1]	P=0.0239	NS	NS	NS
ECB injury (injured vs uninjured plants)[2]	P=0.0007	NS	NS	NS
NBt vs. Bt (non-infested plants)[3]	NS	P<0.0001	NS	NS

* Values followed by different subscripts are statistically different at α=5%

[1] The effect of ECB injury on plant tissue chemistry was determined for NBt corn (n=16) and Bt corn (n=16) that was infested with ECB egg masses

[2] The effect of ECB injury on plant tissue chemistry was compared for NBt corn (n=16) infested with ECB egg masses and all other corn plants (n=48)

[3] The effect of the Bt gene on plant tissue chemistry was compared for NBt corn (n=16) and Bt corn (n=16) plants that were not infested with ECB

[4] *NS* not significant (P>0.05)

Table 3 Total nitrogen content (gkg^{-1}) and C:N ratio of corn leaves and stems as affected by ECB injury and the Bt gene in 2008 and 2009. Data were pooled among four Bt and NBt near-isolines (hybrids). Values are the mean±standard error (n=16)

	Leaves 2008		Leaves 2009		Stems 2008		Stems 2009	
	N	C:N	N	C:N	N	C:N	N	C:N
NBt ECB	$6.70\pm0.66_{a}$	$79.0\pm9.2_{a}$*	$9.28\pm0.65_{a}$	$53.8\pm4.3_{a}$	4.00±0.23	119±6.0	7.52±0.29	$63.0\pm3.0_{a}$
Bt ECB	$9.48\pm0.43_{b}$	$47.2\pm1.9_{b}$	$6.56\pm0.34_{b}$	$72.7\pm3.4_{b}$	3.61±0.14	129±5.2	7.88±0.25	$59.0\pm2.0_{a}$
NBt no-ECB	$7.89\pm0.69_{ab}$	$65.1\pm5.4_{ab}$	$8.72\pm0.41_{a}$	$54.3\pm3.4_{a}$	2.83±0.20	171±8.3	4.62±0.39	$107\pm8.3_{c}$
Bt no-ECB	$7.27\pm0.44_{ab}$	$65.6\pm3.8_{a}$	$9.74\pm0.27_{a}$	$47.9\pm1.2_{a}$	2.84±0.10	163±5.7	5.78±0.33	$85.0\pm5.5_{b}$
Treatment effects (Probability level)								
Hybrid	P=0.0461	NS	NS	NS	P=0.0165	P=0.0077	P=0.0088	P=0.0003
ECB injury	NS	NS	P=0.0046	P=0.0015	P<0.0001	P<0.0001	P<0.0001	P<0.0001
Bt gene	NS	P=0.0061	NS	NS	NS	NS	P=0.0125	P=0.0070
ECB x Bt	P=0.0028	P=0.0046	P<0.0001	P<0.0001	NS	NS	NS	P=0.0486
Contrast Analysis (Probability level)[4]								
ECB injury (infested plants)[1]	P=0.0014	P=0.0019	P=0.0011	P=0.0019	NS	NS	NS	NS
ECB injury (injured vs uninjured plants)[2]	P=0.0316	P=0.0055	NS	NS	P=0.0001	P=0.0001	P=0.0058	P=0.0104
NBt vs. Bt (non-infested plants)[3]	NS	NS	P=0.0417	NS	NS	NS	P=0.0294	P=0.0334

* Values followed by different subscripts are statistically different at α=5%

[1] The effect of ECB injury on plant tissue chemistry was determined for NBt corn (n=16) and Bt corn (n=16) that was infested with ECB egg masses

[2] The effect of ECB injury on plant tissue chemistry was compared for NBt corn (n=16) infested with ECB egg masses and all other corn plants (n=48)

[3] The effect of the Bt gene on plant tissue chemistry was compared for NBt corn (n=16) and Bt corn (n=16) plants that were not infested with ECB

[4] *NS* not significant (P>0.05)

The Bt gene had an inconsistent and often marginal effect on the nitrogen concentration and C:N ratio in corn leaves, whereas Bt stems had a significantly (P= 0.0125) greater N concentration and smaller C:N ratio (P=0.0070) than NBt stems in 2009 (Table 3), which was also true for the subpopulation of non-infested Bt and NBt plants (contrast analysis P<0.05, Table 3).

Decomposition of ECB-infested stems in the field

By the end of the study, the mass loss was 60% for Bt stems, 62% for ECB-infested NBt stems and 55% for non-infested NBt stems, indicating no significant difference in mass loss (Fig. 1) or decomposition rates between the treatments.

The lignin-derived phenols of interest are vanillyls (vanillin, acetovanillone, vanillic acid), syringyls (syringaldehyde, acetosyringone, syringic acid), and cinnamyls (ferulic acid and *p*-coumaric acid). The CuO oxidation results for NBt stems (Table 4) showed that the non-decomposed (time =0) ECB infested stems had less lignin-derived phenols and comparable amounts of vanillic acid and acetovanillone than non-infested stems. On average, the total amount of lignin increased over time within each treatment. The acid to aldehyde ratios of vanillyls (Ad/Al_v) and syringyls (Ad/Al_s) are used as indicators of the degree of degradation of lignin because these compounds are transformed from the aldehyde form to the acid form as they biodegrade (Hedges et al. 1988). In this case, the similar Ad/Al ratios indicate that lignin decomposition during the 5-month period was not extensive enough to induce changes in the Ad/Al ratios. Comparing the profile of the ECB infested versus non-infested stems at 5 months shows that infested stems have more syringic acid and ferulic acid than the non-infested stems, which resulted in an increase in the Ad/Al ratio of the syringyl phenols in those treatments (Table 4).

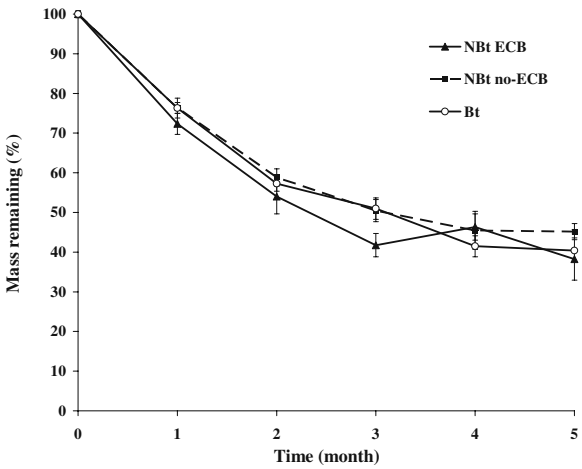

Fig. 1 Mass remaining (%) in ECB injured and non-injured stems from corn hybrids (non-Bt lines, MZ310 and MZ540; Bt lines, MZ3888, and MZ5444) after 5 months of decomposition in field litterbags

Discussion

Effect of ECB injury on chemical composition and decomposition of corn tissue

Injured stems had extensive damage and visible tunnels from ECB feeding, but did not have an elevated lignin content, however leaves from injured plants in 2008 had more lignin than uninjured plants. The reason for this is not clear; it could be that subtle changes in leaf lignin content can be more easily detected with the acid-insoluble fiber method because leaves have originally less lignin (3.4%) than stems (6.4%). Corn leaves are the first part of the plant to get attacked by ECB, as the newly hatched larvae feed on leaves for 2–3 days before moving into the stems (Hyde et al. 1999) and it could be that the specific defense chemicals are produced at the first wounded site, in leaves, rather than in stems. As ECB causes extensive injury in the stems, this could affect translocation of substrates that are involved in the phenylpropanoid pathway or the lignin biosynthesis pathway. Analysis of un-decomposed ECB infested NBt stems revealed more vanillin, syringaldehyde, acetosyringone, syringic acid, ferulic acid and *p*-coumaric acid than in non-infested stems. Thus, the injured stems had a lower concentration of lignin-derived phenols, which indicates that the hypothesis of more lignin deposition as a result of ECB injury is not supported by the results. Our findings appeared to suggest that ECB injury may stimulate lignin deposition in leaves, but not in the stem tissues, which sustain major damage from this herbivore. Further in-depth studies at the enzyme and gene expression level in leaf and stem tissues are needed to test this hypothesis.

Tunnel lengths as a result of stem ECB feeding reached up to a total of 30 cm with individual

Table 4 Amounts (μg compound g^{-1} tissue) of lignin-derived phenols in infested and non-infested MZ540 hybrid stems at time zero and 5 months after decomposition in the field. Values are the means±standard error (n=2)

Lignin-derived compound	Non-infested NBt time=0	Infested NBt time=0	Non-infested NBt time=5 months	Infested NBt time= 5 months
Vanillin	26.9±0.7	15.1±0.8	30.4±2.5	25.5±0.8
Acetovanillone	7.91±0.4	10.4±0.5	9.33±1.0	8.01±2.0
Syringaldehyde	30.0±0.2	11.9±1.2	36.3±3.2	33.5±3.7
Vanillic acid	9.17±0.9	9.00±0.3	9.57±0.7	8.38±3.2
Acetosyringone	23.4±0.3	11.1±1.1	25.8±1.5	26.5±1.4
Syringic acid	3.61±0.1	0.27±0.0	1.36±0.3	10.1±3.5
p-coumaric acid	30.7±3.3	5.64±0.0	26.6±0.9	28.8±10.2
Ferullic acid	3.58±0.6	0.14±0.0	1.14±0.2	6.32±4.6
Ad/Al_S[a]	0.12	0.02	0.04	0.30
Ad/Al_V[a]	0.34	0.59	0.31	0.33

[a] Ad/Al_S=syringic acid/syringaldehyde and Ad/Al_V=vanillic acid/vanillin

continuous tunnels of up to 15 cm. Such damage is bound to have an effect on translocation of water and mobile nutrients between plant parts. The smaller C:N ratio in injured stems could be explained by the disruption of nutrient translocation; if this interpretation is correct, N accumulated in the stems and was not transported to the leaves and grain, contributing to a lower C:N ratio in injured stems than uninjured stems. This is supported by the observation of lower N concentration in leaves of ECB injured NBt plants in 2008, but not in 2009. Although the injured NBt plants accumulated less grain biomass in both 2008 and 2009 (data not shown) compared to the uninjured plants, N concentration in the grain was not affected. Therefore, our results do not provide support for the hypothesis that disruption in nutrient translocation led to N accumulation in the stem of ECB injured plants. It is more likely that herbivory stimulated nutrient uptake to support secondary metabolite production and the formation of defense proteins (Howe and Schaller 2008; Nykanen and Koricheva 2004), which resulted in the elevated N concentration in stems. Both greater and smaller N concentrations in injured tissues compared to non-injured tissue have been reported in the literature (Nykanen and Koricheva 2004) and attributed to processes such as altered nutrient translocation within the plant and synthesis of N-rich proteins and enzymes.

One objective of this study was to access the effect of ECB injury on decomposition of corn residue under field conditions. The ECB infested MZ540 hybrid stems had lower C:N (130.5) and lignin:N (16.4) ratios than the non-injured (C:N =196 and lignin:N=33.8). Regular sampling during a 5-month period (May to October) demonstrated that ECB injury did not affect the decomposition rate of corn stems, which is in agreement with the conclusions of Lehman et al. (2010). The decomposition of corn stems from all treatments was characterized by rapid loss of mass during the first 2–3 months of the study, probably due to decomposition of hemicellulose and cellulose. The Ad/Al ratio of decayed stems collected after 5 months indicates that lignin was not degraded and was likely controlling the decomposition rate of stems during the later part of the decomposition experiment (3–5 months after burying the litterbags). The CuO oxidation results also indicate that the relative contribution of lignin phenols to the total fiber mass increased over time; as cellulose and hemicellulose are degraded by brown-rot fungi (Hedges et al. 1988), lignin becomes the dominant compound remaining in corn residues.

Although mass loss was similar in injured and uninjured stems after 5 months, molecular analysis of the chemical forms of lignin suggest that ECB injury may affect decomposition in the longer-term. Syringic acid was 87% greater in the infested than non-infested stems, suggesting that the former are more susceptible to degradation. The Ad/Al_s ratio of the injured stems at 5 months was 0.30 compared to a ratio of 0.04 in the non-injured stems. In general, Ad/Al ratios less than 0.5 indicate that lignin has not been significantly

altered by microbial degradation (Loh et al. 2008). Based on this criterion, lignin decomposition was not very extensive even after 5 months; however, the high syringic acid concentration suggests that lignin polymers in infested stems are more susceptible to degradation and therefore would be more rapidly decomposed than non-infested stems once lignin degradation begins. Analysis of more replicates, from more points in time, would give a clearer view of molecular-level changes during decomposition.

Effect of the Bt gene on chemical composition and decomposition of corn tissue

In 2009, the Bt leaves accumulated 32% more lignin than NBt leaves, and in the same year the Bt stems also exhibited the same trend, though not statistically different, with 14% more lignin than NBt stems. Although Jung and Sheaffer (2004) argued that there is no reason why the Bt gene would induce more lignin production in corn as the insertion of this gene does not affect the biosynthetic pathway of lignin production, several authors have reported that genetic modification affects the chemical composition of corn tissues. Saxena and Stotzky (2001) reported 33–97% higher lignin content in the stems of 10 Bt hybrids grown in a growth chamber and 8 field-grown Bt hybrids than their non-Bt isolines. Poerschmann et al. (2005) also reported 4–6% more lignin in Bt leaf tissue compared to the NBt isolines, and Bt stems had 18–28% more lignin than NBt stems. We cannot confirm that the Bt gene affects lignin content in corn leaves because the results were not consistent between the study years. There could be an interaction between the physiological maturity level of the plants and lignin content; lignin formation and deposition is completed as the plant matures, which could be why corn harvested at physiological maturity in 2009 had greater lignin content than corn harvested before reaching this developmental stage in 2008.

The Bt gene increased the N concentration of both leaves and stems in 2009 though this was not true in 2008, possibly due to the harvest date as indicated above. In non-injured plants, Bt leaves had 12% more N than NBt isolines and Bt stems had 25% more N than NBt stems. Escher et al. (2000) reported a non-significant difference in N content between Bt and NBt corn leaves of about 9%, which is in agreement with our results. The reason for this difference is not completely clear but one possible explanation could be that the Cry1Ab protein produced in Bt plants contributes to the N concentration in corn tissues. The Bt hybrids used in this study were produced by the Bt11 and MON810 transformation events and those are reported to have 3.3 $\mu g g^{-1}$ to 10.3 $\mu g g^{-1}$ Cry1Ab protein in the fresh weight leaf tissue respectively (US-EPA 2001). No report on the amounts of Cry1Ab in stem tissue were found in the literature but Obrist et al. (2006) reported <1 $\mu g g^{-1}$ stem fresh weight in Bt corn that had the 176 Bt gene insertion event.

The results from this study show that lignin content in corn stems likely is not affected by herbivore injury, whereas leaf lignin content could be affected by injury, although more work is needed to confirm this conclusion. We also demonstrated that the C:N ratio, or more specifically the N concentration in corn tissues was affected by herbivore injury. In theory, this should have implications for corn residue decomposition however our results do not support this hypothesis based on the similarity in decomposition of injured and uninjured corn stems during one field season (5 months). The effect of ECB injury on corn tissue chemistry was subtle and resulted in changes in the chemical form of lignin in stems, which emphasizes the need for future investigations examining plant-insect interactions at the molecular level. Our findings indicate no consistent effect of genetic modification on the lignin content of corn stems and leaves. The small effect of the Bt modification on the N concentration of stems, which is probably linked to the production of the Cry1Ab protein needs further investigation.

Acknowledgements The authors wish to thank Dr. François Meloche, entomologist at Agriculture and Agri-Food Canada, Ottawa, Canada for advice on handling European corn borer. Corn seeds were donated by Maizex and Syngenta. Financial support for this work was from the Green Crop Network, funded by the Natural Sciences and Engineering Research Council of Canada.

References

Baron C, Zambryski P (1995) The plant response in pathogenesis, symbiosis, and wounding: variations on a common theme? Ann Rev Genet 29:107–129

Bergstrom GC, Nicholson RL (1999) The biology of corn anthracnose: knowledge to exploit for improved management. Plant Dis 83:596–608

Bode W, Calvin D, Mason CE (1990) Yield-loss relationships and economic injury levels for European corn borer (Lepidoptera: Pyralidae) populations infesting Pennsylvania field corn. J Econ Entomol 83:1595–1603

Cadisch G, Giller K (1997) Driven by nature: plant litter quality and decomposition. CAB International, UK

Campbell M, Sederoff R (1996) Variation in lignin content and composition. Plant Physiol 110:3–13

Castaldini M, Turrini A, Sbrana C, Benedetti A, Marchionni M, Mocali S, Fabiani A, Landi S, Santomassimo F, Pietrangeli B (2005) Impact of Bt corn on rhizospheric and soil eubacterial communities and on beneficial mycorrhizal symbiosis in experimental microcosms. Appl Environ Microbiol 71:6719–6729

Cheong Y, Chang H, Gupta R, Wang X, Zhu T, Luan S (2002) Transcriptional profiling reveals novel interactions between wounding, pathogen, abiotic stress, and hormonal responses in Arabidopsis. Plant Physiol 129:661–677

Clark T, Foster J, Kamble S, Heinrichs E (2000) Comparison of Bt (Bacillus thuringiensis Berliner) maize and conventional measures for control of the European corn borer (Lepidoptera: Crambidae). J Entomol Sci 35:118–128

Delessert C, Wilson I, Van Der Straeten D, Dennis E, Dolferus R (2004) Spatial and temporal analysis of the local response to wounding. Plant Mol Biol 55:165–181

Dillehay B, Roth G, Calvin D, Kratochvil R, Kuldau G, Hyde J (2004) Performance of Bt corn hybrids, their near isolines, and leading corn hybrids in Pennsylvania and Maryland. Agron J 96:818–824

Dinel HH, Schnitzer MM, Saharinen MM, Meloche FF, Paré T, Dumontet S, Lemee LL, Ambles AA (2003) Extractable soil lipids and microbial activity as affected by Bt and non Bt maize grown on a silty clay loam soil. J Environ Sci Health, Part B 38:211–219

Dixon R, Paiva N (1995) Stress-induced phenylpropanoid metabolism. Plant Cell 7:1085–1097

Douglas CJ (1996) Phenylpropanoid metabolism and lignin biosynthesis: from weeds to trees. Trends in Plant Sci 1:171–178

Ecker J, Davis R (1987) Plant defense genes are regulated by ethylene. Proc Natl Acad Sci USA 84:5202–5206

Escher N, Käch B, Nentwig W (2000) Decomposition of transgenic Bacillus thuringiensis maize by microorganisms and woodlice Porcellio scaber (Crustacea: Isopoda). Basic Appl Ecol 1:161–169

Flores S, Saxena D, Stotzky G (2005) Transgenic Bt plants decompose less in soil than non-Bt plants. Soil Biol Biochem 37:1073–1082

Fogel R, Cromack K Jr (1977) Effect of habitat and substrate quality on Douglas fir litter decomposition in western Oregon. Can J Bot 55:1632–1640

Goering H, Van Soest P (1970) Forage fiber analyses (apparatus, reagents, procedures, and some applications). US Agricultural Research Service, Washington

Goñi M, Montgomery S (2000) Alkaline CuO oxidation with a microwave digestion system: Lignin analyses of geochemical samples. Anal Chem 72:3116–3121

Hahlbrock K, Scheel D (1989) Physiology and molecular biology of phenylpropanoid metabolism. Ann Rev Plant Biol 40:347–369

Hammel KE (1997) Fungal Degradation of Lignin. In: Cadisch G, Giller KE (eds) Driven by Nature: Plant Litter Quality and Decomposition. CAB International, UK, pp 33–45

Hawkins S, Boudet A (1996) Wound-induced lignin and suberin deposition in a woody angiosperm (Eucalyptus gunnii Hook.): histochemistry of early changes in young plants. Protoplasma 191:96–104

Hedges J, Blanchette R, Weliky K, Devol A (1988) Effects of fungal degradation on the CuO oxidation products of lignin: a controlled laboratory study. Geochim Cosmochim Acta 52:2717–2726

Hedges JI, Ertel JR (1982) Characterization of lignin by gas capillary chromatography of cupric oxide oxidation products. Anal Chem 54:174–178

Hopkins D, Webster E, Chudek J, Halpin C (2001) Decomposition in soil of tobacco plants with genetic modifications to lignin biosynthesis. Soil Biol Biochem 33:1455–1462

Howe G, Schaller A (2008) Direct defenses in plants and their induction by wounding and insect herbivores. In: Schaller A (ed) Induced Plant Resistance to Herbivory. Springer, Netherlands, pp 7–29

Hyde J, Martin MA, Preckel PV, Edwards CR (1999) The economics of Bt corn: valuing protection from the European Corn Borer. Appl Econ Perspect Policy 21:442–454

Janzen HH (2005) Soil carbon: a measure of ecosystem response in a changing world? Can J Soil Sci 85:467–480

Jeffries T (1994) Biodegradation of lignin and hemicelluloses. In: Ratledge C (ed) Biochemistry of Microbial Degradation. Kluwer Academic Pub, Dordrecht, Netherlands, pp 233–277

Jenny H, Gessel S, Bingham F (2006) Comparative study of decomposition rates of organic matter in temperate and tropical regions. Soil Sci 171:S116–S129

Johnson JMF, Barbour NW, Weyers SL (2007) Chemical composition of crop biomass impacts its decomposition. Soil Sci Soc Am J 71:155–162

Jung HG, Sheaffer CC (2004) Influence of Bt transgenes on cell wall lignification and digestibility of maize stover for silage. Crop Sci 44:1781–1789

Kessler A, Baldwin I (2002) Plant Response to Insect Herbivory: The Emerging Molecular Analysis. Ann Rev Plant Biol 53:299–328

Lagrimini L (1991) Wound-induced deposition of polyphenols in transgenic plants overexpressing peroxidase. Plant Physiol 96:577–583

Lehman RM, Osborne SL, Prischmann-Voldseth DA, Rosentrater KA (2010) Insect-damaged corn stalks decompose at rates similar to Bt-protected, non-damaged corn stalks. Plant Soil doi:10.1007/s11104-11010-10364-11108

Lehman RM, Osborne SL, Rosentrater KA (2008) No differences in decomposition rates observed between Bacillus thuringiensis and non-Bacillus thuringiensis corn residue incubated in the field. Agron J 100:163–168

Loh P, Miller A, Reeves A, Harvey S, Overnell J (2008) Optimised recovery of lignin-derived phenols in a Scottish fjord by the CuO oxidation method. J Environ Monit 10:1187–1194

Lyons PC, Hipskind J, Vincent JR, Nicholson RL (1993) Phenylpropanoid dissemination in maize resistant or

susceptible to Helminthosporium maydis. Maydica 38:175–181
Martin S, Darrah L, Hibbard B (2004) Divergent selection for rind penetrometer resistance and its effects on European corn borer damage and stalk traits in corn. Crop Sci 44:711–717
Mason C, Rice M, Calvin D, Van Duyn J, Showers W, Hutchison W, Witkowski J, Higgins R, Onstad D, Dively G (1996) European corn borer: ecology and management. North Central Regional Extension Publication No. 327, Iowa State University, Ames, IA.
Melillo JM, Aber JD, Muratore JF (1982) Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. Ecology 63:621–626
Nicholson R, Hammerschmidt R (1992) Phenolic compounds and their role in disease resistance. Ann Rev Phytopathol 30:369–389
Nykanen H, Koricheva J (2004) Damage-induced changes in woody plants and their effects on insect herbivore performance: a meta-analysis. Oikos 104:247–268
Obrist L, Dutton A, Albajes R, Bigler F (2006) Exposure of arthropod predators to Cry1Ab toxin in Bt maize fields. Ecol Entomol 31:143–154
Olson J (1963) Energy storage and the balance of producers and decomposers in ecological systems. Ecology 44:322–331
Ostrander B, Coors J (1997) Relationship between plant composition and European corn borer resistance in three maize populations. Crop Sci 37:1741–1745
Otto A, Simpson M (2006) Evaluation of CuO oxidation parameters for determining the source and stage of lignin degradation in soil. Biogeochem 80:121–142
Pascholati S, Nicholson R, Butler L (2008) Phenylalanine ammonia-lyase activity and anthocyanin accumulation in wounded maize mesocotyls. J Phytopathol 115:165–172
Poerschmann J, Gathmann A, Augustin J, Langer U, Górecki T (2005) Molecular composition of leaves and stems of genetically modified Bt and near-isogenic non-Bt maize-Characterization of lignin patterns. J Environ Qual 34:1508–1518
Poerschmann J, Rauschen S, Langer U, Augustin J, Gorecki T (2008) Molecular level lignin patterns of genetically modified Bt-maize MON88017 and three conventional varieties using tetramethylammonium hydroxide (TMAH)-induced thermochemolysis. J Agric Food Chem 56:11906–11913
Rasse D, Dignac M, Bahri H, Rumpel C, Mariotti A, Chenu C (2006) Lignin turnover in an agricultural field: from plant residues to soil-protected fractions. Eur J Soil Sci 57:530–538
Reay D, Pidwirny M (Lead Authors), Gulledge J, Draggan S (Topic Editor) (2010) Carbon Dioxide. In: Cleveland CJ (ed) Encyclopedia of Earth. Washington, D.C.: Environmental Information Coalition, National Council for Science and the Environment. http://www.eoearth.org/article/Carbon_dioxide. First published in the Encyclopedia of Earth September 27, 2006; Last revised January 3, 2010, Accessed 04 June 2010.
SAS Institute Inc. (2009) SAS Campus Drive, Cary, North Carolina 27513, USA.
Saxena D, Stotzky G (2001) Bt corn has a higher lignin content than non-Bt corn. Am J Bot 88:1704–1706
Stange R Jr, Ralph J, Peng J, Sims J, Midland S, McDonald R (2001) Acidolysis and hot water extraction provide new insights into the composition of the induced "lignin-like" material from squash fruit. Phytochem 57:1005–1011
Tarkalson DD, Kachman SD, Knops JMN, Thies JE, Wortmann CS (2008) Decomposition of Bt and non-Bt corn hybrid residues in the field. Nutr Cycl Agroecosys 80:211–222
Tiwari S, Youngman RR, Laub CA, Brewster CC, Jordan TA, Teutsch C (2009) European corn borer (Lepidoptera: Crambidae) infestation level and plant growth stage on whole plant corn yield grown for silage in Virginia. J Econ Entomol 102:2146–2153
Trofymow JA, CIDET Working Group (1998) The Canadian Intersite Decomposition Experiment (CIDET): Project and site establishment report. Inf. Rep BC-X-378. Natural Resources Canada, Canadian Forest Service, Pacific Forestry Centre, Victoria.126 pp.
US-EPA (2001) Bt Plant-Pesticides Biopesticides Registration Action Document: preliminary risks and benefits sections—Bacillus thuringiensis plant-pesticides. US EPA Office of Pesticide Programs, Biopesticides and Pollution Prevention Division http://www.epa.gov/oscpmont/sap/meetings/2000/october/brad3_enviroassessment.pdf, Accessed 25 April 2010.
Vance C, Kirk T, Sherwood R (1980) Lignification as a mechanism of disease resistance. Ann Rev Phytopathol 18:259–288
Vanlauwe B, Diels J, Sanginga N, Merckx R (1997) Residue quality and decomposition: an unsteady relationship? In: Cadisch G, Giller K (eds) Driven by Nature: Plant Litter Quality and Decomposition. CAB International, UK, pp 157–166
Walter W Jr, Randall-Schadel B, Schadel W (1990) Wound healing in cucumber fruit. J Am Soc Hortic Sci 115:444–452
Wider R, Lang G (1982) A critique of the analytical methods used in examining decomposition data obtained from litterbags. Ecology 63:1636–1642
Zhang S, Yang Q, Ma R (2007) Erwinia carotovora ssp. carotovora infection induced "defense lignin" accumulation and lignin biosynthetic gene expression in Chinese cabbage (Brassica rapa L. ssp. pekinensis). J Integr Plant Biol 49:993–1002
Zwahlen C, Hilbeck A, Nentwig W (2007) Field decomposition of transgenic Bt maize residue and the impact on non-target soil invertebrates. Plant Soil 300:245–257