Crop residue composition and decomposition in transgenic corn agroecosystems: effects of the *Bacillus thuringiensis* gene and herbivory

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ABSTRACT

Bt (*Bacillus thuringiensis*) corn (*Zea mays* L.) is reported to have a higher lignin concentration and be more resistant to degradation compared to conventional non-Bt (NBt) hybrids. NBt hybrids are physically affected by the European corn borer (ECB, *Ostrinia nubilalis* H.), which could increase the deposition of 'stress lignin' in injured tissues and alter the decomposability of corn residue. The objective of this thesis is to test the compositional differences between Bt and NBt corn in terms of fiber, C, and N concentrations and study the decomposition of these residues. The natural variation in lignin between plant parts was also considered to affect the decomposition of corn residue. Field experiments that included 18 Bt and NBt hybrids not infested by ECB showed similarity in lignin and aboveground biomass. Stems from a field-grown Bt hybrid decomposed faster than the NBt near-isoline. In the greenhouse, ECB-infested NBt corn sustained injury, which resulted in lower stem biomass and higher stem N concentration in injured plants. ECB injury did not affect the lignin concentration in stems and CuO oxidation analysis revealed that ECB injury reduced the amount of lignin-derived phenols in stems, which refutes the hypothesis that NBt corn would respond to ECB injury by depositing 'stress lignin'. Infested and non-infested stems buried in the field for five months showed no difference in decomposition due to the Bt gene or herbivory. However, there was 87% more syringic acid in injured NBt stems suggesting that herbivory may enhance lignin decomposition in the longer-term. Under controlled conditions, a 36-week incubation experiment confirmed that Bt and NBt corn tissue decompose at a similar rate, with variation in decomposition rates attributed to the lignin

and N concentration in corn tissues. Soils amended with roots (6.2% lignin) produced significantly lower CO_2 than stems (3.5% lignin) and leaves (3.2% lignin). In conclusion, the Bt gene and ECB infestation do not affect the chemical composition of corn tissue and should not have an effect on residue decomposition in Bt corn agroecosystems. Due to their elevated lignin concentration, corn roots can make an important contribution to the stabilization of C in the soil.

RÉSUMÉ

Le maïs Bt (*Bacillus thuringiensis* - *Zea mays* L.) est reconnu contenir plus de lignine et être plus résistant que les maïs hybrides conventionnels (NBt). Ces derniers sont affectés par la pyrale du maïs (European Corn Borer ou ECB, *Ostrinia nubilalis* H.), qui peut augmenter le dépôt de «lignine causée par le stress» dans les tissus et changer la décomposition des résidus de maïs. L'objective de cette thèse est d'évaluer les différences de composition en fibres, C et N entre les maïs BT et NBt, et d'étudier l'effet de l'herbivorie par ECB sur la décomposition les différentes parties résiduelles du plant. Les expériences menées au champ ont démontré que la biomasse aérienne et le contenu en lignine étaient similaires entre 18 hybrides des types BT et NBt non infestés par l'ECB. Toutefois, les tiges des maïs Bt ont présenté une vitesse de décomposition plus élevée. En serre, les tiges de maïs NBt infesté et meurtri par l'ECB, ont montré une plus faible biomasse et une plus grande concentration en N. Toutefois, les blessures engendrées par l'ECB n'ont pas affectés la concentration de lignine dans les tiges. L'analyse par oxydation au CuO a démontré que les blessures ont réduit la quantité de phénols dérivés de la lignine réfutant ainsi l'hypothèse que le NBt répond aux meurtrissures de l'ECB en produisant un dépôt de lignine. Suite à une décomposition sous terre de cinq mois, aucune différence significative due à la présence du gène Bt ou non, n'a été mesurée entre les tiges infestées et non-infestées. Cependant, il y avait 87% plus d'acide syringique dans les tiges de NBt infestées à l'ECB, ce qui suggère que l'herbivorie pourrait améliorer la décomposition à long terme. Dans des conditions contrôlées, une expérience d'incubation de 36 semaines a confirmé que les maïs Bt et NBt se décomposent à la même vitesse; les différences observées étant dues au contenu en lignine et N dans les tissus. Les sols

fertilisés avec les racines (6.2% lignine) ont produit beaucoup moins de CO_2 que les tiges (3.5% lignine) et les feuilles (3.2% lignine). En conclusion, le gène du Bt ainsi que l'infestation par l'ECB n'affectent pas la composition chimique des tissus de maïs et ne devraient pas affecter la décomposition des résidus de culture. Dû à leur contenu en lignine élevé, les racines de maïs peuvent contribuer de manière importante à la stabilisation du C dans le sol.

PREFACE AND CONTRIBUTION OF AUTHORS

This thesis is composed of five chapters, preceded by a general introduction that includes the objectives and hypotheses, and followed by the overall summary and conclusions and the contributions to knowledge (required sections, according to the guidelines of the Graduate and Postdoctoral Studies Office, McGill University). The first chapter is a review of literature which summarizes the work of previous research. Experimental results are presented in chapter two to four, which are written in manuscript format according to the guidelines of the Graduate and Postdoctoral Studies Office, McGill University. Chapter five constitutes a general discussion and synthesis of results to link the findings of the different experiments, relate them to the thesis objectives and comment on the validity of the *a priori* hypotheses for the thesis. The candidate was the senior author on all manuscripts, and co-authors included Joann K. Whalen, Bao-Luo Ma, Myrna J. Simpson, H. Henry Janzen, and Yves Gelinas. The candidate conducted the thesis research with financial support from project 2d "Transforming plant C into soil C: process-level controls on C sequestration" of the Green Crop Network (GCN), funded by the Natural Sciences and Engineering Research Council of Canada. The GCN proposal, which was funded prior to the candidate's arrival at McGill University, proposed an incubation experiment to test the decomposition of Bt corn residue (suggested by Drs. Joann Whalen and Henry Janzen), which was further developed and expanded by the candidate to assess soil lignin degradation using the cupric oxide oxidation method (chapter four). The candidate was solely responsible for designing the experiments in chapter two (two-year field study on chemical composition of Bt and non-Bt corn tissues

and residue decomposition) and chapter three (greenhouse study on herbivory and lignin content in Bt and non-Bt corn, as well as their decomposition). Those experiments were strategic and novel, in that they were planned to investigate relevant processes that could affect plant C transformation into soil C under field conditions, not mentioned in project 2d of the GCN proposal. The candidate was solely responsible for all day-to-day activities in performing all the experiments, data collection, analysis and interpretation, and writing the manuscripts. Dr. Whalen provided financial support, advisory guidance about the experiments, and editorial assistance with the manuscripts. Dr. Ma provided general guidance and advice on the agronomic aspects of the experiments in chapters one and two. The candidate was trained on the cupric oxide oxidation method presented in chapter four in Dr. Simpson's lab. Dr. Simpson provided advice on this analytical technique and her staff helped with sample analysis. The cupric oxide oxidation analysis presented in chapter three was conducted by the candidate at the lab of Dr. Gelinas. The manuscripts are presented in the following order:

Chapter 1. Yanni, S.F., Whalen, J.K., Ma, B.L. 2010. Crop residue chemistry, decomposition rates and CO_2 evolution in Bt and non-Bt corn agroecosystems in North America: a review. Nutr. Cycling Agroecosyst. 87, 277-297.

Chapter 2. Yanni, S.F., Whalen, J.K., Ma, B.L. 2010. Field-grown Bt and non-Bt corn: yield, chemical composition, and decomposition. Agron. J. 103, 486-493

Chapter 3. Yanni, S.F., Whalen, J.K., Ma, B.L., Gelinas, Y. 2010. European corn borer injury effects on lignin, carbon, and nitrogen in corn tissues. Plant Soil 341, 165-177

Chapter 4. Yanni, S.F., Whalen, J.K., Simpson, M.J., Janzen, H.H. 2011. Plant lignin and nitrogen contents control carbon dioxide production and nitrogen mineralization in soils incubated with Bt and non-Bt corn residues. Soil Biol. Biochem. 43, 63-69.

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TABLE OF CONTENTS

LIST OF TABLES

Chapter 1

Chapter 2

Table 3. Means of grain and stover yield (Mg ha^{-1}) of 18 Bt and NBt 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.

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 and NBt for each corn component are given. Values are the means ± standard error (n=27).

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).

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

Chapter 3

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).

Table 2. Lignin concentration (g kg^{-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).

Table 3. Total nitrogen concentration (g kg^{-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).

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

Chapter 4

Table 1. Organic C, total N, $\delta^{13}C$, $\delta^{15}N$, and lignin concentrations of the incubation soil, indulin lignin and corn residues.

Table 2. Effect of Bt gene modification and addition of alkali lignin to a soil-corn residue mixture on cumulative CO_2 production (g CO_2-C kg^{-1} soil) after 20 weeks. Values are the mean ± standard error (n=8).

Table 3. Effect of the Bt gene and addition of alkali lignin to a soil-corn residue mixture on cumulative mineral N (mg N kg^{-1} soil) produced after 36 weeks and decomposition rate constant (K) from NLIN estimation. Values are the mean ± standard error (n=8).

Table 4. Acid to aldehyde (Ad/Al) ratios and vanillyl to syringyl ratio of root- stem- and leaf-amended soils, with and without added lignin (+L, -L) after 1 week and 36 weeks of laboratory incubation.

Table 5. Proportion residue-C and residue-N retained in soil, calculated from equations 3 and 4, in a sandy loam soil amended with Bt and NBt tissues after 36 weeks of laboratory incubation and ANOVA treatment effects at α=5%. Values are the mean ± standard error (n=8).

LIST OF FIGURES

Chapter 1

Chapter 2

Chapter 3

Chapter 4

LIST OF APPENDICES

Appendix I

Moisture content (g kg^{-1}) of corn grain of field-grown Bt and NBt hybrids in 2008 and 2009. Values are the means ± standard error (n=3).

Appendix II

Hemicellulose concentration (g kg^{-1}) of leaves, stems, and roots of field-grown Bt and NBt hybrids in 2008 and 2009. Values are the means ± standard error (n=3).

Appendix III

Cellulose concentration (g kg^{-1}) of leaves, stems, and roots of field-grown Bt and NBt hybrids in 2008 and 2009. Values are the means ± standard error (n=3).

Appendix IV

Weight (g $plant^{-1}$) of grain+cob of pot-grown Bt and NBt hybrids with or without ECB infestation in 2008 and 2009. Values are the means ± standard error (n=4).

GENERAL INTRODUCTION

A key factor in sustainable crop production is the maintenance of the soil organic carbon (SOC) content; soils lose about 30-50% of their original SOC pool upon conversion to agriculture (Lal 2002) and thus have the potential to accumulate and store carbon (C) from plant residue input. In such soils, the SOC content is linearly related to the residue-C input level (Paustian et al. 2000) until equilibrium between C inputs and outputs are achieved. The resistance of plant residue to biodegradation is an important factor in controlling the soil processes responsible for decomposition and transformation of residue-C inputs into stable SOC. Soluble and labile plant components, such as carbohydrates and amino acids, comprise a rapidly decomposing C and N pool with decomposition rate constants that may exceed 10 y^{-1} (proportion lost per unit time) (Harmon et al. 2009). A second pool with an intermediate decomposition rate is defined, which includes material like cellulose and hemicellulose. Lignin and lignin-like materials are included in a slowly decomposing pool (Herman et al. 1977; Swift et al. 1979; Hadas et al. 2004; Bertrand et al. 2006; Harmon et al. 2009) with rates of decomposition estimated to range between 0.01 and 0.15 y^{-1} (Dignac et al. 2005; Rasse et al. 2006; Harmon et al. 2009). Lignin, the second most abundant compound in vascular plants after cellulose, has a complex polyphenolic structure that makes it recalcitrant, such that lignin can only be completely decomposed by a limited number of microorganisms (white rot fungi and the filamentous bacteria, actinomycetes, to a lesser extent) (Lewis and Yamamoto 1990). With its long residence time in the soil, lignin is more likely than other C compounds of plant origin to become physically and chemically protected in the soil,

allowing it to be transformed into relatively stable forms of SOC. Therefore, plant residues that have elevated lignin concentrations are expected to decompose at slower rates, produce less CO_2 and contribute to the formation of stable SOC compounds.

The interest in corn (*Zea mays* L.) as a source of residue-C soil input comes from the fact that more than 17% of cropland in North America is planted with corn, a highly productive C4 plant that accumulates about 16-24 Mg ha^{-1} in aboveground biomass (Jones, 2003) and 3-5 Mg dry matter ha^{-1} in roots each year (Prince et al. 2001). In addition, Bt (*Bacillus thuringiensis*) corn hybrids now make up more than 60% of the corn acreage in the USA and Canada (Economic Research Service - USDA 2010; Canadian Corn Pest Coalition 2010a). The soil-dwelling bacterium *Bacillus thuringiensis* produces toxins that can be extracted and used as a biological alternative to pesticide. In nature, Bt strains produce crystal (Cry) proteins (δ-endotoxins) encoded by *cry* genes during sporulation, some of which have insecticidal properties. Multiplication and conjugation of *B. thuringiensis* cells are higher in dead or infected insect larvae than in laboratory cultures or in soil, indicating the ecological significance of Cry proteins for survival of this organism (Vilas-Bôas et al.1998; Thomas et al. 2000). When specific insects ingest these proteinaceous toxins, the alkaline pH of their digestive tract activates the toxin, which forms a pore in the gut cell membrane causing cell lysis and eventually insect death. The Bt toxins have specific activities against insects in the orders Lepidoptera, Diptera, Coleoptera, Hymenoptera and Nematodes. Bt corn hybrids are therefore sought as an alternative to pesticides for controlling insect pests such as corn borer (*Ostrinia* spp.) and corn rootworm (*Diabrotica* spp.). The popularity of Bt corn hybrids arises from the fact that these transgenic plants provide more effective insect

protection throughout the growing season than Bt spray formulations. Another advantage is the fact that Bt toxins are highly selective for certain insect groups and species, eliminating the possible harmful effects to non-target organisms. As discussed further in chapter one, the Bt gene is incorporated into the corn genome by transgenic engineering, and the corn types registered in North America are produced through the Bt11 or MON810 transformation events (US-EPA 2001).

Reports that Bt corn has an altered lignin composition, ranging from 33-97% greater lignin concentration than non-Bt (NBt) corn (Saxena and Stotzky 2001), suggests that cropland under Bt corn production could have modified soil ecosystem functions and residue decomposition rates. Bt corn was also reported to produce more aboveground biomass than conventional hybrids in years when herbivore infestation rates were high. This and the altered lignin composition would translate into more decay-resistant residue being incorporated into the soil. It is therefore hypothesized that Bt corn agroecosystems would accumulate more SOC than NBt corn agroecosystems.

The effect of herbivory on corn biomass accumulation and chemical composition is another factor of interest because NBt corn is susceptible to damage by the European corn borer (*Ostrinia nubilalis* H.); one larvae in the stalk can reduce yield by about 8% (Bode et al. 1990). Herbivory could also alter lignin deposition in corn tissue, as many plant species respond to herbivory injury by depositing more lignin or lignin-like materials (including 'stress lignin') in injured tissues. However, it is not known whether this is also true for NBt corn plants. Therefore, ECB infestation and injury could increase the lignin concentration in NBt corn and on the other hand it could reduce biomass accumulation

and damage the tissue, which leads to a more easily decomposable residue. It is therefore hypothesized that the net effect of herbivory on NBt corn (considering both chemical modification and physical damage) would be to increase the decomposition rate of injured tissues. As Bt corn is not affected by ECB due to its genetic modification, the hypothesis is that Bt corn residues are more decay-resistant than NBt corn residues injured by herbivory. This is consistent with the hypothesis that Bt corn agroecosystems would accumulate more SOC than NBt corn agroecosystems.

The main objectives of this study were to test the compositional differences between Bt and NBt corn and the decomposition of Bt and NBt corn residues from trials without herbivory and with deliberate ECB infestation/injury. The specific objectives were:

Objective 1: Determine if Bt corn components differ in lignin and nitrogen (N) concentrations than NBt hybrids. The hypothesis is that Bt and NBt hybrids will be similar based on the fact that the Bt gene does not normally interfere in the lignin biosynthesis pathway and that the amount of crystal-like protein produced in Bt corn is not big enough to cause a difference in N concentration. A secondary objective is to assess differences in yield and biomass production, which affect the amount of residue returned to the field after harvest. Since no ECB infestation is expected in this field, the hypothesis is that biomass production will be similar between Bt and NBt hybrids.

Objective 2: Study the effect of herbivory by the European corn borer on lignin and N concentrations and biomass production in Bt and NBt hybrids. The hypothesis is that lignin concentration will be greater in injured tissue, specifically stems, of NBt corn

hybrids and that Bt hybrids will produce more aboveground biomass compared to injured NBt hybrids.

Objective 3: Test the decomposition rates of Bt and NBt corn stems in the field and test the effect of ECB injury on the decomposition of corn stems. The hypothesis is that Bt stems will decompose at a slower rate based on reported observations that Bt corn is tougher and resists decomposition. Also, NBt stems that have been infested with ECB will show faster decomposition rates than intact Bt stems.

Objective 4: Study the decomposition patterns of different corn residue components, which have different chemical compositions such as roots, stems and leaves, from Bt and NBt corn hybrids. The hypothesis is that tissue with greater lignin concentrations will decompose at a slower rate under laboratory conditions.

FORWARD TO CHAPTER ONE

Chapter one is the literature review, in which previous work on the following will be examined: Bt and NBt grain yield and aboveground biomass production under different herbivory levels, C and N concentrations in Bt and NBt corn hybrids, lignin concentration in Bt and NBt hybrids, decomposition patterns of Bt and NBt corn under laboratory and field conditions, and the differences in composition and decomposition among various corn plant parts. I also present hypothetical scenarios of the amounts of lignin that can be retained in the soil from crop residues with different lignin concentrations.

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CHAPTER 1.

Literature Review

Crop Residue Chemistry, decomposition rates, and CO_2 evolution in Bt and non-Bt corn Agroecosystems in North America: A Review.

1.1 Abstract

Corn is a major cereal crop, with production on more than one fifth of the agricultural land worldwide. In North America, about 50% of corn acreage is planted with transgenic corn hybrids such as those with the gene from Bt that express the insecticidal crystalline protein (Cry1Ab) for the control of European corn borer. Widespread production of Bt corn could affect soil organic carbon storage in agroecosystems if transgenic corn differs from conventional corn in yield and chemical composition. Generally, the yield of Bt corn is greater than NBt corn in years when there is severe infestation of corn insect pests. Some authors report that Bt corn has higher lignin concentration than NBt corn, whereas others found no difference in the chemical composition of near-isolines. Residues with higher lignin concentration are expected to have a slower decomposition rate and release less CO_2 to the atmosphere; however this is not supported by the literature. A few studies have examined decomposition of Bt corn residues in this context, and the findings to date have been inconclusive, perhaps due to the variety of experimental approaches used to study this question. Generally, the literature supports the view that decomposition rates in Bt corn- and NBt corn-amended soils are similar. Whether Bt corn has greater lignin

concentration or slower decomposition rates, the relevant question is whether this will affect the amount of C storage in the soil. A significant gain in SOC requires crop residue inputs with higher lignin concentration than what is realistically expected from Bt corn residue.

Keywords: *Bt corn; European corn borer; Lignin concentration; Residue decomposition; Soil carbon; Zea mays.*

1.2 Introduction

Atmospheric CO_2 concentrations have been increasing exponentially since the Industrial Revolution due to fossil fuel burning, cement production and land use change. The conversion of grasslands and forests to field crop production and deforestation have released about 200 Pg C to the atmosphere since the mid 19^{th} century, with about 90 Pg C from deforestation alone (Reay and Pidwirny 2006). Agricultural soils possess about 128-160 Pg C (Paustian et al. 2000; IPCC 2001) and are perhaps the largest active C reservoir. Agricultural soils may act as a sink or source of CO_2 depending on land management. They can potentially store some of the atmospheric CO_2 fixed by crop plants and hence mitigate greenhouse gas emissions from the agricultural sector.

Soil carbon storage in agroecosystems under corn production has captured my attention for several reasons. Worldwide, more than 20% of agricultural land is planted with hybrids of this adaptable C4 plant (Amos and Walters 2006). In temperate regions, corn plants can achieve a height of more than 2.25 m and an aboveground biomass of

15.9 to 24.0 Mg ha^{-1} (about 6.4 to 9.6 Mg C ha^{-1}) (silage) in a growing season (Jones 2003). An additional 3 to 5 Mg ha^{-1} of biomass is accumulated in the root system (Prince et al. 2001), which is especially interesting in the context of soil C storage because corn roots are not removed at harvest (Amos and Walters 2006). An example based on a corn agroecosystem in southern Ontario, Canada shows that the crop fixes 10 Mg C ha^{-1} y^{-1} through photosynthesis and loses 3 Mg C ha^{-1} y^{-1} through respiration (Fig. 1) (Lal et al. 1997), thus the net primary production (NPP) would be 7 Mg C ha^{-1} y^{-1}. This is consistent with NPP values for corn grown in North America, mostly in the U.S. Midwest, which were calculated to be 7.6 Mg C ha^{-1} y^{-1} using data from Prince et al. (2001). In Fig. 1, grain harvest removes 2.5 Mg C ha^{-1} y^{-1} from the corn agroecosystem and there is a balance between the C entering the soil in crop residues (4.5 Mg C ha^{-1} y^{-1}) and CO_2 respired by soil organisms during crop residue decomposition.

Since the commercial introduction of transgenic crops for field production in 1996, many producers have begun planting transgenic corn hybrids instead of conventional corn hybrids. The first transgenic hybrids were modified at a single locus with genetic material from the bacteria Bt that provided control against Lepidoptera larvae, in particular, European corn borer. While Bt corn is still popular, the "stacked" transgenic hybrids that provide resistance to insect attack and tolerance of herbicide damage are favoured in some regions. At present, Bt corn accounts for about 63% of corn production in the US (Economic Research Service/USDA 2010) and 93.8% of the corn grown in Canada (Canadian Corn Pest Coalition 2010a).

It has been suggested that Bt corn differs from NBt corn hybrids in yield and chemical composition, which could affect residue decomposition and CO_2 emissions from the SOC pool. The objectives of this review are to (1) examine the C input from residues in corn agroecosystems, (2) compare the yield and chemical characteristics of residues from Bt corn and NBt corn, and (3) evaluate the decomposition rates of Bt corn and NBt corn residues.

1.3 Carbon Input from Corn Residues

1.3.1 Root residue

Roots interact with the soil environment, assimilating minerals and water from the soil solution and releasing organic materials. They play an important part in C flow in the soil-plant system, since 16-33% of the C assimilated by plants through photosynthesis is transferred into the soil through the roots (Heal et al. 1997). Corn has a more extensive root system than most other annual crops, thus providing a greater root C input. A three-year field study showed that the post-harvest C input from corn roots was more than double the C input of wheat or soybean roots (Buyanovsky and Wagner 1986).

Root C inputs to the soil come from rhizodeposits and root biomass (dead roots). There is great variation in the reported values of C input through rhizodeposits and root biomass in the literature due to differences in experimental design and study conditions (Amos and Walters 2006) and the difficulty of quantifying rhizodeposits due to their

labile nature. While root exudation is probably a major soil C input, there is very little data on flux rates and chemical composition of exudates under field conditions.

Rhizodeposits include root cap cells, mucilage and exudates, a diverse group of substances excreted actively or secreted passively by growing roots. While it is not known precisely how much rhizodeposition occurs under field conditions, values from growth chamber and greenhouse studies provide some insight into the magnitude of C transferred from corn roots to the rhizosphere soil. Molina et al. (2001) reported that rhizodeposits constitute an estimated 24.4% of the C fixed by plants through photosynthesis and that they are rapidly assimilated by microorganisms for metabolic processes (respiration, growth). Buyanovsky and Wagner (1997) estimated that rhizodeposit C constitutes 40% of the total root-derived C (root biomass + rhizodeposits). Rhizodeposits recovered in soil microbial biomass and SOC pools represented 5.2 to 61.8% with an average of 29% of total root-derived C (root biomass and rhizodeposits) of corn plants at different growth stages grown in the field (2 studies) and growth chambers (10 studies) (Amos and Walters 2006). The C contribution of rhizodeposits based on 29% of total biomass and an average corn root biomass of 2.1 Mg ha^{-1} (averaged from field grown corn data at late growth stages based on 75,000 plants ha^{-1} from Amos and Walters 2006) would be 0.6 Mg ha^{-1}.

As for the root biomass remaining in the soil after harvest, it represents an average of 15% of the corn aboveground biomass (Prince et al. 2001). The decomposition of dead root residues by soil microorganisms produces CO_2 and leads to the eventual humification and physical stabilization of C from root biomass. Molina et al. (2001) estimated that root

residues account for about 50% of the SOC pool and Johnson et al. (2006) proposed that 1.5 to 3 times more root C than shoot C is stabilized in the SOC pool, which suggests that root biomass makes a greater contribution to soil C sequestration than aboveground residues. Root biomass has considerable value for SOC storage because of the amount of C contained in these residues and the fact that they are less easily mineralized than rhizodeposits, thus more likely to become chemically or physically stabilized in deeper soil layers (Bolinder et al. 1999).

1.3.2 Shoot residue

For the purpose of this review, the shoot is considered to be the stem and leaves of the corn plant excluding the cob and grain. On average, a whole corn plant at physiological maturity contains 436 kg C per 1000 kg dry matter, distributed as follows: 26.6% in the leaves, 24.5% in the stem, 32% in the grain, 7% in the roots, and 9.8% in the cob (Latshaw and Miller 1924). There have been many studies that examined the C input and decomposition of aboveground corn residues; an estimated 3 to 4 Mg C ha^{-1} y^{-1} are added to the soil from aboveground crop residue in corn agroecosystems in the USA (Johnson et al. 2006). About 7.7 to 20% of the corn shoot residues are retained in the SOC pool in long-term field experiments (Bolinder et al. 1999).

Many producers in North America are planting Bt corn, but the consequence of this activity on SOC pools is not known. High-yielding Bt corn is expected to produce more grain and plant biomass than conventional corn, which could increase the C input from shoot residues left behind at harvest.

1.4 Transgenic Bt Corn

1.4.1 Bt corn types

Bt corn is a transgenic corn hybrid containing a gene from *Bacillus thuringiensis*, which is a naturally occurring gram positive aerobic bacterium that produces spores. During sporulation Bt produce crystal-encrusted proteins, known as δ-endotoxins, which are toxic to the larva of some insects and thus have been isolated and their proteins used in insecticide formulations since 1961 (Gill et al. 1992; US-EPA 1998). As noted by Wei et al. (2003), the reason why this soil bacterium has evolved toxins against insects that are not complete soil-dwellers is not clear. They suggested that these Bt species have evolved from ancestors that targeted soil-borne nematodes since there are a number of Cry proteins that have been identified as affecting nematodes. As well, it has been shown that the ability of conjugal plasmid transfer in Bt is greater in infected or dead insect larvae than in soils (Vilas-Bôas et al. 1998; Thomas et al. 2000) which might indicate a possible ecological significance of the Cry protein for survival and spread of these Bt strains. *Bacillus thuringiensis* was first discovered and isolated in the early 1900s by a Japanese biologist, Shigetane Ishiwatari, who was investigating a disease that kills silkworms and later work by several researchers led to the identification of the toxin and its source in the bacterium. With advances in DNA technology, genes from several Bt subspecies (also known as varieties) that encode for the production of the toxin have been cloned and incorporated into plant genomes (eg., cotton, corn, potatoes, soybeans) for protection against Coleopteran, Dipteran, and Lepidopteran herbivores. Production of the toxin by

the plants as opposed to spray formulations solves problems like application management and pesticide degradation upon exposure to sunlight.

The toxicity of Cry proteins is species-specific and they are activated inside the target species upon ingestion. When the crystal-encrusted proteins reach the gut of the larva, the alkaline pH solubilizes the crystals and releases the protoxins, which are activated by proteases (trypsin) produced by the host. The active toxin attaches to receptors in the midgut and forms pore structures that penetrate into the membrane thus causing the cells to lyse (Gill et al. 1992; Pigott and Ellar, 2007) leading to the death of the insect within two days of ingestion. Details and the exact mode of action of the toxin are still not completely identified. The insect specificity and mode of action of the different Cry proteins are related to their three dimensional structure; the structure of the Cry1Ab make it specific to Lepidoptera. The specific Bt subspecies used in corn against the European Corn Borer, the major Lepidopteran corn pest in North America, is the Bt *kurstaki* (US-EPA 1998) from which the gene encoding for the production of the Cry1Ab is taken. Expression of the transgene depends on the transformation event used to incorporate the Bt gene into the corn genome. The Bt corn types registered with the US Environmental Protection Agency (EPA) are shown in Table 1. The Bt11 (transformed with plasmid pZO1502) and MON810 (transformed with plasmids PV-ZMBK07 & PV-ZMGT10 ballistically introduced together) (US-EPA 2001) events produce Bt toxin in all plant parts and are effective against 1st, 2nd, and 3rd generation of ECB larvae. Table 2 shows the amounts of the Cry1Ab protein produced in each of the Bt11 and MON810 corn plant components.

There are concerns about the development of resistance to the Bt toxin especially in crop monocultures. In Bt corn fields, it is mandatory to plant 20% of the acreage in non-Bt (Canadian Corn Pest Coalition 2010b), which acts as refuge for non-resistant insects providing mates for surviving individuals that have resistant alleles. Environmental concerns relate to the persistence of the toxin in the soil, and in water (Tank et al. 2010), and its effects on non-target organisms however, it has been generally shown that the effects are minimal.

After ECB, corn rootworm (*Diabrotica* spp.) has now become the most destructive and widespread insect pest of corn in North America. Yield and quality losses, harvest time delays, and insecticide costs, in the USA and Canada are substantial (Metcalf 1986; Ostlie 2001). A genetically modified corn with rootworm resistance (CRW-Bt) (MON 863) was released in Canada (DKC42-23) in 2003. The gene encompasses the coleopteran specific insecticidal δ-endotoxin (Cry3Bb) from *B. thuringiensis*. At a clay loam site and under heavy infestation, it was also observed that the CRW-Bt hybrid had a yield advantage of 10 to 66% compared to the NBt control hybrid (Ma et al. 2009). Bt corn hybrids targeting rootworm are being used in Canada and the US (Table 1) but a full discussion on rootworm resistant corn is beyond the scope of this review.

1.4.2 Fate of Cry proteins in the soil and their effect on soil organisms

Cry proteins from Bt transgenic crops are added to the soil through root exudation when the plants are alive and through crop residues when they are incorporated in the soil (Stotzky 2000) and there are concerns that they might have adverse effects on non-target

soil organisms, which in turn will affect decomposition and other biological processes in the soil ecosystem. Cry proteins have been shown to bind to clay particles and humic substances (e.g. Tapp and Stotzky 1998; Saxena and Stotzky 2000) and persist in the soil (e.g. Zwahlen et al. 2003) retaining their insecticidal activity (e.g. Tapp and Stotzky 1995) but in general have no persistent negative effects on soil organisms. Readers are referred to a comprehensive review conducted by Icoz and Stotzky (2008) about the fate and effects of Bt crops in soil ecosystems. Their general findings indicate no effect of the Bt toxins on earthworms, woodlouse, collembola or mites from field experiments and few negative effects on nematodes. Two studies reported fewer mycorrhizal fungi colonization of Bt roots with the 176 Bt insertion event, which is not registered for use at present.

1.4.3 European corn borer infestation effects

The infestation of corn with ECB fluctuates from year to year and differs among geographical regions. ECB can produce as many as three generations of larvae per year, with an average of two generations in cooler temperate regions like southern Manitoba, Ontario and Quebec, Canada. Losses from ECB include stalk injury by first and second generation larvae, stalk lodging, ear drop due to second generation larvae, and enhancement of stalk rot in the injured stalks (Willson and Eisley 2001). In addition, ECB injury lowers grain yield because damaged stalks reduce the translocation of photosynthates within the plant (Martin et al. 2004), and the combine harvestor is unable to pick up the ear from the ground, i.e. reduces harvestable yield. Yield loss depends upon the growth stage when the plant is infested (Bode et al. 1990) and on the number of larvae

in the stalk. On average, a yield loss of 5.5% from first generation larvae and 2.8% from second generation larvae is expected when the infestation rate is one larva per stalk (Bode et al. 1990), and a combined corn yield loss as high as 12% could occur from first and second generation larvae (Hagerman 1997).

Grain yield losses are not expected to influence the amount of residue that is returned to the soil after harvest, however injury to stalks could affect the amount of aboveground biomass produced and the rate of crop residue decomposition. The healthy stalks of Bt corn could translocate photosynthates through the plant and increase the C input through rhizodeposition, compared to ECB infested corn. Insect wounding triggers a biochemical cascade that modifies cellular and physiological responses through induced systemic resistance, and could lead to differences in the chemical composition of residues from Bt corn and conventional corn hybrids (Lewis and Yamamoto, 1990; Smith et al., 1994). These hypothesized differences between Bt and NBt corn would affect decomposition rates and SOC pools in corn agroecosystems of North America. The next sections of this review will provide a critical analysis of findings from studies that evaluated the yield and residue composition of Bt corn and NBt corn, as well as the decomposition of residues from these hybrids.

1.5 Yield of Bt Corn

The yield of Bt and NBt corn near-isolines from seven studies conducted at experimental field sites in the USA and Canada is summarized in Table 3. Soil texture ranged from silt loam to clay loam, and a variety of cultivation practices (plant

populations, tillage, herbicides, etc.) were used. The mean grain yield from five studies with 18 Bt/NBt pairs was 8,829 kg ha^{-1} for Bt corn and 8,367 kg ha^{-1} for NBt corn (Folmer et al. 2002; Dillehay et al. 2004; Ma and Subedi 2005; Mungai et al. 2005; Subedi and Ma 2007). The Bt corn produced 1 to11% more grain than NBt corn, except in the study by Ma and Subedi (2005) where there was 3% less grain from Bt than NBt corn (Table 3).

The silage yield/aboveground biomass from six studies ranged from 11,350-36,315 kg ha^{-1} for Bt corn and 9,910-39,453 kg ha^{-1} for NBt corn (Table 3). Silage yields ranged from 17% lower to as much as 47% higher in agroecosystems with Bt corn, compared to NBt corn (Folmer et al. 2002; Jung and Sheaffer 2004; Motavalli et al. 2004; Ma and Subedi 2005; Fang et al. 2007; Subedi and Ma 2007). This wide range is partly due to variations in experimental conditions, yield potentials of selected hybrids pairs, and measurements among studies, as well as differences in the ECB infestation in the reviewed studies.

It is generally accepted that yields are greater with Bt corn than NBt corn in years with high ECB infestation (more than 2 larvae per plant) (Dillehay et al. 2004; Ma and Subedi 2005). Variability in the results presented here suggests there were phenotypic and genotypic differences between corn types and hybrids unrelated to the Bt gene effect, an issue which can be overcome by testing a large number of hybrids under the same conditions. It would be informative to compare Bt corn and NBt corn with ECB infestation and analyse specific plant components (grain, cobs, stems, leaves and roots) as well as the usual measures of biomass production. At least we need to know the annual

input of C in Mg C ha^{-1} y^{-1} of the non-harvested corn components that contribute to the SOC pool.

1.6 Soil Carbon Inputs from Bt Corn

1.6.1 Chemical composition of Bt corn

Table 3 shows that the average amount of C returned to the soil from the Bt and NBt residue (aboveground biomass minus grain, assuming 40% C content) is 4,937 kg C ha^{-1} and 4,753 kg C ha^{-1}, respectively. The decomposition of the non-harvested corn residues (cobs, stems, leaves and roots) by soil microorganisms results in some CO_2 loss via respiration as well as C stabilization within the SOC pool. Decomposition is affected by the physical integrity and the chemical composition of the residue. Lignin and C:N ratio are especially important because lignin is the most recalcitrant component in the plant tissue and C/N ratio exerts an important control on residue decomposition (Cadish and Giller 1997), which is why my focus will be primarily on these measurements.

The lignin, organic C and organic N concentrations of corn residues in seven studies from North America and three studies from Europe are summarized in Table 4. The lignin concentration of corn residues was most frequently quantified with the Goering and Van Soest (1970) gravimetric method, which gives the Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), and Acid Detergent Lignin (ADL) of plant tissues (Masoero et al. 1999; Rossi et al. 2003; Jung and Sheaffer 2004; Mungai et al. 2005; Fang et al. 2007; Lehman et al. 2008a; Tarkalson et al. 2008). Other analytical techniques to quantify lignin

content in corn residues included the Klason method (Jung and Sheaffer 2004; Poerschmann et al. 2005), the acetyl bromide (AcBr) method (Saxena and Stotzky 2001b; Jung and Sheaffer 2004; Flores et al. 2005), and off-line and on-line thermochemolysis (Poerschmann et al. 2005). Generally, it is not possible to directly compare the values obtained from one method with another. As Poerschmann et al. (2005) pointed out, the similarity between the AcBr and Klason values reported by Jung and Sheaffer (2004) is unusual and suggests underestimation of the Klason lignin fraction. Corn residues are generally separated into stems, leaves, stem internodes, shoots (stems plus leaves), and in some cases whole plants, roots and cobs. In most experiments, plants were harvested at physiological maturity, so the lignin content of plant components would be representative of residues found in corn agroecosystems.

More data is available on the chemical composition of corn stems and shoots than other plant components (Table 4). In three studies, the lignin concentration of stems and shoots was statistically greater in Bt corn than in NBt isolines (Masoero et al. 1999; Saxena and Stotzky 2001b; Poerschmann et al. 2005). The average lignin concentration in the corn stalk from eight studies was between 5.1 and 10.8% for Bt corn and from 3.8 to 8.8% for NBt corn (Table 4). The lignin concentration in Bt corn stems was as much as 63.2% greater or 3.8% lower than the NBt isoline (Masoero et al. 1999; Saxena and Stotzky 2001b; Rossi et al. 2003; Jung and Sheaffer 2004; Mungai et al. 2005; Poerschmann et al. 2005; Lehman et al. 2008a; Tarkalson et al. 2008). Significant differences in lignin concentration were reported by Saxena and Stotzky (2001b), based on the analysis of 11 Bt hybrids and 10 NBt hybrids grown in the field and in a growth chamber. In that study, the lignin content was analyzed by fluorescence microscopy and

staining with toluidine blue in addition to the acetyl bromide method. In three studies where Bt corn stems had less lignin than NBt corn (Table 4), the lignin concentration did not differ statistically between Bt and NBt corn stems (Rossi et al. 2003; Jung and Sheaffer 2004; Mungai et al. 2005).

On average, the lignin concentration in leaves was 4.9% for Bt corn and 4.8% for NBt (Poerschmann et al. 2005; Fang et al. 2007; Tarkalson et al. 2008). Single values for cobs and roots are included in Table 4. The nutrient concentration of corn stems and leaves, averaged from five studies, was 42.8% C and 1.0% N for Bt corn, with 42.3% C and 0.9% N in NBt corn (Table 4).

What if Bt corn, or any corn type, has an elevated lignin concentration? Would that result in an increase in the amount of stable C in the soil? In theory, we can estimate the transformation of plant lignin into stable SOC from a simple decomposition model. For the sake of argument, I hypothesized that Bt corn has an elevated lignin concentration, compared to NBt corn, and tested the effect this might have on residue decomposition and C storage in the soil. Decomposition can be described by an exponential first order equation consisting of two litter pools, one that decomposes rapidly and one that decomposes slowly (Johnson et al. 2007; Bahri et al. 2008). In this example, I considered decomposition as the loss of lignin, rather than the mass loss of litter where:

$$L_t = L_1 \exp(-k_1 t) + L_2 \exp(-k_2 t) \quad (1)$$

L_t is the total lignin (in percent) remaining after time t (days), L_2 is the slow decomposing pool, which is equated with the lignin (in percent) decomposing at the slow constant rate

of K_2 (% d^{-1}), and L_1 is 100-L_2, which represents the non-lignin components that decompose rapidly at the constant rate of K_1. The values for the constants K_1 and K_2 (Table 5) are based on the decomposition rate of corn stems from the study of Johnson et al. (2007), determined from a 498-day decomposition experiment.

Two scenarios representing the range of differences between the lignin concentration of Bt and NBt corn in Table 4 were tested. The Bt lignin concentration was assumed to be 6% or 60% greater than NBt, which was assigned a value of 6% (average lignin concentration in NBt stems, Table 4). The results in Table 5 show that after 200 days of decomposition, the amount of lignin in the undecomposed residue was 7% greater in Bt corn residue than NBt corn when the Bt corn contains 6% more lignin. If these results can be directly extrapolated to the field scale, an agroecosystem where 10,000 kg ha^{-1} y^{-1} of residue is returned to the soil (50% of the 20,000 kg ha^{-1} of NBt aboveground biomass from Table 3), would have up to 38 kg ha^{-1} y^{-1} more lignin remaining from Bt corn after 200 days of decomposition. Since this residue is made up mainly of undecomposed lignin, I propose that it is likely to be stabilized and become part of the slow turnover SOC pool. These calculations show that a meaningful increase in the amount of stable SOC requires the input of corn residues having 6% more lignin concentration than conventional corn residues, a scenario that seems very unlikely.

1.6.2 Lignin content of corn

The above example is based on generalized assumptions on the lignin content of corn. However, lignin is a highly heterogeneous compound, randomly formed with variable

subunit composition and intermolecular linkages (Lewis and Yamamoto 1990; Campbell and Sederoff 1996). Lignin content varies among plant species and between plant cells, meaning that plant components could have different lignin contents and thus decompose at different rates. The data presented in Table 4 shows that the lignin concentration of corn plants was greatest in roots (one study), followed by stems, cobs (one study) and leaves.

The lignin composition and concentration in plant cells is regulated by enzymes in the biosynthetic pathway and gene expression. Environmental stress affects lignin formation and deposition in vascular tissues (Campbell and Sederoff 1996), as does wounding, such as that caused by feeding insects (Lewis and Yamamoto 1990; Smith et al. 1994). Furthermore, wound-induced lignin deposition may cause lignification of the whole cell, in contrast to normal lignification, which occurs only in the cell wall (Lewis and Yamamoto 1990). Although ECB damage was reported in some studies reviewed in Table 4, there was no analysis of the lignin deposition in relation to ECB wounding. I suspect that corn plants infested with ECB, causing tunneling and partial decomposition of the stalk, may differ in lignin concentration from uninfested corn plants but there has been no literature published to support this yet.

1.6.3 Qualitative measurement of molecular lignin composition

Cupric oxide oxidation and degradative thermal methods among others release the vanillyl (V), syringyl (S), and cinnamyl (C) phenols from the lignin molecules, which can then be measured by the gas chromatography-mass spectrometry (GC-MS). The sum of

the three phenols known as VSC has been used as an indicator of total lignin but is not a quantitative measure of lignin especially when using the CuO oxidation method. In CuO oxidation, the lignin molecule is not completely depolymerised (Otto and Simpson 2006) and there are interferences from side-chain reactions (Poerschmann et al. 2005). In addition, the ratio of acid to aldehyde of vanillyl and syringyl is used as an indicator of the degree of decomposition of organic matter, as this ratio increases as decomposition progresses (Poerschman et al. 2005; Otto and Simpson 2006). Poerschman et al. (2005) used these methods to study the difference between Bt and NBt corn lignin at the molecular level. In their study, two pairs of Bt and NBt corn hybrids, Novelis (MON810 event) and its isoline Nobilis, and Valmont (176 event) and its near-isoline Prelude, were field grown in Germany where no ECB infestation was observed. The Bt components had significantly higher lignin concentrations than the NBt components; the average ratio of lignin concentration in Bt/NBt stems (between the 4^{th} and 5^{th} internodes) and leaves was 1.23 and 1.18, respectively. The increase in lignin was mainly due to an increase in the guaiacyl-type lignin (made from vanillyl precursors) and to a lesser extent to an increase in the hydroxyphenyl-type lignin (made from cinnamyl precursors). This was true for both stems and leaves, but was more pronounced in stems. The guaiacyl-type lignin monomers are considered more recalcitrant than the other two lignin monomers (syringyl and cinnamyl derived), which reinforces the belief that Bt corn residue especially the stems are stronger and more resistant to degradation.

1.7 Decomposition of Bt Corn Residues

Corn residues constitute a soil C input, and plant components with high lignin concentrations are expected to have an extended residence time in the soil and therefore contribute to soil C sequestration (Zibilske and Materon 2005). The reviewed studies suggest that there may be more corn residues left in agroecosystems with Bt corn than NBt corn (Table 3). In addition, the lignin concentration tends to be greater in the stems and shoots of Bt corn (Table 4). These factors are expected to slow decomposition rates and increase the stabilization of the C inputs from transgenic corn hybrids (Saxena and Stotzky 2001b; Hopkins and Gregorich 2005). Yet, the experimental evidence does not support this hypothesis unequivocally. Three decomposition studies with controlled-environment conditions showed lower CO_2 production in Bt compared to NBt corn residue amended soils (Dinel et al. 2003; Castaldini et al. 2005; Flores et al. 2005). In contrast, field decomposition litterbag studies showed either no difference in mass loss between Bt and NBt corn residues buried in the field (Lehman et al. 2008a; Tarkalson et al. 2008) or faster decomposition of Bt leaves compared to NBt leaves during early decomposition, after which the two hybrids levelled off at the end of the study (Zwahlen et al. 2007).

In their laboratory experiment, Flores et al. (2005) reported significantly lower CO_2 production (20 to 39% less decomposition during a 32-42 day incubation period) from soils amended with Bt residue than NBt residues of a corresponding crop. They tested transgenic and unmodified hybrids of corn, rice, potato, cotton, canola and tobacco. The lower CO_2 production was not related to differences in C:N ratio or lignin concentration,

or of the inhibition of the activity of soil microbiota (Table 6). Similarly, Castaldini et al. (2005) found 10% less CO_2 respiration from soil incubated with Bt corn residue, but the comparison was done with one Bt11, one 176 Bt and one NBt variety that was not an isoline or near isoline to either of the Bt varieties. It is possible that the results were influenced by corn varieties selection. Dinel et al. (2003) incubated a silty clay loam soil from Sainte-Barbe in Quebec, Canada, with Bt shoots (Pioneer 38W36) and with NBt shoots (Pioneer 3893) and measured 30.5% more cumulative CO_2-C production from the NBt amended soil after 51 days of incubation.

In contrast, Hopkins and Gregorich (2003) reported no difference between CO_2 evolutions in Bt vs. NBt corn residue amended soils. The plant residues used for this study were the vegetative shoots of corn harvested at the 6-leaf stage (Hopkins and Gregorich 2003), while Castaldini et al. (2005) used 10 to 12-week old corn shoots as residue and Flores et al. (2005) used the aboveground residues from plants that had achieved physiological maturity. Some of the difference in the results may arise from the phenological stage at which corn residues were collected. Fang et al. (2007) obtained root, stem, and leaf residue from Bt and NBt corn, and plant parts were added separately to laboratory microcosms containing soils with different textures. The Bt factor had no effect on CO_2 efflux even though the Bt components had higher lignin and C:N than the NBt components. The residue component and soil texture significantly affected CO_2 efflux.

In the field, Tarkalson et al. (2008) studied decomposition of two Bt corn hybrid residues and their isolines in litterbags and reported that the decomposition rates for

leaves, cobs, and stalks were similar for Bt and NBt hybrids. Lehman et al. (2008a) also used the litter bag technique in the field, but found no differences in decomposition of Bt and NBt corn residue even though the Bt corn tissue had higher lignin concentration. They commented on the fact that there was no insect stress during the experiment and that the presence of such stress might result in weaker NBt stalks that are less resistant to decay. It was also noted that the authors were unable to measure mass loss after about one year of the litter burial, which includes the last 20% of the litter residue (presumably the more recalcitrant portion). The reason for this could be due to the increase in the soil to litter ratio which made differences in mass difficult to detect.

Lehman et al. (2008a) measured the mechanical strength of the chopped corn tissue and found that all Bt types tended to have stronger residues, more physically resistant, although not significantly different from NBt types. They suggested that similar analysis be conducted on the un-chopped tissue to see if the chopping had an effect on strength and consequently on decomposition in the field. This point suggests that the tissue size and preparation for decomposition/incubation studies might have an effect on the results.

Results from the above reviewed laboratory and field studies suggest that decomposition rates and CO_2 evolution from Bt corn residue-amended soils are often similar to those amended with NBt residue. This is true even where the Bt corn had higher concentration of the more recalcitrant cell constituents such as cellulose and lignin (Flores et al. 2005; Fang et al. 2007; Lehman et al. 2008a). It is noted that almost all the studies focused on the decomposition of aboveground crop residues (leaves, stems and cobs), and root decomposition was seldom examined.

1.7.1 Decomposition of corn components

Tarkalson et al. (2008) found that corn components differed significantly in their decomposition. After 23 months in the field, the leaves, stalks, and cobs retained 5.5%, 17.7%, and 38.6% of their total C amount respectively, indicating that cobs were the slowest to decompose. Table 7 shows the average chemical composition of these plant components. The soluble fraction appears to control the decomposition rate in addition to the C:N ratio of the plant components, since leaves, having the highest percentage of the soluble fraction and the lowest C:N ratio, decomposed rapidly even though they contained higher lignin than stalks or cobs (Table 7). In the Merschman hybrids M-0012Bt and M-00110, Fang et al. (2007) reported that stems had the highest C concentration and C:N ratio followed by leaves then roots. Decomposition was not correlated to the C:N ratio, as leaves decomposed more quickly than stems and roots, suggesting that leaf tissues contained more soluble than structural carbon than stems and roots. They also noted that after 73 days of incubation, the factor that most significantly affected cumulative decomposition was the soil texture. In the silty clay soil Bt roots, which had the highest lignin concentration among all Bt and NBt components, decomposed slower than Bt leaves and Bt stems whereas the NBt roots had a faster decomposition rate. This may be related to the presence of soil microbial communities capable of degrading lignin and other resistant compounds in roots. Filamentous fungi (mainly basidiomycetes) are key organisms for lignin degradation (Hammel, 1997). As discussed earlier, the decomposition of roots from Bt corn has not received much attention, although roots constitute a considerable soil C input and may be more slowly

decomposed than other corn residues. Studies are needed to consider the decomposition of Bt corn roots in the context of SOC dynamics and soil C sequestration.

1.8 Conclusions

Given that Bt corn production is increasing globally and accounts for about 50% or more of the corn production in North America if stacked hybrids (e.g., Bt plus Roundup Ready genes) are included, it is worth examining the effects of this crop on SOC dynamics. There is evidence that higher Bt corn yields produce more residues than NBt corn, constituting a greater C input to agroecosystems. Some studies suggested that the insertion of the Bt transgene into the corn genome could affect the lignin concentration of Bt corn, however it should be noted that the Bt transgene does not affect the biosynthetic pathway of lignin production in the plant (Jung and Sheaffer, 2004) and should not affect the amounts of lignin produced. Since only three out of the seven reviewed studies had significantly greater lignin concentration in the Bt corn tissue, it could be argued that the greater lignin is due to difference in the phenotypic characteristics of the selected hybrids. In most of the studies the Bt and NBt hybrids were not isolines but rather near-isolines, which means that they are not exactly the same and may vary in their crop heat units requirement, among other traits. In addition, there appears to be no difference in the decomposition rates of Bt and NBt corn residues even when Bt residues exhibited greater lignin concentrations and no lasting adverse effects on soil decomposers. This suggests that the effect of Bt corn production on the soil ecosystem is minimal, though this may be more appropriately stated for soils that are not continuously under Bt crop production for the long term.

Finally, corn plants for many experiments were grown in greenhouses or growth chambers without the introduction of the ECB larvae, which are common in the field. The response of corn to stress conditions and injury of the vascular tissue from the ECB could lead to differences in chemical composition and should be considered as a factor when conducting experiments that compare Bt corn and NBt corn.

Table 1 Bt corn types classified by gene transformation event. Data from Hagerman (1997), Hyde et al. (1999), US-EPA (2001), Baute (2004), Dow Agrosciences (2007), and Icoz and Stotzky (2008).

Event	Cry Protein	Brand Name
176	Cry1Ab	KnockOut® - Novartis† (field and popcorn)
176	Cry1Ab	NatureGard® - Mycogen†
Bt11	Cry1Ab	YieldGard® ® or Attribute™- Northrup King/Novartis Seeds (field and sweet corn)
MON810	Cry1Ab	YieldGard® - Monsanto
TC1507	Cry1F	Herculex1 – Dow AgroSciences/Pioneer Hi-Bred
MIR604	mCry3a (modified Cry3a)	SYN-IR604-8 (rootworm) – Syngenta
CBH-351	Cry9C	StarLink™ - Aventis Crop Science (corn for feed or industrial uses only) (not approved in Canada)
MON863	Cry3Bb	YieldGard® Rootworm- Monsanto
DAS-59122-7	Cry34Ab1 + Cry35Ab1	Herculex RW (rootworm) – Dow AgroSciences/Pioneer Hi-Bred

† EPA Registration expired and not renewed.

Table 2 Expression of Cry Protein in plant tissue, on a fresh weight basis unless otherwise noted (Canadian Food Inspection Agency 1996, 1997, 2007; Dow Agrosciences 2007; US-EPA 2001).

Active Ingredient	Leaf (ng mg^{-1})	Root (ng mg^{-1})	Pollen (ng mg^{-1})	Grain (ng mg^{-1})
Cry1Ab (176)	0.44 - 0.47†	0.008	1.14 - 2.35	<0.005 (kernel)
Cry1Ab (Bt11)	3.3	2.2 - 37 ng mg^{-1} protein	<0.09 dry wt. pollen	1.4 (kernel)
Cry1Ab (MON810)	7.9 - 10.3	Not available	0.09	0.19 - 0.39
Cry1F	111	Not available	136	90
mCry3a	5 - 26 (dry wt.)	7 - 25 (dry wt.)	0	0.85 (dry wt. kernel)
Cry9C	44	25.9	0.24	18.6 (kernel)
Cry3Bb1	30 - 93	3.2 - 66	30 - 93	49 - 86
Cry34Ab1 + Cry35Ab1	67.4 + 43.3	54.9 + 10.4 (R1 stage root)	68.1 + 0.14	45.7 + 1.61

† At physiological maturity.

Table 3 Yield of Bt and NBt corn from selected studies in North America. I assumed that yield values were reported on a dry matter basis. Aboveground biomass, plant yield, and silage refer to all aboveground parts, including grain. Calculations for silage/aboveground biomass yield included whole plant data (Jung and Sheaffer 2004) as well as silage and aboveground biomass estimates. The Bt and NBt corn yields (average ± standard error) from these studies are provided at the end of the table.

Study Paper	Experimental Conditions	Description	Yield Range (kg ha^{-1})	Yield Average (kg ha^{-1})	Difference between Bt & NBt (%)
Mungai et al., 2005 Missouri, USA	5 Bt and 5 NBt isolines no-till planted for 2 years. ECB infestation observed.	Bt grain	4835-8102	6704	
		NBt grain	5239-6704	6075	10.4
Dillehay et al., 2004 Pennsylvania & Maryland, USA	1 Bt, 1 NBt isoline, & 1 lead NBt hybrid grown in 15 experiments on 2 sites (Pennsylvania & Maryland) over 3 years. ECB infestation observed.	Bt grain	3900-13300	9100	
		NBt grain	4000-13500	8600	5.8
Ma and Subedi, 2005 Ottawa, Ontario, Canada	Average of 3 Bt/NBt pairs (2000), 6 Bt/NBt pairs (2001), 7 Bt/NBt pairs (2002) (total hybrids used over years is 7). ECB infestation observed.	Bt grain	4170-9310	6570	
		NBt grain	4350-9250	6780	-3.1

Subedi and Ma, 2007 Ottawa, Ontario, Canada	Average of 1 Bt/NBt pairs over 2 years. No significant ECB infestation observed.	Bt grain	NA[†]	10050	10.7
		NBt grain	NA	9075	
Folmer et al., 2002 Nevada, USA	Early maturing N4242Bt, N4242 NBt,and late maturing N7333Bt, N7333 NBt grown without irrigation for silage at one field and N7333Bt, N7333 grown with irrigation for grain at another field. 33-56% ECB infestation in NBt corn observed.	Bt grain (early maturing)	NA	8323	8.7
		NBt grain (early maturing)	NA	7658	
		Bt grain (late maturing)	NA	9509	6.2
		NBt grain (late maturing)	NA	8957	
		Bt grain (late maturing, irrigated)	NA	11549	1.1
		NBt grain (late maturing irrigated)	NA	11424	
		Bt silage yield (early maturing)	NA	31608	16.5
		NBt silage yield (early maturing)	NA	27125	

		Bt silage yield (late maturing)	NA	36315	-8.0
		NBt silage yield (late maturing)	NA	39453	
Ma and Subedi, 2005 Ottawa, Ontario, Canada	Average of 3 Bt/NBt pairs (2000), 6 Bt/NBt pairs (2001), 7 Bt/NBt pairs (2002) (total hybrids used over years is 7). ECB infestation observed.	Bt aboveground biomass (stalk, leaf, ear)	8010-14100	11350	-2.0
		NBt aboveground biomass	7820-14010	11580	
Subedi and Ma, 2007 Ottawa, Ontario, Canada	Average of 1 Bt/NBt pairs over 2 years. No significant ECB infestation observed.	Bt aboveground biomass (stalk, leaf, kernel)	NA	18315**	8.2
		NBt aboveground biomass	NA	16928‡	
Motavalli, 2004 Missouri, USA (range from 2 years)	5 Bt and 5 NBt isolines planted on one site over 2 years. ECB infestation observed.	Bt aboveground biomass	14568-28889	18568	15.2
		NBt aboveground biomass	10864-21728	16123	
Jung and Sheaffer, 2004 Minnesota, USA	Six hybrids (3 MON810 & 3 Bt11) and the NBt isolines, field grown at four locations. ECB infestation observed.	Bt plant yield (average 2 DKC hybrids over 4 locations)	NA	21225‡	6.8

		NBt plant yield (average 2 DKC hybrids over 4 locations)	NA	19875‡	
		Bt plant yield (N3030Bt one location)	NA	17400‡	
		NBt plant yield (N3030 one location)	NA	21000‡	-17.1
Fang et al., 2007 Missouri, USA	1 Merschman Bt and NBt hybrid pair, field grown. ECB damage observed in NBt.	Bt aboveground biomass	11270-17910	14590	
		NBt aboveground biomass	5750-14070	9910	47.2
† NA = data not available ‡ Conversion from g/plant to kg/ha based on estimate of 75,000 plant/ha density.		Average Bt grain yield		8829 ± 678	
		Average NBt grain yield		8367 ± 664	
		Average Bt silage/aboveground biomass yield		21171 ± 3009	
		Average NBt silage/aboveground biomass yield		20249 ± 3346	

Table 4 Lignin, organic C and total nitrogen concentration of plant components originating from Bt corn and NBt corn from selected studies. Averages (± standard errors) do not include the stem values (in italics) from Fang et al. (2007), which were already included by Mungai et al. (2005).

Study	Experimental conditions	Description	Component	Bt Corn			NBt Corn			Difference between Bt and NBt lignin (%)
				Lignin (%)	Organic Carbon (%)	Total Nitrogen (%)	Lignin (%)	Organic Carbon (%)	Total Nitrogen (%)	
Tarkalson et al., 2008 (Nebraska, USA)	2 Bt hybrids (MON810) and the NBt isolines. In the field for 1 year.	ADL Lignin (harvested at physiological maturity)	Leaves	7.8	37.9	1.6	8.8	37	1.5	-11.4
			Stems	6.5	43.6	0.8	6.5	43.5	0.7	0.0
			Cobs	5	46.3	0.6	4.6	45.9	0.7	8.7
Jung & Sheaffer, 2004 (Minnesota, USA)	Six hybrids (3 MON810 & 3 Bt11) and the NBt isolines, field grown at four locations. 23-35% ECB damage in NBt hybrids.	ADL Lignin (harvested at physiological maturity)	Whole plant	2.2	NA[†]	NA	2.2	NA	NA	0.0
			Stem Internode	6.4	NA	NA	6.5	NA	NA	-1.5
Poerschmann et al., 2005 (Aachen, Germany)	2 Bt hybrids (MON810 & 176) and the NBt isoline. No ECB infestation.	Off-line thermochemolys-is Lignin confirmed with Klason lignin (harvested at BBCH 75 growth stage)[‡]	Leaves	3.7	NA	NA	3.5	NA	NA	5.7
			Stems	10.8	NA	NA	8.8	NA	NA	22.7

Mungai et al., 2005 (Missouri, USA)	5 Bt the NBt isolines field planted for 2 years. ECB infestation observed.	ADL Lignin	Stems	6.8	41.9	0.54	7.0	42.3	0.60	-2.9
Lehman et al., 2008a (South Dakota, USA)	2 Bt (one MON810 and 1 stacked) and 1 NBt isoline field planted for 2 years. No ECB infestation.	ADL Lignin (harvested at full maturity)	Shoots	5.6	42.9	0.78	5.3	42.7	0.77	6.1
Fang et al., 2007 (Missouri, USA)	1 Merschman Bt and NBt hybrid pair, field grown. ECB damage observed in NBt.	One hybrid (ADL Lignin) (stem component included in data of Mungai et al., 2005)	Leaves	3.3	48.9	0.71	2.1	48.3	0.96	57.1
			Stems	*7.8*	*50.6*	*0.52*	*4.3*	*49.8*	*0.66*	*80.2*
			Roots	11.7	43.5	1.2	9.9	41.7	1.15	18.2
Flores et al., 2005 (Long Island, N.Y., USA)	3 Bt (2 field and one sweet corn) and the NBt isolines in pots in plant growth room. No ECB infestation.	AcBr Lignin (harvested at production of seeds) Lignin data is included in Saxena and Stotzky, 2001b.	Stems	-	41.8	1.4	-	39.7	1.1	
Masoero et al., 1999 Northern Italy)	2 Bt and the NBt isolines field grown at 3 locations. 50.2% stalk breakage in the NBt due to ECB (7% in Bt).	Average of 2 hybrids (ADL Lignin)	Stover	6.2	NA	NA	5.8	NA	NA	7.8

Rossi et al., 2003 (Northern Italy)	2 Bt and the NBt isolines field grown at 4 locations. Breakage observed in the NBt due to ECB.	Average of 2 hybrids (ADL Lignin)	Shoots (stover)	5.1	NA	NA	5.3	NA	NA	-3.8
Saxena & Stotzky, 2001b	11 Bt and 10 NBt hybrids in growth chamber.	Range (average) of 11 Bt and 10 NBt hybrids in growth chamber (AcBr Lignin)	Stems	7	NA	NA	4.9	NA	NA	42.9
Saxena & Stotzky, 2001b (New York, USA)	8 Bt and 7 NBt hybrids in field.	Range (average) of 8 Bt and 7 NBt hybrids in field (AcBr Lignin)	Stems	6.2	NA	NA	3.8	NA	NA	63.2
		Average of all values		6.3 (±0.6) ‡	43.4 (±1.1)	1.0 (±0.1)	5.7 (±0.6)	42.6 (±1.2)	0.9 (±0.1)	
		Average values for stems and shoots		6.7 (±0.5)	42.6 (±0.4)	0.9 (±0.2)	6.0 (±0. 5)	42.1 (±0.8)	0.8 (±0.1)	
		Average values for leaves		4.9 (±1.4)	43.4 (±5.5)	1.2 (±0.4)	4.8 (±2.0)	42.7 (±5.6)	1.2 (±0.3)	

† NA = data not available.
‡ cob content milky, about 40% dry matter, http://en.wikipedia.org/wiki/BBCH-scale_(maize).

Table 5 Amounts of lignin remaining after 200 days of decomposition under three scenarios in which Bt corn had greater lignin concentration than NBt corn, calculated from the double exponential decomposition model (Johnson et al. 2007; Bahri et al. 2008). The amount of corn residue returned to the soil is assumed to be 50% of the aboveground biomass = 10,000 kg ha^{-1} y^{-1}

	Time (days)	L_t† (%)	$L_1 exp(-kt)$‡ (%)	$L_2 exp(-kt)$ⱷ (%)	Amount lignin remaining (Kg ha^{-1} y^{-1})	Difference between amount lignin remaining compared to NBt residue (Kg ha^{-1} y^{-1})
Average lignin of NBt corn stems	1	94.701	88.703	5.998		
	200	5.682	0.001	5.681	568	-
Scenario 1 Lignin is 6% greater in Bt vs. NBt corn	1	94.724	88.326	6.398		
	200	6.061	0.001	6.060	606	38
Scenario 2 Lignin is 60% greater in Bt vs. NBt corn	1	94.903	85.306	9.597		
	200	9.091	0.001	9.090	909	341

†total lignin remaining after 200 days of decomposition, ‡fast decomposing non-lignin fraction, ⱷ slow decomposing lignin fraction. K_1=0.06% d^{-1}, K_2=0.000273% d^{-1}.

Table 6 Carbon dioxide (CO_2) evolution from soils amended with 0.5% (by weight) residue from Bt corn and NBt near-isolines (data from Flores et al. 2005).

Bt isoline	Residue type and % added (w w^{-1}, dry tissue)	CO_2 evolution (mg C 100 g $soil^{-1}$)	C:N (leaf)	C:N (stem)	NBt isoline	CO_2 evolution (mg C 100 g $soil^{-1}$)	C:N (leaf)	C:N (stem)
NK4640Bt	stem + leaf at 0.5%	52.3	79	149.9	NK4640	73.1	80.3	171.8
966	stem + leaf at 0.5%	55.2	-	-	Prime Plus	76.3	-	-
DK647Bty	stem + leaf at 0.5%	58.3	-	-	DK647	75.3	-	-
NK6800Bt	leaves at 0.5%	91	38.4	26	NK6800	108	26.6	17.5
NK6800Bt	stem at 0.5%	112	38.4	26	NK6800	181	26.6	17.5

Table 7 Chemical composition of the fiber fraction (decreasing order) in leaves, stalks, and cobs of corn hybrids (average of four hybrids) (data from Tarkalson et al. 2008).

Leaves	Stalks	Cobs
Soluble fraction (40.3%)	Cellulose (36.9%)	Hemicellulose (42.8%)
Hemicellulose (28.6%)	Soluble fraction (34.5%)	Cellulose (35.1%)
Cellulose (23.2%)	Hemicellulose (21.8%)	Soluble fraction (17.3)
Lignin (8.0%)	Lignin (6.5%)	Lignin (4.8%)

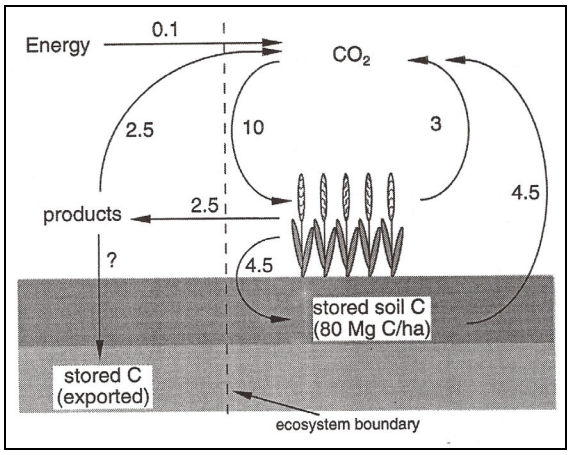

Fig. 1 Approximate annual carbon fluxes in a typical corn agroecosystem in southern Ontario. All fluxes are in Mg carbon ha^{-1} y^{-1} (Lal et al. 1997).

FORWARD TO CHAPTER TWO

In the literature review, it is evident that there is still some controversy about the variations in lignin between Bt and NBt corn hybrids and between the amounts of residue produced from those hybrids. The objective of the first experiment is to test the agronomic performance and chemical composition of several Bt and NBt hybrids pairs that are suitable for the Quebec growing conditions in order to assess possible effects on the soil ecosystem.

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CHAPTER 2.

Field-grown Bt and non-Bt corn: yield, chemical composition, and decomposition.

2.1 Abstract

Bt corn 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. My objectives were to evaluate the biomass of field-grown Bt and NBt 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/NBt corn pair were buried in the field and sampled periodically during one year. 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 NBt stems in one year of the two-year 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 NBt stems. I 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-NBt corn residue.

2.2 Introduction

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 2010a). Bt corn hybrids are popular with farmers in this region because the gene from the Bt gram positive bacterium produces crystal-like proteins (Cry protein) for protection against European corn borer (*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 non-target 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 to 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 non-grain components. Greater stover and above ground 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 ten Bt hybrids with different insertion events (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; Zurbrugg et al. 2010). 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 0.44–111 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 above mentioned 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 20-39% less CO_2 evolution from soils amended with Bt corn residue compared to 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, 2008b; Tarkalson et al. 2008; Lehman et al. 2010; Zurbrugg et al. 2010) even when the Bt residue had significantly greater lignin concentration (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/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 eight month period.

This study was predicated on the hypothesis that Bt corn produces more grain and stover biomass under field conditions, but I did not expect differences in lignin or C and N concentrations 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 NBt corn differed and could therefore alter the residue decomposition rates. Decomposition of Bt and NBt corn residues was tested with intact corn stems in a one-year buried litterbag experiment; based on previous litterbag experiments, the hypothesis was that Bt and NBt stems would have similar decomposition rates in the field.

2.3 Materials and Methods

2.3.1 Location, experimental design, soil and corn hybrid characteristics, harvesting

The experiment was carried out in two growing seasons during 2008 and 2009 in a field located at the Emile A. Lods Agronomy Research Centre of McGill University in Ste-Anne-de-Bellevue, Quebec, Canada (45^{o}24'N, 73^{o}56'W). The field was planted with continuous corn prior to 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 classified as a fine-loamy, mixed, frigid Typic Hapludalf (Grey Brown Luvisol) 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 m x 19 m, was divided into 54 plots, each 5 m x 3 m, and each plot contained 4 rows with 75cm row spacing. Pre-seeding 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 April 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}. Post-emergence 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 (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 re-seeding 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 cm x 2 cm mesh) to protect young seedlings, which was removed when the plants were at the 5-6 leaf stage, and ear netting (0.7 cm x 0.7 cm mesh) was also placed on 10 corn ears per plot at the blister (R2) development stage.

Harvesting was done 126 days 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 days 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), stems (including the first node above the soil surface), and the ears into grain + 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 25-30 cm within a week of the aboveground harvesting and thoroughly cleaned to remove adhering soil particles. All collected material was dried at 60^oC for 48 hours and ground using a Wiley mill to pass through a 1mm mesh sieve.

2.3.2 Plant tissue analysis

Ground leaves, stems, and roots were analyzed for fiber concentration (hemicellulose, cellulose, lignin), C, and N. 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).

2.3.3 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 stems, up to 5 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 6 replicates and 7 sampling dates. Eighty-four litterbags, 10 cm x 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 6 bags from each treatment were collected monthly for the first 6 months, and one set of bags kept over the winter and collected one year after they were buried. Litter bags were buried in no-till plots with low residue input in a long-term corn agroecosystem on the Macdonald Research Farm in Ste-Anne-de-Bellevue, Quebec, Canada (45^{o}30'N, 73^{o}35'W). Soil at this site was a sandy loam, frigid Typic Endoaquent (Dystric Gleysol) of the St-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^{o}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 months 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.

2.3.4 Statistical analysis

Analysis of variance (ANOVA), using the Proc GLM procedure on SAS software (SAS Institute Inc. 2009), was done to test the effects of hybrid, Bt gene modification (GM), and their interaction on yield, fiber concentration, and C and N concentrations of field-grown corn. Because the hybrids had different CHU requirements, I calculated the difference between CHU requirement and actual CHU acquired in each year and used it 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. 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 Inc. 2009) was used to fit the mass loss data into a single exponential decomposition model (Olson 1963).

2.4 Results

2.4.1 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 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 about 1 Mg ha^{-1} more grain than the NBt hybrids in both years, but the difference was not statistically significant. Grain moisture content (Appendix 1) 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.

2.4.2 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 (Appendix 2) and cellulose (Appendix 3) in corn tissues were not affected by Bt gene modification or hybrid type. In 2008 and 2009 respectively, hemicellulose concentration was between 22-32% and 26-35% in leaves, 17-28% and 15-25% in stems, and 24-31% and 26-35% in roots. Cellulose concentration in 2008 and 2009 respectively, was 24-39% and 28-40% in leaves, 26-50%

and 27-54% in stems, and 35-48% and 34-48% 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). 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).

2.4.3 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 one year in the field, the Bt stems lost 56% of their mass compared to 43% for NBt stems (Figure 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 5 (P=0.0105) but total mass loss after one year was not different between Bt and NBt stems. Decomposition rate constants were k=0.0871 ± 0.0054 for the Bt stems and k=0.0912 ± 0.0054 for the NBt stems.

2.5 Discussion

The experiments that were designed to test differences between Bt and NBt hybrids in yield and chemical composition were carried out in two years with a relatively large number of hybrids so that variability in hybrid types could be accounted for however, growing the corn in the same field during the two seasons creates a limitation in 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 South-Eastern Quebec and Eastern Ontario regions and can be used to reasonably 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 South-Eastern Quebec and Eastern Ontario (CEROM, 2009; OMAFRA, 2010).

Since no ECB stress was observed in either study year, I 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} more grain (data not shown) 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 I 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, I would expect smaller stover yield from NBt hybrids with some ECB infestation due to the tunneling damage of the feeding larvae. My 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 above-ground 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 my results; however they reported greater dry matter in kernels and leaves in the Bt hybrids for two years when moderate ECB damage occurred (i.e.,

stems were infested by corn borers with 3 or more holes but no stalk lodging was observed).

Fiber concentration 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 my results do not agree with Saxena and Stotzky (2001), who reported up to 97% greater lignin concentration in Bt corn stem sections, between the 3rd and 4th nodes, than in NBt corn stems. The corn stem sections taken in this study (1st to 14th nodes in 2008, 1st to 7th nodes in 2009) differed from those selected in the Saxena and Stotzky (2001) study, but other work suggests that such large differences in lignin concentration between Bt and NBt corn stems is unusual. Lehman et al. (2010) reported similar lignin concentrations between Bt and NBt corn stems, based on stem sections (2nd to 3rd 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 to 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 (1st to

7^{th} node) to whole stems (1^{st} to 14^{th} 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 to 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 one year 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 concentrations (Mungai et al. 2005; Fang et al. 2007). From the data presented by Tarkalson et al. (2008) and Lehman et al. (2010), I estimated that stalk/stem mass loss from litterbags accounted for 58% of the initial weight (after 11 months) and 70% of the initial weight (after 8 months), respectively. In contrast, mass loss from litterbags in this study accounted for 56% or less of the initial weight after 12 months, indicating a lower decomposition rate, which was likely due to cooler weather conditions at my 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} y^{-1}; at about 46% C, this constitutes an

addition of 2.3 Mg C ha^{-1} y^{-1} and half of the residue-C is expected to remain in the soil after one year in this 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 one year 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.

2.6 Conclusions

I 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 one year, which in addition to root input, constitute an annual input of about 1.1 Mg C ha^{-1} y^{-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 x 10^4 Mg C from corn land (1.3 million ha) in Eastern Canada, would be retained in SOM, contributing to the estimated potential of 0.08 Pg C y^{-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 NBt residue after 6-12 months 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.

Table 1 Mean annual temperature, total precipitation, and Crop Heat Units during the 2008 (May-Sep.) and 2009 (May-Oct.) growing seasons at Ste-Anne-de-Bellevue, QC, Canada (Environment Canada 2010a).

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

†Acquired Crop Heat Units calculated from emergence day till harvesting day.

‡Values in parentheses are normals for 1971 – 2000 in Ste-Anne-de-Bellevue (Environment Canada, 2010b).

Table 2 Characteristics of 18 hybrids used in the field experiment. Source: (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

† CB = Corn Borer Protection, GT = Glyphosate Tolerant, LL = Gluphosinate Herbicide Resistant, RR = Roundup Ready.

‡ Strength 1-9, 1=best.

ⁿ 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.

Table 3 Means of grain and stover yield (Mg ha^{-1}) of 18 Bt and NBt 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.

		2008		2009	
Hybrid	**Bt/NBt**	**Grain yield (Mg ha^{-1})**	**Stover Yield (Mg ha^{-1})**	**Grain yield (Mg ha^{-1})**	**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 x GM	NS	NS	NS	NS

† NS = Not Significant.

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

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 and NBt 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 x 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

†Hybrid and Hybrid x GM were not significantly different for N and C:N ratio for any component in any year.

‡NS = Not Significant.

§Log transformation done for 2009 root lignin, 2009 leaf N and C:N, and 2009 root C:N.

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. Stems represent below ear sections in 2009. Values are the means ± standard error (n=54).

Component	2008		2009	
	Lignin (g kg^{-1})	**C:N**	**Lignin (g kg^{-1})**	**C:N**
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.

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

	Treatment	**C (g kg^{-1})**	**N (g kg^{-1})**	**C:N ratio**
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

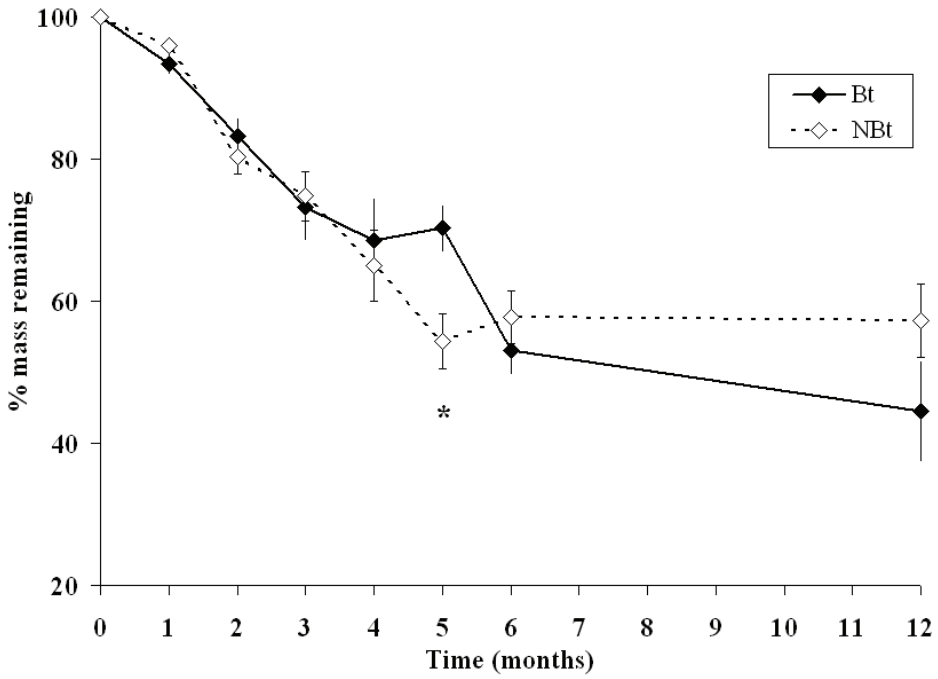

Figure 1 Stem mass remaining of Pioneer 38W22 (Bt) and Pioneer 38W21 (NBt) after one year in the field. Error bars are the standard error (n=6). Significantly different values at α=5% are marked with an asterisk.

FORWARD TO CHAPTER THREE

In chapter two, there was no difference between Bt and NBt corn hybrids in terms of yield and chemical composition under field conditions where no herbivory was observed. It is hypothesized that the chemical composition of NBt corn will be affected by injury from the European corn borer under relatively high infestation rates and that the yield and biomass accumulation will be less than non-injured Bt corn or NBt corn. A greenhouse pot experiment where plants are infested with ECB was designed to test this hypothesis. The results from a 2-year pot experiment are presented next.

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CHAPTER THREE

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

3.1 Abstract

Plant herbivores often stimulate lignin deposition in injured plant tissue, but it is not known whether corn reacts to ECB injury in this manner. Bt gene 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 non-NBt) were grown in pots and half were infested with ECB. The experiment was repeated in two years. ECB injury increased the lignin concentration in corn leaves in one of two years and lowered the C:N ratio in injured stems. Lignin concentration in leaves was greater in Bt than NBt corn in one year and Bt stems had greater N concentration than non-NBt stems in one year of the two 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*

3.2 Introduction

European corn borer is a tunneling insect that bores into corn 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 (Ecker and Davis 1987;

Baron and Zambryski 1995; Dixon and Paiva 1995). 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 (Vance et al. 1980; Walter Jr et al. 1990; Nicholson and Hammerschmidt 1992; Stange Jr et al. 2001; 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 the 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 (Jeffries 1994; Campbell and Sederoff 1996; Hammel 1997; Hopkins et al. 2001; 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 concentration than NBt corn (Saxena and Stotzky 2001; Poerschmann et al. 2005) and decomposed more slowly when residues were mixed with soil (Dinel et al. 2003; Castaldini et al. 2005; 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 concentration, lignin:N ratio and the C:N ratio (Fogel and Cromack Jr 1977; Melillo et al. 1982; Cadisch and Giller 1997; Vanlauwe et al. 1997; Johnson et al. 2007). Damage to leaves and vascular tissue by herbivory affects translocation of photosynthates and nutrients within the plant (Mason et al. 1996; Martin et al. 2004) 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 (Kessler and Baldwin 2002; Howe and Schaller 2008), 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. Zwahlen et al. (2007), Lehman et al. (2008a), and Tarkalson et al. (2008) 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 one 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 (Hedges and Ertel 1982; Hedges et al. 1988; Goñi and Montgomery 2000; Poerschmann et al. 2005, 2008; Otto and Simpson 2006; Loh 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, C and N 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 are not greenhouse-protected there is a possibility of fungal infection through the injury sites however, no differentiation is made between direct ECB infestation effects or indirect fungal infection effects and the measured lignin deposition response will represent 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, I also considered the effect of ECB injury on the chemical composition of leaf tissue.

3.3 Materials and Methods

3.3.1 Greenhouse pot 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, C and N concentration 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 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 an outside 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 kg 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 g kg^{-1} clay, 14.1 g organic C kg^{-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 two months after seeding, and provided a total of 460 mg N per pot, 117 mg K 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 (during the V6 growth stage), three egg masses (each mass consisting of about 50 eggs) were placed in the leaf whorl. In the second infestation (during the tasseling stage), 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 three 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 and stems (without the tassel). 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^{o}C for 48 hours and ground using a Wiley mill to pass through a 1 mm mesh sieve prior to analysis.

To estimate lignin concentration, 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 concentrations were measured with a CN Flash EA Analyzer (Carlo-Erba, Milan, Italy).

3.3.2 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 x 4 corn hybrids), five replicate stem sections were 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 = 3-5mm, medium =6-9mm, and thick = 10-13mm. Weighed stem pieces were placed in mesh nylon/polyester bags, 10 cm x 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 1m 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.

Stems from litterbags were cleaned with distilled water within hours of sampling and dried at 50°C for 48 hours. 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.

3.3.3 Lignin molecular characterization

Lignin molecular characterization by the alkaline CuO oxidation method was used to assess 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^{o}C for 1.5 h to allow the oxidation reaction to occur. The supernatant was then transferred to clean tubes, 0.1 mL (580 μg 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^{o}C for 1 hour. The samples were analyzed on an Agilent 6890N 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 x 0.25 mm x 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 sample volume was 1 μL, injected in splitless mode, with an initial injector temperature of 120ºC, followed by a 10ºC sec^{-1} ramp to a final temperature of 300ºC that was held for 10 min. The initial oven temperature was set at 100ºC and maintained for 10 min, followed by a ramp of 20ºC min^{-1} to a final temperature of 320ºC 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. Recovery of the internal standard, ethyl vanillin, was between 70% and 98%.

3.3.4 Statistical analysis

The effect of hybrid, genetic modification (Bt, NBt), ECB injury and the interaction of Bt and ECB on lignin concentration, 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.

3.4 Results

3.4.1 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 tables 1 to 3, I 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 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 I 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, N and C:N ratio was attributable to hybrids, as indicated in Tables 2 and 3.

Leaf lignin concentration was greater in ECB-injured NBt plants in 2008 (P=0.0074) but not in 2009 (Table 2). Leaf lignin concentration 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 concentration 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 concentration (37.8 g kg^{-1}) was in the non-infested Bt leaves and the smallest lignin concentration (28.7 g kg^{-1}) was in the non-infested NBt leaves. There was no effect of ECB infestation or ECB injury on lignin concentration in the stems in both years. Table 2 also shows that lignin concentration in the stems was not affected by the Bt gene in both years. I 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 N 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 N concentration and C:N ratio in corn tissue during this study (Table 3). No ECB effect was observed on leaf N concentration 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 x ECB interaction effect on N concentration 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).

The Bt gene had an inconsistent and often marginal effect on the N 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).

3.4.2 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 five 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).

3.5 Discussion

3.5.1 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 concentration, 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 concentration 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. My findings appear 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 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 (Appendix 4) compared to the uninjured plants, N concentration in the grain was not affected. Therefore, the 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 (Nykanen and Koricheva 2004; Howe and Schaller 2008), 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 MZ310/MZ540 hybrid stems had lower C:N (115) and lignin:N (16.4) ratios than the non-injured (C:N = 177 and lignin:N = 29.0). 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.

3.5.2 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 concentration in the stems of 10 Bt hybrids grown in a growth chamber and 8 field-grown Bt hybrids than their NBt 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. I cannot confirm that the Bt gene affects lignin concentration 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 concentration; lignin formation and deposition is completed as the plant matures, which could be why corn harvested at physiological maturity in 2009 had greater lignin concentration 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 near-isolines and Bt stems had 25% more N than NBt stems. Escher et al. (2000) reported a non-significant difference in N concentration between Bt and NBt corn leaves of about 9%, which is in agreement with

my 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 μg g^{-1} to 10.3 μg 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 μg g^{-1} stem fresh weight in Bt corn that had the 176 Bt gene insertion event.

3.6 Conclusions

The results from this study show that lignin concentration in corn stems likely is not affected by herbivore injury, whereas leaf lignin concentration could be affected by injury, although more work is needed to confirm this conclusion. I 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 my 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. My findings indicate no consistent effect of genetic modification on the lignin concentration 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.

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	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)[d]						

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)†	NS	NS	NS	*P*=0.0475	*P*<0.0001	*P*<0.0001
Bt gene (uninjured plants)‡	NS	NS	NS	NS	n.d.	n.d.

† 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).

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

ⁿ NS = not significant (P>0.05), n.d. = not determined.

Table 2 Lignin concentration (g kg^{-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 g kg^{-1}		
NBt ECB	39.3 ± 1.8	33.2 ± 0.8_a †	67.7 ± 3.0	56.7 ± 3.0
Bt ECB	33.6 ± 1.6	33.5 ± 0.8_a	63.2 ± 2.5	58.6 ± 2.5
NBt no-ECB	34.4 ± 1.5	28.7 ± 0.8_b	72.2 ± 3.1	55.3 ± 3.2
Bt no-ECB	31.6 ± 0.9	37.8 ± 1.3_c	72.4 ± 2.5	63.2 ± 2.7
		Treatment effects (Probability level) #		
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)‡	*P*=0.0239	NS	NS	NS
ECB injury (injured vs uninjured plants)ⅆ	*P*=0.0007	NS	NS	NS
NBt vs. Bt (non-infested plants)¶	NS	*P*<0.0001	NS	NS

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

‡ 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.

ⅆ 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).

¶ 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.

NS = not significant (P>0.05).

Table 3 Total nitrogen concentration (g kg^{-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	$6.70 \pm 0.66_{a}$	$79.0 \pm 9.2_{a}$ †	$9.28 \pm 0.65_{a}$	$53.8 \pm 4.3_{a}$	4.00 ± 0.23	119 ± 6	7.52 ± 0.29	$63.0 \pm 3.0_{a}$
Bt ECB	$9.48 \pm 0.43_{b}$	$47.2 \pm 1.9_{b}$	$6.56 \pm 0.34_{b}$	$72.7 \pm 3.4_{b}$	3.61 ± 0.14	129 ± 5	7.88 ± 0.25	$59.0 \pm 2.0_{a}$
NBt no-ECB	$7.89 \pm 0.69_{ab}$	$65.1 \pm 5.4_{ab}$	$8.72 \pm 0.41_{a}$	$54.3 \pm 3.4_{a}$	2.83 ± 0.20	171 ± 8	4.62 ± 0.39	$107 \pm 8.3_{c}$
Bt no-ECB	$7.27 \pm 0.44_{ab}$	$65.6 \pm 3.8_{a}$	$9.74 \pm 0.27_{a}$	$47.9 \pm 1.2_{a}$	2.84 ± 0.10	163 ± 6	5.78 ± 0.33	$85.0 \pm 5.5_{b}$
	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)#							
ECB injury (infested plants)‡	P=0.0014	P=0.0019	P=0.0011	P=0.0019	NS	NS	NS	NS
ECB injury (injured vs uninjured plants)ⅆ	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)¶	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%

‡ 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.

ɖ 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).

¶ 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.

NS = not significant (P>0.05).

Table 4 Amounts (μg compound g^{-1} tissue) of lignin-derived phenols in infested and non-infested MZ540 hybrid stems at time zero and five 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[†]	0.12	0.02	0.04	0.30
Ad/Al_V[†]	0.34	0.59	0.31	0.33

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

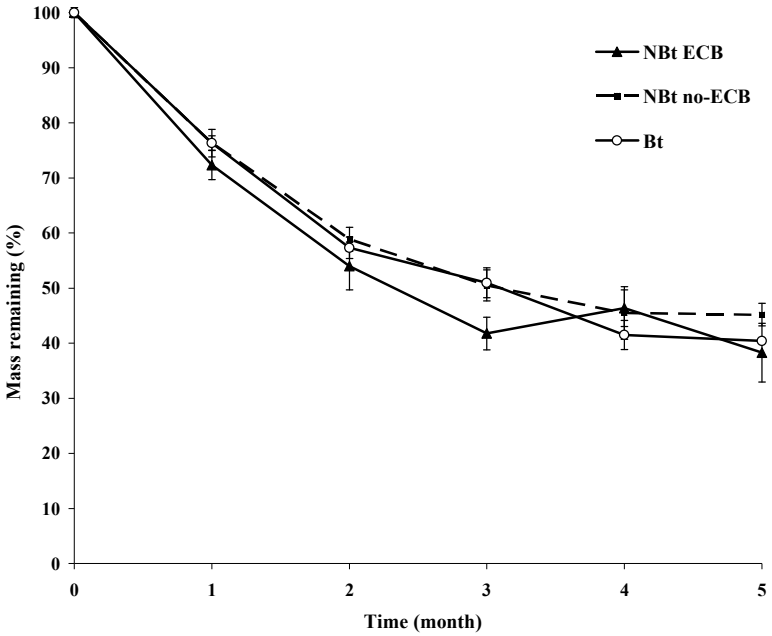

Fig. 1 Mass remaining (%) in ECB injured and non-injured stems from corn hybrids (NBt hybrids, MZ310 and MZ540; Bt hybrids, MZ3888, and MZ5444) after 5 months of decomposition in field litterbags.

FORWARD TO CHAPTER FOUR

The experiments described above have shown that the Bt gene did not have a consistent effect on the chemical composition of corn tissue nor was lignin concentration affected by ECB infestation. More pronounced differences are evident between corn components whereas roots seemed to have greater lignin concentrations than aboveground plant parts. Molecular characterization of stems after five months in the field demonstrated the resistance of lignin to decomposition. For this reason I was interested in examining the decomposition patterns of different plant components under controlled conditions to assess how lignin and C:N ratio control decomposition rates. The use of stable isotopes is ideal for tracing the carbon from the plant residue to the soil as it gets mineralized. Chapter four describes an incubation study that includes leaves, stems, and roots from Bt and NBt corn plants; an exogenous lignin source was added to some samples to allow the assessment of the effect of elevated lignin concentration on decomposition.

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CHAPTER FOUR

Plant lignin and nitrogen contents control carbon dioxide production and nitrogen mineralization in soils incubated with Bt and non-Bt corn residues

4.1 Abstract

Bt corn is reported to produce lignin-rich residues, compared to NBt corn, suggesting it is more resistant to decomposition. As the Bt gene is expressed selectively in stem and leaf tissue, it could affect lignin distribution in corn, which naturally has greater lignin concentration in roots than in stems and leaves. My objective was to evaluate the effects of corn plant components, the Bt gene and elevated lignin inputs on decomposition. Roots, stems and leaves from Bt corn and NBt corn near-isolines enriched with ^{13}C and ^{15}N were finely ground and mixed separately with soil, then incubated at 20^{o}C for 36 weeks. The effect of elevated lignin on decomposition was tested by adding a commercial lignin source (indulin lignin) to half of the samples. In addition to weekly CO_2 analysis and regular measurement of N mineralization, the degree of lignin degradation was evaluated at 1 and 36 weeks from the acid to aldehyde ratio (Ad/Al) of vanillyl and syringyl lignin-derived phenols. The CO_2 production and N mineralization was lower in root-amended soils than stem- and leaf-amended soils. The Bt genetic modification increased CO_2 production from stem-amended soils (P<0.05) and decreased N mineralization in root-amended soils. The ^{13}C and ^{15}N results also showed more residue-C and -N retained in soils mixed with NBt stem residues. After 36 weeks leaf- and stem-amended soils with indulin lignin had a lower Ad/Al ratio and were less degraded than

soils without exogenous lignin. In conclusion, plant lignin and N concentrations were good predictors of CO_2 production and N mineralization potential. Corn roots decomposed more slowly than aboveground components emphasizing the importance of recalcitrant root residues in sustaining the organic matter content of soil.

Keywords: *Bacillus thuringiensis; corn; carbon dioxide production; incubation; lignin; lignin-derived phenols; nitrogen mineralization*

4.2 Introduction

The use of Bt corn to avoid damage from the European corn borer has been increasing since its commercialization in 1996. In 2009, Bt corn hybrids were planted on more than 50% of land under corn in North America (ERS-USDA, 2009). There have been reports that Bt corn differs chemically from corn hybrids without the Bt gene (NBt) and some farmers state that Bt corn residues are tougher than NBt residues and decompose more slowly (Lehman et al. 2008a). Such differences could affect soil microbial activity and residue decay, altering N mineralization and CO_2 emitted from agroecosystems where Bt corn is grown.

Lignin content of residue is one of the main factors affecting decomposition due to the recalcitrance of this complex molecule and its resistance to degradation by soil microorganisms and extracellular enzymes (Melillo et al. 1982; Cadisch and Giller 1997; Austin and Ballare 2010). Residues with high lignin content are expected to decompose

more slowly, and persist longer in soils than residues with low lignin content. Studies comparing the lignin concentration of Bt and NBt corn are contradictory; some report that lignin concentration is higher in Bt residues than NBt residues (Saxena and Stotzky 2001; Poerschmann et al. 2005), others find little effect (Masoero et al. 1999; Jung and Sheaffer 2004; Mungai et al. 2005; Lehman et al. 2008a, 2010; Tarkalson et al. 2008; Zurbrugg et al. 2010). If lignin concentration is affected by the Bt gene, which is expressed selectively in leaf and stem tissue, then the extra lignin might not be distributed uniformly in the plant. In addition, lignin content of plants varies naturally, with greater lignin concentration in roots and stems than in leaves (Abiven et al. 2005; Mungai et al. 2005), so lignin concentration of different plant parts needs to be considered in residue decomposition experiments (Abiven et al. 2005).

If some Bt hybrids have greater lignin concentrations than NBt isolines, this would be expected to slow the decomposition of Bt corn residues, as shown in some laboratory studies (Dinel et al. 2003; Castaldini et al. 2005; Flores et al. 2005) that found lower CO_2 production from soils amended with Bt corn residue, but other authors reported no differences in CO_2 production from soils receiving Bt vs NBt corn residues in the field (Lehman et al. 2008a; Tarkalson et al. 2008; Zurbrugg et al. 2010) and laboratory (Hopkins and Gregorich 2003; Fang et al. 2007). Even when the Bt corn residue had greater lignin and lignin:N ratio (Fang et al. 2007), implying lower decomposability, there was no difference between the two residue types. A silt-loam soil amended with NBt corn roots (Merschman-00110) had 2.7 times more N mineralization than soil mixed with Bt roots (M-00112Bt), but this was not observed when roots were incubated in a silty clay or sandy loam soil (Mungai et al. 2005). In addition, the Bt gene did not affect N

mineralization from leaves and stems in any soil in this incubation experiment (Mungai et al. 2005).

As the difference in the lignin concentration of mixed residues from Bt and NBt corn may be too slight to have an effect on decomposition, it would be informative to compare CO_2 production and N mineralization of corn components, namely roots, stems and leaves. Reports from experiments comparing the decomposition of Bt corn, which had 18-80% more lignin than the NBt corn (Fang et al. 2007), showed no difference in CO_2 production whereas lower CO_2 production was reported when the Bt corn had 90% more lignin than the NBt hybrids (Flores et al. 2005). Lignin concentration of the substrate has a direct effect on decomposition rates and therefore, to be able to test the effect of elevated lignin concentrations on decomposition rates it would be useful to add an exogenous lignin source to some treatments for comparison.

The types of phenylpropanoid monomers that make up lignin molecules also affect the recalcitrance of residues. Lignin is formed from the radical coupling of three alcohol monomers, *p*-coumaryl, coniferyl, and sinapyl alcohols incorporated into the macromolecule as the vanillyl (V), syringyl (S), and cinnamyl (C) phenols and their derivatives (Otto and Simpson 2006). The CuO oxidation method combined with gas chromatograph-mass spectrometry (GC-MS) is used to measure these lignin-derived phenols. Lignin residues that contain more vanillin are more difficult to degrade because vanillin is derived from guaiacyl phenylpropanoid which has a free C_5 position on the aromatic ring, producing more condensed lignin molecules. Brown-rot and white-rot fungi have been shown to selectively degrade syringyl phenols over vanillin phenols

(Hedges et al. 1985; Eckardt 2002; Boerjan et al. 2003; Christiernin et al. 2009), which are considered to be the most recalcitrant of the lignin monomers (Feng and Simpson 2008). Therefore, plant material with a high vanillyl to syringyl ratio (V:S) can be expected to be more resistant to degradation. The acid to aldehyde ratios (Ad/Al) of vanillin and syringyl can also be used as an indicator of the degree biodegradation or diagenetic alteration of lignin by microorganisms specifically white-rot fungi; as biodegradation proceeds, the monomers are transformed from aldehyde forms to their acid forms, which results in an increase in the Ad/Al ratio (Hedges et al. 1988; Opsahl and Benner 1995; Poerschmann et al. 2005; Otto and Simpson 2006; Loh et al. 2008). Poerschmann et al. (2005) reported that total lignin concentration in stems and leaves was greater in two Bt corn hybrids than in NBt hybrids. This was attributed to an increase in guaiacyl-type lignin (specifically, *trans*-3-(3,4-Dimethoxyphenyl)-3-propenoic acid ME) in the Bt corn hybrids, evaluated by thermochemolysis and CuO oxidation methods. However, the Ad/Al ratio implies that Bt corn stems are more susceptible to oxidation than NBt stems, which suggests that molecular-level transformations of lignin-derived organic matter occur during the decomposition perhaps explaining CO_2 production and the eventual stabilization of plant-derived C in soils.

The first objective of this study was to compare the decomposition of corn roots, stems and leaves with the expectation that roots would decompose at slower rates due to their elevated lignin concentration compared to other plant components. The second objective was to determine if residue decomposition differs between Bt corn and NBt corn; the hypothesis is that CO_2 production would be similar between Bt and NBt since the hybrids used in this experiment had a similar chemical composition. I was also

interested in assessing the effect of elevated lignin concentration of soil-residue mixtures on decomposition, which was tested by the addition of an exogenous lignin source to simulate elevated lignin concentration in the added substrate. It is expected that the addition of exogenous lignin would hinder decomposition from those treatments. Correlations between decomposition (CO_2 production) and chemical attributes of corn residues (lignin and N concentrations, C:N and lignin:N ratios), as well as changes in the Ad/Al ratios of lignin-derived phenols from Bt and NBt corn residue during decomposition were also measured.

4.3 Materials and Methods

4.3.1 Soil and corn litter

Soil was collected in September 2007 from the 0-20 cm layer of a long-term corn experiment at the Macdonald Research Farm, Ste-Anne-de-Bellevue, Quebec, Canada (45°30'N, 73°35'W). The soil was a Dystric Gleysol (815 g sand kg^{-1}, 96 g clay kg^{-1}, with a pH of 6.0, 17.6 g organic C kg^{-1} and 1.6 g kg^{-1} N). The soil was air-dried and passed through a 2 mm mesh sieve. A Bt corn hybrid (DKC 38-33, MON810 Bt insertion event) and an NBt near-isoline (DKC 38-32) were grown in this sandy-loam soil in pots in the greenhouse and enriched with the ^{15}N and ^{13}C according to the method of Bromand et al. (2001). Briefly, this involved weekly pulse-labeling with ^{13}C-CO_2 beginning at the V2 growth stage and adding ^{15}N-KNO_3 fertilizer after each pulse-labeling event. The resulting ^{13}C and ^{15}N enrichment in the corn tissue was above the enrichment of the background soil and corn tissue (Table 1). Corn was harvested at the V9-V10 growth

stage (Ritchie et al. 1986) and separated into leaves, stems, and roots. Corn components were dried at 50^{o}C for 24 h and ground to pass through a 1 mm mesh sieve. The isotope enrichment levels, C and N, and fiber contents of soil, corn and indulin lignin are shown in Table 1.

4.3.2 Aerobic soil incubation

The experiment was a complete factorial design, with three plant components (roots, stems and leaves) from two near-isolines (Bt and NBt corn), added to soil, from the same site where the corn was grown, with and without indulin lignin (Sigma Chemical Co., St. Louis, MO, 1-6384), for a total of 12 experimental treatment combinations. Four replicates of each treatment were prepared for each of 10 sampling dates (1, 2, 4, 8, 12, 16, 20, 24, 30 and 36 week after the beginning of the study) to permit destructive sampling, for a total of 480 jars. Each replicate consisted of 50 g of air-dried soil placed in an acid-washed 120 cm^3 plastic vial with 0.5 g of ground corn tissue. Plastic vials that received the lignin treatment were amended with 0.1 g of indulin lignin (+L). The contents of each vial were mixed thoroughly, moistened to 40% water-filled pore space, and placed inside 1-liter Mason jars, along with 10 mL distilled water to maintain soil humidity. Jars were capped with an air-tight lid, incubated in the dark at 20^{o}C, and lids were removed to aerate the jar for 15 min every week. Air-tight rubber septa were fitted into the lids of the replicates designated for weekly CO_2 sampling. The gas samples were injected into pre-evacuated 12 mL exetainers (Labco, Wycombe, UK) with an extra 60 mil teflon-silicone septa (National Scientific, Rockwood, TN, USA) containing a small amount of magnesium perchlorate to absorb moisture, for short-term storage. The CO_2

concentration was measured by thermal conductivity detector using a gas chromatograph (Hewlett-Packard 5890 Series II, Hewlett-Packard Company, Avondale, PA, USA) equipped with a Porapak Q column (ethylvinylbenzene and divinylbenzene copolymer beads; 80-100 mesh; length, 25 m; internal diameter, 0.20 mm; Supelco 20331). The carrier gas was helium (50 mL min^{-1}). Oven and detector temperatures were 120°C and 250°C, respectively.

CO_2 gas sampling was continued until week 20 at which time CO_2 production had stabilized. The CO_2 value was converted from volume to mass units following the ideal gas equation (Livingston and Hutchinson 1995):

$$_{sample}CO_2\text{-}C = \frac{C_m \times M \times P}{R \times T} \quad (1)$$

where $_{sample}CO_2$-C is the amount of C in the sample (in mg L^{-1}), C_m is the measured CO_2 (in μL L^{-1}), M is the atomic weight of C (12 g C mol^{-1} CO_2^{-1}), P is the atmospheric pressure (1 atm), R is the universal gas constant (82.06 atm mL mol^{-1} $^oK^{-1}$), and T is the room temperature (298 ^oK). Then, the amount of CO_2 produced (g CO_2-C kg^{-1} soil) was calculated as follows:

$$g\ CO_2\text{-}C\ kg^{-1}\ soil = \frac{_{sample}CO_2\text{-}C \times V}{W} \quad (2)$$

where V is the volume of the headspace in the jar (0.96 L) and W is the weight of the soil used in the incubation (50 g).

On the designated weeks, vials were removed for destructive sampling, the amended soils were thoroughly mixed, and a 5 g sub-sample was immediately extracted with 2 M

KCl solution (Maynard et al. 2008) for NH_4-N and NO_3-N analysis with a Lachat Quik Chem Flow Injector Autoanalyzer (Lachat Instruments, Milwaukee, WI 53218, USA). An additional 5 g sub-sample was taken for moisture determination (dried at 50^{o}C) and kept for ^{13}C and ^{15}N measurements. The plastic vial was sealed with a screw-on lid and the remaining soil was stored at –15^{o}C for CuO oxidation analysis.

The amount of mineral N (NH_4-N plus NO_3-N) produced during the 36 week incubation was used to calculate the mineralization rate constant (k, in week^{-1}) by non-linear regression analysis according to the following equation: $N_0=N_{min}(1 - e^{-kt})$, where N_0 is the cumulative N mineralized (mg N kg^{-1}) after time t (in weeks) and N_{min} is the potentially mineralizable N (mg N kg^{-1}) under optimum temperature and moisture (Curtin and Campbell, 2008).

4.3.3 Lignin-derived phenols

CuO oxidation was carried out on pooled soil samples representing the four replicates of the Bt and NBt near-isolines of each corn component, with and without added lignin (+L, –L), from week 1 and week 36. Lignin-derived phenols were liberated from soil using CuO oxidation (Hedges and Ertel 1982; Otto and Simpson 2006). Briefly, 0.2-0.4 g of soil was weighed into Teflon-lined bombs with 1 g CuO, 100 mg ammonium iron (II) sulfate hexahydrate, and 15 mL of 2 M NaOH. The bombs were heated at 170^{o}C for 2.5 hours, then cooled immediately under running water. The supernatant was transferred into a teflon centrifuge tube and acidified with 6 M HCl to pH 1, then centrifuged and kept in the dark for 1 hour. The supernatant was then transferred into a separation funnel and

extracted with diethyl ether, then concentrated and dried under N_2. The CuO oxidation products were derivatized to trimethylsilyl (TMS) derivatives by reaction with 90 mL N,O-bis- (trimethylsilyl)-trifluoroacetamide (BSTFA) and 10 mL pyridine for 3 h at 70^{o}C before GC/MS analysis. Lignin-derived phenols were measured using an Agilent model 6890N GC with an HP-5MS fused silica capillary column (30 m x 0.25 μm x 0.25 μm thickness) coupled to an Agilent model 5973N quadrupole mass selective detector. The GC operating conditions were as follows: temperature at 65^{o}C for 2 min, then increased to 300^{o}C at 6^{o}C min^{-1}, held at 300^{o}C for 20 min. The injected sample volume was 3μL splitless and the run time was 62 min. Agilent Chemstation G1701DA software was used to process the data and identify the compounds by comparison of the mass spectra with the Wiley MS library data. Vanillic acid was used as an external quantification standard and individual compounds were normalized to the amount of organic C in each sample. The eight lignin-derived phenols identified were vanillin, acetovanillon, vanillic acid (V units), syringealdehyde, acetosyringone, syringic acid (S units), and *p*-coumaric acid and ferulic acid (C units) (Hedges and Ertel 1982; Poerschmann et al. 2005; Otto and Simpson 2006; Loh et al. 2008).

4.3.4 Stable isotope analysis

Sub-samples of soils from the incubation jars were analyzed for ^{13}C and ^{15}N stable isotopes using a Vario EL III elemental analyzer (Elementar, Germany) with a Conflo II interface (Thermo, Germany) and a Delta XP Plus Advantage isotope ratio mass spectrometer (Thermo, Germany). The international standards Vienna Pee Dee Belemnite (VPDB) ^{13}C with an absolute isotope ratio $^{13}C/^{12}C = 0.0112372$ and Air with an absolute

isotope ratio $^{15}N/^{14}N = 0.003676$ were used for $\delta^{13}C$ or $\delta^{15}N$ analysis. The proportion of residue-C or residue-N (P, in %) that was recovered in the soil after 36 weeks was calculated as follows:

$$P_{\text{C or N}} = \frac{\delta_{tr} - \delta_{\text{C}}}{\delta_R - \delta_C} \times 100 \quad (3)$$

where δ_{tr} is the $\delta^{13}C$ or $\delta^{15}N$ measured in the treatment soils, δ_C is the $\delta^{13}C$ or $\delta^{15}N$ measured in the control soil which received no residue ($\delta^{13}C = -24.00$ or $\delta^{15}N = 14.12$), and δ_R is the $\delta^{13}C$ or $\delta^{15}N$ of the corn residue + exogenous lignin where applicable (Table 1) weighted for the proportion of residue/exogenous lignin. The proportion of residue-C and residue N retained in soil from the added residue (+ exogenous lignin where applicable) was calculated as:

$$\text{Proportion residue} - \text{C or} - \text{N retained} = \frac{P_{C \text{ or } N} \times OC \text{ or } N}{C_i \text{ or } N_i} \times 100 \quad (4)$$

where OC is the organic C content of soil (g C kg^{-1} soil), which was assumed equal to total C since no carbonates were detected upon treatment of soil with 1 M HCl, N is the total N content of the soil (in g residue-N kg^{-1} soil), C_i and N_i are the initial amounts of C (g C kg^{-1} tissue) and N (g N kg^{-1} tissue) which was added in the 0.5 g residue and 0.1 g exogenous lignin. Organic C and total N were measured in each treatment replicate at 36 weeks.

4.3.5 Statistical analysis

All data was checked for normality and log transformed where needed based on the Shapiro-Wilk test. The effects of the plant component (leaves, stems, and roots), corn

near-isolines (Bt, NBt) and lignin addition (+L, –L) on cumulative CO_2 production and N mineralization were tested using the GLM procedure on SAS software (SAS Institute Inc. 2009). Pre-planned orthogonal contrasts were used to evaluate differences between corn components. When effects of corn near-isolines and lignin addition were significant ($P<0.05$), mean values were compared with a post-hoc LSMeans test at the 95% confidence level. Decomposition rate constants (k) were calculated by least-squares iteration using the NLIN procedure on SAS software (SAS Institute Inc. 2009). Pearson correlation coefficients (Proc Corr procedure) were used to examine the relation of CO_2 with corn chemical parameters %N, %lignin, C:N ratio, and lignin:N ratio. The Proc Reg procedure (SAS Institute Inc. 2009) was used to relate CO_2 production to the total amount of lignin that was added to each treatment (based on the initial lignin concentration of the specific plant component and the added exogenous lignin). No statistical analysis was conducted on CuO analyses, performed without replication. Values presented in tables and figures are untransformed means with the standard error of the means (SE).

4.4 Results

Roots tended to have a greater lignin concentration, a smaller C concentration, and a greater lignin:N ratio than leaves and stems (Table 1). The C:N and lignin:N ratios tended to be lower in the Bt tissues than in NBt tissues (Table 1).

Decomposition reached a constant rate after 20 weeks of incubation. There were no significant interactions between lignin and the Bt effect so these results are not presented. Cumulative CO_2 was lower ($P<0.0001$ at $\alpha = 5\%$) from soils amended with roots than

from soils with stems or leaves (Table 2). In leaf- and root-amended soils, there was no difference between Bt and NBt treatments (Table 2), but in stem-amended soil, there was greater CO_2 production from the Bt treatments (P=0.044 at α=5%) and from the soils without the added lignin (P=0.040). Regression analysis indicated a strong inverse relationship between the amount of lignin in residue and the amount of CO_2 produced in the –L samples (R^2=0.9610). The slopes of the regression lines (Fig. 1), –1.6535 (SE = 0.7097) for the +L samples and –1.9725 (SE = 0.1986) for the –L samples, indicate no difference in the rates of decomposition with the addition of the exogenous lignin. Pearson correlation coefficients showed that CO_2 inversely correlated with lignin:N ratio (r= –0.933, P<0.0001, n=12) and indigenous residue lignin (r= –0.843, p=0.0006, n=12). CO_2 also correlated well with initial plant N (r= 0.799, P=0.0018, n=12) but not with the C:N ratio (r= –0.456, P=0.1363, n=12).

Total N mineralization (NH_4-N + NO_3-N) after 36 weeks was lowest in root-amended soil and highest in leaf-amended soil (Table 3). Root-amended soils showed the smallest amount of mineralized N followed by stem- and leaf-amended soils (Table 3). The Bt genetic modification negatively affected the amount of N mineralized in root-amended soils (Table 3). The addition of indulin lignin had no effect on the amount of mineralized N in all residue-soil mixtures. Fitting the N mineralization data with a first order equation revealed that the mineralization rate constant k was lowest in root-amended soils (k=0.06-0.07, Table 3). Leaf- and stem-amended soils had comparable k values except for NBt stems that had k values comparable to the root-amended soils (Table 3).

The Ad/Al ratios of vanillin and syringyl increased from 1 to 36 weeks within each treatment suggesting enhanced lignin oxidation over the course of the experiment (Table 4). At 36 weeks, the +L samples had smaller Ad/Al ratios than –L samples in the leaf-amended soils however, this trend was not observed in the stem- and root-amended soils. Comparisons between the Bt and NBt treatments at the beginning of incubation and at the end of the 36 weeks shows that the Bt-amended soils had lower Ad/Al ratios than the NBt-amended soils in all but two treatments within the leaf- and stem-amended soils. This result indicates that the lignin in the NBt residues was initially more susceptible to biodegradation and that it has undergone more modifications to the phenylpropanoid units than the lignin in the Bt residues. The addition of lignin to residue-soil mixtures increased the V:S ratio (Table 4). The V:S ratio increased between week 1 and week 36 of the incubation in leaf- and root-amended soils, but not in stem-amended soils (Table 4).

The amounts of residue-C and residue-N that were retained in the soil after 36 weeks are given in Table 5. There were no differences in the retained residue-C between the +L and –L or the Bt and NBt treatments, however more residue-C was retained from roots (P=0.0004 at α=5%) than from leaves and stems. The Bt genetic modification and the addition of exogenous lignin significantly affected the proportions of residue-N retained in the soil after 36 weeks of incubation and had a significant interaction between component and genetic modification and between component and exogenous lignin. More residue-N was retained from leaves and stems from NBt plants than Bt plants (P<0.0001 at α=5%) and more residue-N retained from +L stems than –L stems (P=0.0064 at α=5%) (Table 5).

4.5 Discussion

The results support the observation that corn roots have greater lignin concentrations than stems and leaves, at least at stages before maturity, in agreement with Fang et al. (2007), who reported a lignin concentration of 11.7% (Bt corn roots) and 9.94% (NBt corn roots). As plants reach maturity and their constitutional lignin deposition is completed, the lignin concentration of stems is expected to increase to levels close to that of roots (Forbes and Watson 1992). Johnson et al. (2007) and results from chapter two of this thesis reported that mature stems from field-grown corn had a similar lignin concentration as roots, and these components contained more lignin than leaves. It is also possible that stems of field-grown plants may have higher lignin than those from greenhouse plants because more structural rigidity is required in the field. Roots, both Bt and NBt, had lower C concentration than leaves and stems. This observation agrees with Fang et al. (2007) and Johnson et al. (2007), and can be explained by the fact that only about 25% of the C assimilated by plants is transported to the roots (Mooney 1972). However, it should be noted that there is always a risk of soil contamination in root samples, which could have contributed to the lower C concentration in the roots compared to other plant parts. Corn leaves contained more C and N than stems or roots.

There tended to be smaller C:N and lignin:N ratios in Bt tissue than NBt tissue, due to greater N concentration in leaves and stems or lower C concentration in roots. This leads to the question why does Bt tissue tend to have more N than NBt isolines? The presence of the Cry1Ab protein in Bt corn could be the reason. Escher et al. (2000) reported slightly more N concentration in leaves of X4334-EPR Novartis Bt hybrid (1.2% N) than

in a corresponding NBt hybrid (1.1% N) where the Bt hybrids contained 0.46-0.51 mg Cry1Ab g^{-1} dry leaf tissue. The hybrid used in this study was a MON810 Bt, which is reported to contain 7.9 - 10.3 mg Cry1Ab g^{-1} fresh weight leaf (Canadian Food Inspection Agency 1997); there was 26% more N in leaves and 48% more N in stems of the Bt hybrid than the NBt isoline, which is consistent with these reports.

Based on lignin concentration, the roots are expected to have slower decomposition rates than other components and this is evident in the CO_2 and N mineralization data. In this study lignin:N ratio, lignin concentration, and N concentration of corn tissue were good predictors of decomposition (cumulative CO_2 production during 20 weeks) and of cumulative N mineralization. Material with a high lignin:N ratio was reported to have slower decomposition rates than material with a low lignin:N ratio (Mellilo et al. 1982; Taylor et al. 1989; Johnson et al. 2007), which is consistent with my results. The slower decomposition of roots is also confirmed by the ^{13}C data which shows that more residue-C was retained from roots than other components. It should be noted that loss of ^{13}C from the soil (less residue-C recovery) indicates loss of CO_2, which can be directly linked to the decomposition of organic materials in the jars and a greater amount of ^{13}C recovery in some treatments indicates less degraded plant material. Soil N cycling on the other hand provides several pathways for ^{15}N loss from the soil; when plant material degrades, the released N (and ^{15}N) can be transformed into mineral N forms, incorporated into microbial biomass, or lost from the soil through nitrification (N_2O) and denitrification (NO_x, N_2O, N_2). However, when comparing treatments and when corroborated by CO_2 and N mineralization results, a soil that has more residue-N recovery (less ^{15}N loss) can

be said to have less degraded plant material and less gaseous N lost from the system compared to other treatments indicating less decomposition of tissue in the former.

It was hypothesized that there would be no differences in decomposition rates between Bt and NBt corn residue based on the lack of differences in lignin concentration, which have been confirmed by the CO_2 evolution results and is in agreement with laboratory incubation results reported by Hopkins and Gregorich (2003) and Fang et al. (2007). The only effect of the Bt genetic modification on CO_2 production was in the stem-amended soils, where it exerted a positive effect on the rate of decomposition and which is reflected in the smaller amount of residue-C retained in Bt stem samples and is also corroborated with the much smaller residue-N in those samples. A closer look at the initial chemical composition of the stem residue shows that this effect can be related to the elevated N concentration, and consequently smaller C:N ratio of the Bt stems (31 g kg^{-1}) compared to the NBt stems (16 g kg^{-1}) rather than to a difference in lignin concentration. An effect of the Bt genetic modification on N mineralization was observed in the root-amended soil samples; smaller mineralization from Bt roots cannot be explained by the lignin concentration or the C:N ratio but seems related to the smaller N concentration in Bt roots compared to NBt roots. However, no definite conclusions can be drawn from this result due to the possibility of soil contamination in root samples and the fact that the Cry1Ab protein is not produced in the roots and should not have affected the composition of the root tissue. The numerically greater ^{13}C recovery from NBt stem residues, strongly suggests that up to 21% of stem C from the NBt hybrids were transformed into a more stable form of soil C compared to 12% of stem C from the Bt residues. It can be assumed that the apparent differences between treatments are based on

the mineralization and loss of the labile material in the residue, rather than to the decomposition of lignin, since it has been strongly related to the lignin:N ratio and N concentration. On the other hand, the increase in the Ad/Al ratios of the lignin phenols have indicated that lignin has been altered by microbial activity by the end of the nine-month experiment and that the NBt residue was more susceptible to degradation, apparently in disagreement with the CO_2 results indicated above. Ertel and Hedges (1984) and references therein, attribute an elevated Ad/Al ratio to microbial oxidation of the phenylpropanoid units to carboxylic acid without necessarily causing cleavage of the lignin aromatic ring. An increase in carboxyl content could lead to greater solubility and suggests that samples with higher Ad/Al ratios, such as the NBt corn residues, would be more susceptible to degradation once the lignin molecules start to decompose.

A change in the V:S ratio over time is expected when the syringyl units are preferentially degraded over the vanillyl units by the brown-rot and white-rot fungi. My results indicate that the increase in the V:S ratio between week 1 and week 36 was similar for Bt and NBt treatments providing an added indication that the decomposition of these residues was not different during this time frame.

The addition of exogenous lignin increased the V:S ratio suggesting that this type of lignin had an elevated vanillyl content and is more stable compared to the indigenous corn-derived lignin, and is therefore not expected to decompose or contribute to the production of CO_2 in the +L samples. This was confirmed by the CO_2 production and N mineralization results, which showed no difference between +L and –L treatments except in the +L stem-amended soils where the addition if indulin lignin decreased the

production of CO_2 and is supported by the greater residue-N retained from +L stems compared to –L stem treatments, which lost more ^{15}N through nitrification and/or denitrification processes consistent with more decomposition in the –L stem treatments. It is evident from Figure 1 that this is caused by the NBt +L stems which produced less CO_2 than the other stem treatments; it seems likely that biodegradation of the NBt stems, which had the smallest N concentration (16 g kg^{-1}), was affected by the addition of the exogenous lignin in those treatments. The observation that indulin lignin apparently did not decompose during the incubation period is not surprising since it is a recalcitrant material and in a different chemical form than lignin in corn tissue. Indigenous lignin is bound to other fibers and proteins and forms complexes with hemicellulose and cellulose; plant lignin undergoes biochemical modifications and is released gradually as cells are broken down by decomposers, which is not the case for indulin lignin. This is probably why the total amount of lignin (+L samples) showed a non-significant regression relationship with CO_2 production whereas indigenous lignin (–L samples) significantly related to CO_2 production.

4.6 Conclusions

I conclude that lignin and N concentrations, and consequently the lignin:N ratio, of corn tissue control its decomposition. The C:N ratio of stem residue appears to explain the positive effect of the Bt genetic modification on decomposition and N mineralization from these plant components. Corn roots, which accumulate a biomass of 3000-5000 kg ha^{-1} (Prince et al. 2001) and have an average of 62 g kg^{-1} lignin, could make an important contribution to soil C because of their inherent resistance to decay. Aboveground corn

residues (stems and leaves) were more susceptible to decomposition in this study. This might not be the case in corn agroecosystems due to the similarity in lignin concentration of corn roots and stems at physiological maturity. In addition, the effect of herbivory by ECB on the physical strength and integrity of NBt corn residues needs to be considered under field conditions. Since exogenous lignin was not susceptible to decomposition and generally did not contribute to more CO_2 production and N mineralization, I was only able to observe the effect of elevated-lignin residue on decomposition through the root decomposition patterns, which exhibited about 50% slower decomposition rates. The results strongly suggest that Bt corn does not differ from NBt corn in terms of decomposition and should have no effect on the soil C dynamics in Bt corn agroecosystems.

Table 1 Organic C, total N, $\delta^{13}C$, $\delta^{15}N$, and lignin concentrations of the incubation soil, indulin lignin and corn residues.

Sample	Organic C ($g\ kg^{-1}$)	$\delta^{13}C$	Total N ($g\ kg^{-1}$)	$\delta^{15}N$	Lignin ($g\ kg^{-1}$)	C:N	Lignin:N
Soil	16.4	-24.0	1.20	14.12	n.d.†	13.7	n.d.
Unlabeled corn tissue (average of shoots and roots)	n.d.	-12.3	n.d.	1140	n.d.	n.d.	n.d.
Indulin lignin	567	-27.1	7.40	137.5	1000‡	76.6	135
Bt Leaves	452	9.80	35.5	4386	28.5	12.7	0.80
NBt Leaves	431	16.1	26.2	3882	35.9	16.5	1.37
Bt Stems	401	2.52	30.8	5721	36.2	13.0	1.18
NBt Stems	412	7.25	15.9	5131	34.6	25.9	2.18
Bt Roots	245	1.79	14.8	4138	54.2	16.6	3.66
NBt Roots	383	10.1	17.8	4288	69.6	21.5	3.91

† n.d. = not determined.

‡ Assumed but not measured.

Table 2 Effect of the Bt gene and addition of alkali lignin to a soil-corn residue mixture on cumulative CO_2 production (g CO_2-C kg^{-1} soil) after 20 weeks. Values are the mean ± standard error (n=8).

Corn residue	**Cumulative CO_2 (g CO_2-C kg^{-1} soil)**					
	Bt	**NBt**	Pr>F at α=0.05	**+L**	**-L**	Pr>F at α=0.05
Leaves	2.47 ± 0.11	2.39 ± 0.10	NS†	2.41 ± 0.11	2.46 ± 0.10	NS
Stems	2.35 ± 0.06	2.14 ± 0.09	0.044	2.14 ± 0.10	2.36 ± 0.05	0.040
Roots	1.77 ± 0.17	1.79 ± 0.07	NS	1.72 ± 0.10	1.83 ± 0.15	NS
	Contrast analysis (significance probability)					
Leaves vs. Stems	*P*=0.5233	*P*=0.0557		*P*=0.0931	*P*=0.5280	
Leaves vs. Roots	*P*=0.0007	*P*<0.0001		*P*=0.0002	*P*=0.0006	
Stems vs. Roots	*P*=0.0032	*P*=0.0084		*P*=0.0110	*P*=0.0029	

† Not Significant (*P*>0.05).

Table 3 Effect of the Bt gene and addition of alkali lignin to a soil-corn residue mixture on cumulative mineral N (mg N kg^{-1} soil) produced after 36 weeks and decomposition rate constant (K) from NLIN estimation. Values are the mean ± standard error (n=8).

Corn residue	**Cumulative mineral N (mg N kg^{-1} soil)**					
	Bt	**NBt**	Pr>F at α=0.05	**+L**	**-L**	Pr>F at α=0.05
Leaves	153 ± 2.03	150 ± 1.09	NS	151 ± 1.95	151 ± 1.50	NS
Stems	110 ± 1.58	109 ± 1.76	NS	110 ± 2.06	109 ± 1.11	NS
Roots	85.0 ± 0.82	89.8 ± 1.26	0.0064	87.9 ± 0.77	86.7 ± 1.68	NS
	Contrast analysis (significance probability)					
Leaves vs. Stems	*P*<0.0001	*P*<0.0001		*P*<0.0001	*P*<0.0001	
Leaves vs. Roots	*P*<0.0001	*P*<0.0001		*P*<0.0001	*P*<0.0001	
Stems vs. Roots	*P*<0.0001	*P*<0.0001		*P*<0.0001	*P*<0.0001	
	Weekly decomposition rate constant (k)					
Leaves	0.15 ± 0.00	0.14 ± 0.00		0.16 ± 0.00	0.13 ± 0.00	
Stems	0.16 ± 0.01	0.08 ± 0.01		0.14 ± 0.01	0.10 ± 0.01	
Roots	0.07 ± 0.00	0.06 ± 0.00		0.07 ± 0.01	0.06 ± 0.00	

Table 4 Acid to aldehyde (Ad/Al) ratios and vanillyl to syringyl ratio of root- stem- and leaf-amended soils, with and without added lignin (+L, -L) after 1 week and 36 weeks of laboratory incubation.

Ratio	Bt+L Week1	Bt+L Week36	Bt-L Week 1	Bt-L Week 36	NBt+L Week 1	NBt+L Week 36	NBt-L Week1	NBt-L Week36
				Leaf-amended soil				
Ad/Al_s	1.49	1.57	1.32	2.03	1.45	2.20	1.71	2.26
Ad/Al_v	1.55	1.34	1.68	2.23	1.15	1.75	2.46	3.65
V:S	1.22	1.59	1.02	1.07	1.28	1.15	1.01	1.09
				Stem-amended soil				
Ad/Al_s	1.05	1.79	1.01	1.31	1.57	1.35	1.08	1.58
Ad/Al_v	0.71	1.14	0.95	1.31	1.44	1.65	1.01	1.39
V:S	1.38	1.21	0.71	0.61	1.79	1.30	0.66	0.69
				Root-amended soil				
Ad/Al_s	0.88	1.40	0.96	1.13	1.18	1.55	1.36	1.42
Ad/Al_v	1.09	1.23	1.58	1.58	1.21	1.31	1.87	2.03
V:S	0.80	1.21	0.69	0.77	1.14	1.25	0.80	0.75

Values are based on lignin phenols yields that were normalized to sample mass. For the determination of the V:S ratio, V is the sum of vanillin + vanillic acid + acetovanillon and S is the sum of syringyl + syringic acid + syringaldehyde. Ad/Al_v is the ratio of vanillic acid to acetovanillon and Ad/Al_s is the ratio of syringic acid to syringaldehyde (Otto and Simpson, 2006).

Table 5 Proportion residue-C and residue-N retained in soil, calculated from equations 3 and 4, in a sandy loam soil amended with Bt and NBt tissues after 36 weeks of laboratory incubation and ANOVA treatment effects at α=5%. Values are the mean ± standard error (n=8).

	Bt	**NBt**	**+L**	**-L**
	Retained Residue-C (%)†			
Leaves $_{a}$	17.7 ± 2.4	16.8 ± 2.1	14.5 ± 1.8	20.0 ± 2.2
Stems $_{a}$	12.8 ± 2.6	21.1 ± 2.0	17.6 ± 3.0	16.2 ± 2.5
Roots $_{b}$	31.7 ± 5.8	30.6 ± 5.4	26.5 ± 6.0	35.8 ± 4.6
	Retained Residue-N (%)			
Leaves	66.6 ± 4.3	91.7 ± 2.9*	83.3 ± 4.7	75.0 ± 6.7
Stems	42.8 ± 3.3	100 ± 6.0*	81.1 ± 12.8	62.0 ± 9.6*
Roots	75.3 ± 5.0	71.8 ± 6.2	75.4 ± 6.4	71.7 ± 4.7

Treatment Effects		
	Retained residue-C treatment effects at α=5%	Retained residue-N treatment effects at α=5%
Bt	NS	*P*<0.0001
Lignin	NS	*P*=0.0064
Component	*P*=0.0004	NS
Bt x Component	NS	*P*<0.0001
Lignin x Component	NS	*P*=0.0147

† Values with different subscripts within a column are statistically different at α=5%. Values with an asterisk within a row (Bt vs NBt and +L vs -L) are statistically different.

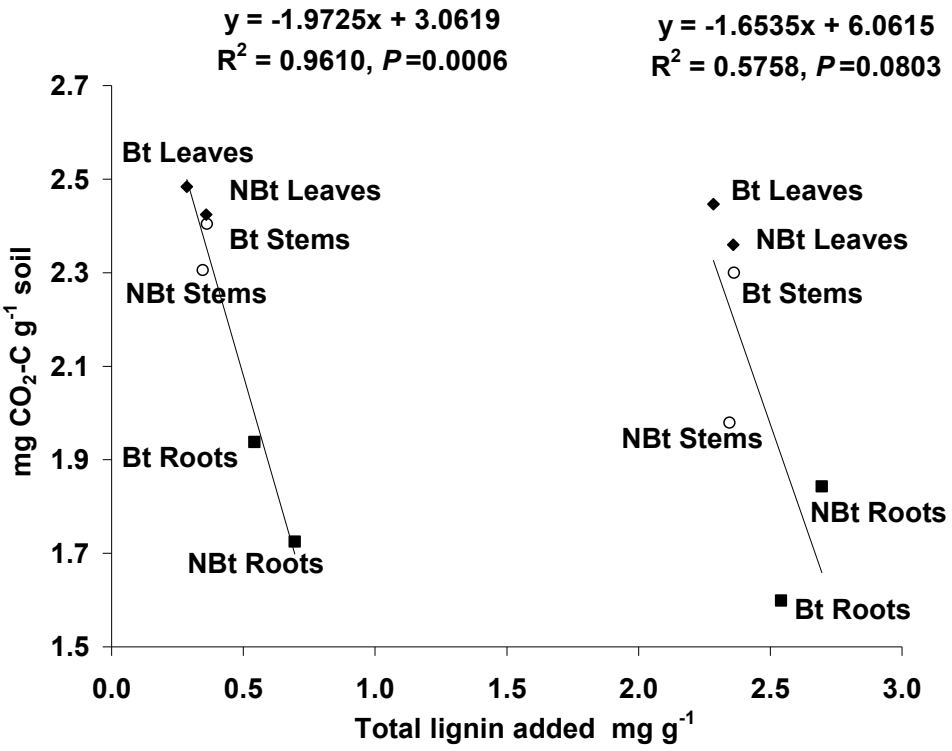

Fig. 1. Cumulative CO_2-C produced after 20 weeks of incubation in relation to total amount of lignin (mg g^{-1}) added to treatments.

FORWARD TO CHAPTER FIVE

I conducted five experiments that tested several hypotheses about the composition and decomposition of corn residue as affected by plant part, the addition of the Bt gene modification, and herbivory in the field and under controlled conditions. In the following chapter, I link the individual experiments and discuss the implications of my findings.

CHAPTER 5.

General Discussion and Synthesis of Findings

Concerns about the ecological effects of transgenic corn production are related to the amount and composition of the resulting residue and the effects of the added toxins on soil microbial processes. The current work was aimed at investigating the occurrence and degree of change that occurs in corn plants as a result of the Bt gene modification and the effects of those changes on residue decomposition. The primary focus was on the measurable differences in chemical composition, biomass production, CO_2 production and mass loss upon residue decay, rather than on the direct effects on the microbial community. In addition to the fundamental research questions, I also aimed to relate the findings to practical field applications that would be of interest to producers who are dealing with the corn residue and managing the soil in sustainable ways to conserve C stocks for the long-term profitability of their corn production systems.

5.1 Effect of the Bt Gene Modification on Nitrogen and Lignin Concentrations

I hypothesized that N concentrations would be similar between Bt and NBt hybrids but found some differences. The effect of the Bt gene modification on corn chemistry was mainly manifested as an increase in the N content in the field-grown corn, which was also true in some cases in the pot-grown corn (chapters three and four). The likely explanation is an increase in N uptake in the Bt plants rather than a direct effect of the Cry1Ab protein

production. I based this explanation on the observation of an increased N concentration in the below-ear portion of the Bt stems and a decrease in N concentration in the roots of the Bt plants, suggesting this effect was attributed to more efficient N translocation within the corn plant.

As well, there was no consistent evidence that the Bt gene affected lignin concentrations. The only observed effect was an increase in lignin concentration in the leaves of pot-grown experiments in one of the two-year study. In chapter three, I noted that the plants in that year (2009) were more physiologically mature than the previous year and suggested that Bt plants could have deposited some extra lignin at that maturity stage. However, the results from the field experiment (chapter two) do not show a similar trend noting that the corn plants had reached physiological maturity in the field. In chapter two I also suggested that the gravimetric lignin measurement method used in this work might not be sensitive enough to detect small depositions of lignin however, for practical applications, such small depositions would not be expected to affect residue 'toughness', thereby having no impact on farmers' tillage practices or residue decomposition at the agroecosystem level.

5.2 Effect of the Bt Gene Modification and Herbivory on Yield and Biomass

The effect of the Bt gene modification on biomass was of interest because it could affect the amount of residue returned to the field after grain harvest. The general consensus was that, without ECB infestation, Bt plants do not produce more biomass and this was clearly shown in the field experiment over two years. Since leaf, stem, and stover

biomass (chapters two and three) were not affected by the Bt modification, there would be no extra residue generated in Bt corn agroecosystems and there should be no difference in decomposition resulting from mass differences. As well, some reports had previously shown higher moisture contents in Bt plants compared to the near-isolines, which would require that the crop stay longer in the field to reach the minimum harvest moisture (20-26%) or an increased drying cost, but this was not the case in this field experiment. These findings indicate that there should be no significant agronomic differences between Bt hybrids that produce the Cry1Ab protein and the non-Bt near-isolines, making the farmers' decision to purchase transgenic seed a purely economical one.

As for the effect of herbivory on biomass accumulation, it was hypothesized that ECB injury would cause a reduction in leaf biomass because of a hindrance in nutrient translocation and in stem biomass because of the physical damage. The results showed that hybrid type was a more influential factor controlling biomass production, which is related to genotypic and phenotypic hybrid differences. The results of this experiment (chapter 3) showed that when physical damage to stems is extensive, as in the year 2008 of the experiment, stems lost mass but otherwise there was no effect on leaf biomass. One thing that is not clearly explained is the higher mass accumulation in the injured stems compared to un-injured stems in the year where damage was relatively less extensive (2009). One possibility is that injured stems formed a more dense tissue as a protective measure, but it cannot be concluded from this experiment. In the field, herbivory usually causes stem breakage and lodging leading to nutrient leaching and exposure of plant

tissue to the decomposing microorganisms, which means that in a real life situation, mass loss is the likely outcome when corn stems are infested with ECB.

5.3 Effect of ECB Herbivory on Nitrogen and Lignin Concentrations

Herbivory was identified as a factor in nitrogen translocation and a potential inducer of lignin deposition, both of which could affect the decomposition of residue. ECB infestation occurs regularly in corn fields in Quebec and generally in North America, which has lead to the increased use of Bt hybrids that provide a high percentage of protection. Damage to stems can be extensive, as shown in chapter three, and normal vascular nutrient translocation is expected to be affected directly by the damage. Though I found that the N concentration was higher in injured stems, I was unable to relate this to a disruption in translocation (as discussed in chapter three). It is more likely that injury caused an increase in N uptake (and possibly other nutrients) to support the production of defense and healing compounds. Therefore, both the Bt modification and herbivory had an effect on N concentration; Bt caused about 40% increase in N and infestation caused 30-40% increase in N. In a field where ECB infestation is expected, planting Bt or non-Bt corn hybrids will result in the same increase in N concentration in plant tissues, in response to herbivory. Whether these plants would require additional N fertilizer to avoid compromising yield objectives is beyond the scope of this work, however from an agronomic point of view there would be no need to adjust the fertilization regime. Ma and Subedi (2005) reported that the response to N additions was similar in Bt and NBt hybrids and that the Bt hybrids tended to accumulate more N in the stalk but not in the grain. The

increase in N, known to control decomposition among other factors, is expected to lead to faster initial decomposition rates of corn residues, as discussed in section 5.4.

I expected to find greater lignin concentration in injured stems but not in leaves since leaves showed minor signs of ECB damage. Contrary to expectations, there was no difference in gravimetrically-measured 'bulk lignin' in stems and some lignin increase in leaves of injured plants. Also, data from molecular lignin characterization does not support the hypothesis of lignin increase as a result of injury. Since leaf lignin was not measured by the CuO oxidation method I cannot confirm the gravimetrically higher lignin found in leaves of injured plants, but this finding is plausible under the hypothesis of stress-lignin deposition. The same was not true in stems because stems exhibited more obvious (visual) damage, which could have hindered the production of compounds and processes needed for stress-lignin deposition. If we accept that injury caused an increase in leaf lignin (14-18% within the NBt leaves) would this affect decomposition? This cannot be answered from my results, but the literature suggests that there would be less decomposition in the long-term and I have calculated that a 6% increase in lignin responds to a 7% increase in the amount of lignin remaining in residue after 200 days of decomposition (chapter one). Such an accumulation of lignin in residue with time allows for plant C to be transformed into soil C, or at least increases the residence time of plant C in the soil. Therefore, when injured corn plants are left in the field, the stems are expected to stimulate *initial* decomposition because of the higher N concentration and the leaves are expected to hinder *late-stage* decomposition because of the higher lignin. High concentrations of N have a suppressing effect on lignin degradation due to the suppression of ligninase (Berg and McClaugherty, 2003), therefore the combination of a

high-N stem residue with a high-lignin leaf residue retards lignin decomposition during the late stages of decay, especially that accumulation of N normally occurs at this late stage (Berg and McClaugherty, 2003). The combined effect of ECB injury on corn stems and leaves remains to be investigated.

5.4 Effect of the Bt Gene Modification and Herbivory on Field Decomposition of Corn Stems

Corn stem residue from Bt and NBt plants, having similar initial lignin and C:N ratio, showed similar decomposition rates after one year in the field but had different C:N ratios at the end of that time. As discussed in section 5.3, N accumulation in decomposing residue is a well known phenomenon and this was shown here as well; N concentration of Bt stems increased by 400% while that of NBt stems increased by 220% resulting in a significantly lower C:N ratio of Bt compared to NBt stems. This is consistent with the numerically bigger mass loss of the Bt stems but the reason behind it is not clear. It was expected that Bt stems would decompose slower because they were reported to be 'tougher' and more resistant to decomposition but I found no evidence to support that. Based on my results, there are no indications of a suppression of microbial activity in decomposing Bt corn stems. Initial mass loss, which is dictated by the C:N ratio, was similar for up to four months and later, after the winter period, mass loss suggested slightly faster decomposition of Bt stems. It would have been interesting to test the decomposition of residue that had initially different C:N ratios but limitations in material prevented this test. I had the opportunity to test this factor under controlled conditions (chapter four), and found that stems with a high N concentration (Bt) produced more CO_2

than stems with a low N concentration after an incubated period of 20 weeks. However, in the field the results differ; ECB-injured stems (chapter three) that did have a higher N and smaller C:N ratio than the non-injured stems buried in the soil for a period of nine months showed similar decomposition patterns. As well, Zurbrugg et al. (2010) recently tested the field decomposition of Bt and NBt corn leaves and showed that decomposition rates were similar and that the C:N ratios, though initially different between isolines, were similar after five months in the field. They also showed that 99% of the initial concentration of Cry1Ab proteins from two Bt hybrids had degraded after eight months in the field.

Though ECB infestation did not cause a variation in lignin deposition or affect decomposition rates, it did however result in a change in lignin chemistry in fresh and decomposed residue, indicating the potential for faster long-term decomposition of infested stems, as shown by the higher syringic acid concentration in infested stems than non-infested stems. For short-term decomposition there were no differences in mass loss related to ECB infestation, which is supported by the findings of Lehman et al. (2010) from a one year decomposition experiment with Bt and NBt corn residues.

These results and others cited in the literature (chapter one), in combination with my findings support the suggestion that soil microbial activity is unlikely to be affected by the presence of the Bt residue and that infestation does not affect the decomposition rate (mass loss). A slower decomposition rate would have affected nutrient cycling and nutrient availability for plants and the microbial community and would have had a

positive effect on soil C storage but no such effects are expected in a Bt or a non-Bt corn agroecosystem based on this work.

5.5 Decomposition Patterns of Corn Residue Under Controlled Conditions: Effect of Lignin

The effect of lignin on decomposition was evaluated in an incubation experiment using plant parts that exhibited natural differences in lignin concentrations and adding an exogenous lignin source to the soil. The selected exogenous lignin, indulin lignin a by-product from the paper mill industry, did not decompose during incubation, which I attributed to its recalcitrant vanillyl-rich form. However, the effect of lignin was evident from the lower CO_2 production from root-amended soils that had 75% and 92% more lignin than stems and leaves, respectively. There was also an average of 82% more C retained in the soil from roots than from stems and leaves consistent with the values proposed by Johnson et al. (2006) of 1.5 to 3 times more root C than shoot C being stabilized in the SOC pool. Roots are always left in the field after harvest and constitute an important source of relatively lignin-rich C, which has the potential to be stabilized in an intermediate or slow decomposing C pool. This effect will be pronounced when fields are under low tillage management, leaving the roots relatively undisturbed. In another scenario, genetically engineered plants modified to produce more lignin in their roots for support and insect resistance would have added benefits in terms of low CO_2 production and soil C sequestration potentials.

SUMMARY AND CONCLUSIONS

There is evidence from the scientific literature that Bt corn has elevated lignin concentrations compared to NBt hybrids and that in some cases they exhibit slower decomposition due to greater concentrations of lignin. This is supported with anecdotal information from agricultural producers that Bt corn residue is tough and not as easily decomposed as NBt residue. However, other scientific reports state that Bt and NBt corn hybrids are not compositionally different and that they exhibit similar decomposition patterns. Therefore, this work was aimed at testing the compositional differences between Bt and NBt corn in order to assess the effects of extensive Bt corn production on decomposition, a fundamental soil ecosystem process.

Four experiments were conducted to test the proposed hypotheses. In the first experiment, 18 Bt and NBt corn near-isoline hybrids, suitable for Quebec growing conditions, were grown in the field in two years where no herbivory was detected. The results indicated no difference in lignin or C concentrations and showed a possible increase in N concentration in Bt stems compared to NBt stems. The second experiment tested the effect of ECB injury with the hypothesis that injured NBt stems would either exhibit greater stress-lignin deposition causing them to be more resistant to decomposition or would be physically damaged and thus more susceptible to decomposition. Eight Bt/NBt hybrid pairs were grown in pots and half of the pots were infested with ECB. Lignin concentration did not differ in NBt and Bt stems, however leaves from injured plants showed more lignin deposition in the year that had extensive

ECB damage. This result indicated that stress-lignin deposition likely occurred in injured tissue, but was detected only in the leaves, which had lower lignin concentration than stems. The N concentration in injured stems was also greater than non-injured stems, which seemed to be a result of more N uptake by injured plants. Greater N concentration and lower C:N ratio in NBt stems from injured plants was hypothesized to speed decomposition of these residues. However, in a 5-month litterbag field decomposition experiment, there was no difference in decomposition between Bt and NBt hybrids or between ECB injured and non-injured stems. Molecular characterization of lignin-derived phenols in decomposed stems after 5 months indicated that lignin degradation had not begun after this time interval but stems from injured plants had 87% more syringic acid, which strongly suggests that those stems would decompose faster in the long term. Decomposition of field-grown Bt and NBt stems showed that there was a tendency for Bt stems to decompose faster than non-ECB affected NBt stems when buried in the field for one year, and this seemed to be related to the greater N concentration and lower C:N ratio in Bt stems than in NBt stems. Finally, I investigated the decomposition patterns of ^{13}C and ^{15}N labeled corn components (leaves, stems, and roots) that have natural differences in lignin concentrations and added an exogenous lignin source to half the samples in an effort to simulate a high-lignin residue addition to the soil. The results clearly showed that root residue, which had a relatively high lignin concentration, decomposes slower than leaves and stems that have less lignin and that more residue-C is retained in the soil from roots, increasing the possibility that residue C will be transformed into stable soil C. This experiment corroborates the findings of the field decomposition experiments, as it also showed that N and lignin concentrations in the residue are good indicators of short-term

decomposition. The exogenous lignin was not decomposing during this incubation, which is likely due to the recalcitrance of this type of lignin compared to plant-associated lignin.

Results from this work indicate no agronomic benefit from planting Bt corn when no ECB infestation is expected and all indicators point to the similarity in composition and decomposition of Bt and NBt corn tissue. In fact, Bt residue could potentially decompose faster than NBt residue if the N concentration in Bt residue is elevated due to production of the Cry1Ab protein, but this remains to be quantified using ELISA (enzyme-linked immunosorbent assay) and tested in long-term field experiments. Therefore, the decision to plant Bt corn hybrids should be based on the incidence of ECB. NBt corn is still widely used and agronomic losses occur due to stalk lodging and tissue damage but no effect on decomposition is expected as a result of this damage. While there is little effect of herbivory on the total lignin concentration in NBt hybrids, my findings suggest compositional changes in lignin phenols due to ECB infestation. Further work is needed to test the effect of ECB injury on lignin deposition at the injury site using more sensitive techniques such as bioassays for the detection of lignin enzyme precursors (e.g. PAL) or lignin measured by thioglycolic acid reaction (LTGA). In this study stable isotope tracers, predicted that 31% of root-C is retained in the soil compared to an average of 17% from aboveground residue-C in the short term stressing the contribution of corn roots to the maintenance of SOC. The lignin and N concentration of residues control decomposition and can be used to predict decomposition of different corn tissues in multiple-phase exponential decay models after those have been calibrated for specific soils and environmental conditions.

CONTRIBUTION TO KNOWLEDGE

The experiments conducted to answer the objectives of this work provide the following contributions to knowledge:

1. I have shown that the lignin concentration in Bt and NBt corn tissue does not differ, based on 18 near-isoline hybrids grown under Eastern Canadian field conditions.

2. I estimated that for a 7% increase in soil lignin retention after one growing season, a 6% increase in the lignin concentration of the residue is required. This is unlikely to come from a Bt versus NBt scenario, however, such variation in lignin concentration could exist naturally for roots versus aboveground residue inputs.

3. I have found repeated indications that Bt tissues (leaves and stems) have greater N concentrations than corresponding NBt tissues, which warrants further investigation to determine if the Cry1Ab protein is expressed constitutively in these tissues and thus increases their N concentration.

4. The effect of ECB injury on lignin, C, and N concentrations was tested for the first time and the results show that injury does not affect lignin deposition in corn stems when assessed by the ADL method (Goering and Van Soest, 1970). This method was able to detect greater lignin concentration in leaves of heavily infested plants,

5. CuO oxidation method was used for the first time to characterize lignin-derived phenols in ECB infested and non-infested NBt corn stems and to assess the changes in these phenols before and after tissue decomposition. Results from this method indicated that lignin in injured tissue is more susceptible to degradation than that from non-injured tissue.

6. CuO oxidation method was used for the first time as an indicator (analytical tool) to compare the decomposition of lignin from Bt and NBt leaves, stems and roots over time.

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APPENDIX I

Moisture content (g kg^{-1}) of corn grain of field-grown Bt and NBt hybrids in 2008 and 2009. Values are the means ± standard error (n=3).

Hybrid	Bt/NBt	Year 2008	Year 2009
N23-F7	Bt	399 ± 60	272 ± 15
N25N-GT-CB-LL	Bt	336 ± 39	297 ± 13
N29-A2	Bt	370 ± 37	296 ± 32
N34-F1	Bt	379 ± 26	319 ± 13
N45-A6	Bt	446 ± 44	285 ± 35
MZ2263	Bt	405 ± 35	289 ± 8
MZ3877	Bt	416 ± 18	288 ± 11
MZ3888	Bt	409 ± 27	297 ± 28
MZ5444	Bt	483 ± 15	353 ± 24
N23-F9	NBt	386 ± 18	271 ± 14
N25N-GT	NBt	351 ± 14	303 ± 12
N29-G7	NBt	402 ± 38	289 ± 19
N34F-GT	NBt	419 ± 20	314 ± 10
N45A-LL	NBt	364 ± 4	323 ± 18
MZ226	NBt	346 ± 29	277 ± 12
MZ27-00RR	NBt	403 ± 31	286 ± 8
MZ310	NBt	411 ± 22	288 ± 23
MZ540	NBt	458 ± 24	369 ± 30

APPENDIX II

Hemicellulose concentration (g kg^{-1}) of leaves, stems, and roots of field-grown Bt and NBt hybrids in 2008 and 2009. Values are the means ± standard error (n=3).

Hybrid	Bt/NBt	Leaves		Stems		Roots	
		2008	2009	2008	2009	2008	2009
MZ3888	Bt	273 ± 3	327 ± 13	215 ± 10	186 ± 7	273 ± 9	284 ± 15
MZ5444	Bt	266 ± 17	288 ± 10	201 ± 10	172 ± 13	278 ± 12	314 ± 7
MZ3877	Bt	285 ± 13	304 ± 8	257 ± 13	207 ± 5	266 ± 9	297 ± 14
MZ2263	Bt	269 ± 10	318 ± 7	218 ± 4	203 ± 9	292 ± 4	306 ± 9
N25N-GT-CB-LL	Bt	248 ± 13	297 ± 19	219 ± 13	194 ± 12	272 ± 10	286 ± 10
N34-F1	Bt	288 ± 3	282 ± 5	226 ± 18	230 ± 10	277 ± 3	295 ± 3
N23-F7	Bt	273 ± 9	325 ± 10	217 ± 7	202 ± 9	274 ± 13	305 ± 8
N45-A6	Bt	264 ± 11	292 ± 4	212 ± 3	199 ± 9	265 ± 3	287 ± 15
N29-A2	Bt	259 ± 8	300 ± 11	200 ± 5	187 ± 14	278 ± 4	308 ± 15
MZ310	NBt	278 ± 1	320 ± 11	226 ± 14	209 ± 13	257 ± 11	295 ± 14
MZ540	NBt	266 ± 11	298 ± 17	221 ± 16	188 ± 11	269 ± 14	294 ± 9
MZ27-00RR	NBt	255 ± 20	306 ± 10	237 ± 8	220 ± 16	263 ± 2	306 ± 5
MZ226	NBt	289 ± 13	321 ± 5	234 ± 16	214 ± 11	290 ± 12	314 ± 5
N25N-GT	NBt	287 ± 6	291 ± 10	217 ± 16	193 ± 17	291 ± 7	297 ± 10
N34F-GT	NBt	272 ± 14	307 ± 2	207 ± 24	215 ± 7	287 ± 7	295 ± 15
N23-F9	NBt	273 ± 4	319 ± 11	225 ± 1	212 ± 15	274 ± 16	294 ± 11
N45A-LL	NBt	271 ± 17	297 ± 5	229 ± 17	184 ± 8	279 ± 9	316 ± 15
N29-G7	NBt	272 ± 16	313 ± 5	244 ± 18	222 ± 19	267 ± 14	318 ± 7

APPENDIX III

Cellulose concentration (g kg^{-1}) of leaves, stems, and roots of field-grown Bt and NBt hybrids in 2008 and 2009. Values are the means ± standard error (n=3).

Hybrid	Bt/NBt	Leaves		Stems		Roots	
		2008	2009	2008	2009	2008	2009
MZ3888		303 ± 10	322 ± 12	349 ± 48	353 ± 34	405 ± 32	381 ± 21
MZ5444		283 ± 13	312 ± 15	349 ± 17	350 ± 41	433 ± 28	412 ± 7
MZ3877		302 ± 22	338 ± 14	434 ± 26	393 ± 8	379 ± 8	420 ± 10
MZ2263		262 ± 8	344 ± 8	278 ± 12	361 ± 7	388 ± 22	433 ± 23
N25N-GT-CB-LL	Bt	340 ± 26	360 ± 21	393 ± 52	478 ± 32	460 ± 17	436 ± 17
N34-F1		327 ± 8	333 ± 5	380 ± 35	434 ± 31	436 ± 19	392 ± 30
N23-F7		323 ± 12	377 ± 3	390 ± 40	416 ± 20	446 ± 5	401 ± 25
N45-A6		336 ± 17	342 ± 3	413 ± 7	392 ± 17	434 ± 36	385 ± 7
N29-A2		333 ± 22	344 ± 17	329 ± 36	342 ± 48	428 ± 11	414 ± 3
MZ310		306 ± 21	335 ± 10	376 ± 46	379 ± 6	404 ± 38	405 ± 24
MZ540		314 ± 13	291 ± 5	400 ± 28	358 ± 18	430 ± 6	385 ± 14
MZ27-00RR		323 ± 13	340 ± 12	413 ± 36	417 ± 11	446 ± 9	423 ± 8
MZ226		297 ± 31	353 ± 14	335 ± 45	401 ± 30	411 ± 28	445 ± 10
N25N-GT	NBt	327 ± 18	357 ± 21	385 ± 50	434 ± 17	439 ± 9	412 ± 12
N34F-GT		322 ± 28	345 ± 12	416 ± 45	431 ± 10	424 ± 14	416 ± 7
N23-F9		302 ± 24	348 ± 29	372 ± 43	401 ± 15	394 ± 25	382 ± 18
N45A-LL		346 ± 23	344 ± 10	402 ± 51	410 ± 18	445 ± 15	408 ± 8
N29-G7		314 ± 17	365 ± 7	410 ± 29	397 ± 46	439 ± 5	422 ± 16

APPENDIX IV

Weight (g plant^{-1}) of grain+cob of pot-grown Bt and NBt hybrids with or without ECB infestation in 2008 and 2009. Values are the means ± standard error (n=4).

Hybrid	Bt/NBt	ECB/no-ECB	Year 2008	Year 2009
MZ 310	NBt	ECB	40.0 ± 8.0	60.2 ± 1.4
MZ 3888	Bt		55.2 ± 1.3	63.4 ± 1.4
MZ 540	NBt		45.1 ± 8.5	73.6 ± 4.1
MZ 5444	Bt		60.8 ± 1.0	74.3 ± 4.6
N45ALL	NBt		41.2 ± 2.2	66.6 ± 3.5
N45A6	Bt		51.1 ± 2.4	69.8 ± 2.9
N33H6MF1	NBt		31.3 ± 4.0	51.0 ± 12
N33D2MF2	Bt		44.3 ± 1.9	67.4 ± 2.2
MZ 310	NBt	No-ECB	57.4 ± 2.7	63.5 ± 3.8
MZ 3888	Bt		63.0 ± 3.9	58.5 ± 2.7
MZ 540	NBt		70.8 ± 3.6	61.9 ± 17
MZ 5444	Bt		75.0 ± 2.2	74.4 ± 1.6
N45ALL	NBt		64.1 ± 4.9	69.9 ± 2.1
N45A6	Bt		65.6 ± 4.9	61.6 ± 5.2
N33H6MF1	NBt		44.4 ± 9.9	54.4 ± 15
N33D2MF2	Bt		52.7 ± 6.1	53.8 ± 12