Decomposition and Composition Analysis of sibling *Bt* and Non-*Bt* Corn

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Abstract:

Literature suggested that *Bt* corn has higher lignin concentration compared to its sibling non-*Bt* corn. Increase in lignin concentration might result in a decreased rate of *Bt* corn decomposition compared to that of non-*Bt* corn. Reduced decomposition may result in increased residue persistence. This study determined the biochemical composition (soluble sugars, starch, hemicellulose, cellulose, lignin, total C and total N) and the rate of decomposition of stover from *Bt* and non-*Bt* corn on stover. Ground stover (2 mm) was incubated in a Barnes soil at 25^{o} C and 60% water filled pore space. Gas chromatography was used to measure the total respired CO_2 of amended soil periodically over 120 days. A two-component exponential decay model was used to describe the decomposition of the corn residue. The more quickly decomposing materials are referred to as the active fraction and the slowly decomposing component as the passive fraction. The half-life of the active component was about 11 days for both *Bt* and non-*Bt* corn. The passive component had a half-life of 1070 days for *Bt* corn and 1370 days for the non-*Bt* corn. Suggesting that the *Bt* corn may decay slightly faster than the non-*Bt* corn. In the field corn stover would be subjected to predation by macro and meso-fauna, which could alter rate of decomposition.

Introduction:

Yield loss from European corn borer (*Ostrinia nubilalis)* infestation is valued in excess of $1 billion annually in North America (Huang et al., 1999; Saxena and Stotzky, 2000). Corn genetically modified to express the toxin from *Bacillus thuringiensis* (*Bt*) is grown in areas at risk for infestation European corn borer.

Bt is a rod shaped, spore-forming, gram-positive bacterium marketed for its ability to work as a biopesticide to control lepidopteran pests (Saxena and Stotzky, 2000). *Bt* produces a crystalline inclusion during sporulation, which may contain more than one type of insecticidal crystal protein (Stotzky, 2000). The toxin may exhibit activity against several orders of insects (Stotzky, 2000), however, it has no apparent effect on earthworms, nematodes, protozoa, bacteria and fungi in soil (Saxena and Stotzky, 2001a)

Anecdotal evidence from farmers suggested that *Bt* varieties of corn appeared to decompose more slowly in the field compared to their non-*Bt* corn counter-parts. Decomposition occurs in several steps. The corn leaves and stalks are broken down through weather or consumption by macro (herbivores) and meso fauna (e.g. insects). Microbes (fungi and bacteria) decompose plant material. Differences in decomposition can reflect the palatability of the corn residue to the insects and other consumers. It also reflects the basic composition of the material. For instance, the initial rate of decomposition corresponds to the initial concentration of N and readily available C, but as decomposition progressed, the rate reflects the lignin content (Berg and Matzner, 1997).

Saxena and Stotzky, (2001) reported a larger concentration of lignin in *Bt* compared to non-*Bt* corn isolines. An increase in lignin was hypothesized to decrease the

decomposition rate of *Bt* corn compared to its non-*Bt* isoline. Reduced decomposition may result in increased residue on the soil, which could have the indirect benefit of reducing erosion. However, persistence of *Bt* corn in the soil may also mean an increase in residence time of the toxin, thereby increasing the potential hazard to non-target organisms and increasing selection pressure for toxin-resistant target insects. This study determined the biochemical composition and the rate of decomposition of stover from *Bt* and non-*Bt* corn.

Materials / Methods:

Corn hybrids Pioneer 3893 (Non-*Bt*) and isoline Pioneer 38W36 (*Bt*) were field grown in west central Minnesota. There were four replications. Each plot was 18.3 m long by 6.1 m wide, using 76 cm row spacing. Four plants were selected randomly and harvested from each field plot at maturity. The plant material from each plot was pooled. The corn stover (leaves and stems) was dried at 45°C, stored at -80°C, and ground to pass through a 2mm sieve.

Decomposition conditions.

Barnes soil was used for all incubations. The soil was collected from the surface 15 cm, air-dried, and passed through a 2 mm sieve. About 0.2 g dried and ground plant was mixed thoroughly with 50-g soil in a 230-mL bottle. Unamended soil was used as a control. The experiment was initiated with the addition of water to achieve about 60% water-filled pore space (WFPS). Soil and residue mixtures were incubated in the dark for

120 days at constant temperature (25°C) with 49% humidity. Water was added as necessary to maintain the desired soil moisture.

Measuring CO_2 evolution monitored decomposition. Gas samples were removed from the jars each weekday during the first two weeks. Subsequently, gas samples were taken on days 15, 18, 24, 31, 45, 66, 94, and 120. During the initial week of sampling, the jars were capped between 3 and 6 hours prior to taking a gas sample. The CO_2 accumulation time was increased to about 48 hours by 60 days after initiation as the flux rate had decreased. The accumulation time was recorded and used to calculate the rate of CO_2 flux. After the prescribed accumulation time, a 2.5 mL gas sample was removed from the jar using a gas-tight syringe and 2 mL of the gas was injected into an evacuated 1.8 mL amber sample vial. The sample vials had Teflon/red rubber lined aluminum caps. Duplicate 50-µL gas samples were injected within 8 hours of sample collection using a CP8200 Varian autosampler into a Porapak Q column (injection temperature, 150ºC; column temperature 80ºC; detector temperature 130ºC; filament temperature 250ºC) on a 3800 Varian gas chromatograph equipped with a thermal conductivity detector. The flow rate was 32 mL min^{-1} using helium as the carrier gas. A three-point standard CO_2 curve was used at each sampling date.

Composition analysis

The composition of plant material was determined using a sequential extraction of 0.5 g plant material for soluble sugars, starch, hemicellulose, cellulose and acid soluble and acid-insoluble lignin (Martens and Loeffelmann. 2002; NREL, 1995; NREL, 1996a; NREL, 1996b; Tarpley et al., 1993). Soluble sugars, starch, hemicellulose and cellulose

were determined by HPLC. Standards were included and concentrations adjusted to reflect recovery rates. Acid soluble lignin concentration was determined spectrophotometrically (NREL, 1996b) and acid-insoluble lignin was determined by proximate method (NREL, 1995). The total C and N of the plant material were determined with a LECO CN-2000 (LECO Corporation, St. Joseph, MI).

Data was analyzed using analysis of variance, step-wise regression analysis, and non-linear regression.

Results and discussion:

The variability was large for all components; the non-*Bt* and Bt corn had similar amounts of soluble sugars, starch, hemicellulose, cellulose and lignin (Table 1). In contrast, Saxena and Stotzky's (2001b) reported that *Bt* isolines had 33 to 97% more lignin in their stems between the 3rd and 4th nodes compared to non-*Bt* isolines. The plant material in Saxena and Stotzky's (2001b) study was either grown for 97 d in a growth room or 90 d in the field. In our current study field grown Pioneer 3893 (Non-*Bt*) and isoline Pioneer 38W36 were used, which is not one of the hybrids used by Saxena and Stotzky (2001b). In addition, our samples were collected at harvest and contained leaves as well as nodes and inter-nodes from stalks.

The total C evolved reflects both the C from the residue added and C released that had previously been in the soil (Figure 1). The C flux was similar on most sampling dates, with a slightly larger flux from the *Bt* corn residue compared to the non-*Bt* residue. Small differences in instantaneous fluxes can result in measurable differences in total flux.

A cumulative net flux was calculated. It was assumed that the amount of C originating from the soil was the same for amended and control soils. Therefore, the C evolved from the control was subtracted from the amended soil. This calculated net C evolved represents only the C from the corn residue added to the soil. This assumption neglects any potential priming effects. The net cumulative C evolved as a function of time was calculated by assuming linearity between sampling points and calculating the area under the rate curve (Fig. 2).

On average, 31 mg of C from the *Bt* residue was released after 120 day, which corresponds to 33% of the C added (Fig. 2). In comparison, 26 mg of C from the non-*Bt* isoline was released after 120 day, which corresponds to 27% of the C added.

Decomposition can be described biologically and mathematically using an exponential function (Wieder and Lang, 1982). We used a two-component model assuming a rapidly decomposing or active fraction (C_a) and a slowly decomposing fraction (C_p) equation 1 (Fig. 3).

$$C_t = C_a \times \exp(-k_a \times t) + (100 - C_a) \times \exp(-k_p \times t) \quad \textit{Equation 1.}$$

The percent C remaining at time (t) is C_t, C_a is the percent C in the active fraction, k_a is the rate for the active component, (100-C_a) is the percent C in the passive fraction (C_p) and k_p is the decomposition rate of the passive component. The half-life for the two fractions is calculated with equation 2.

$$\text{Half life} = t_{1/2} = \frac{0.693}{k_t} \qquad \textit{Equation 2.}$$

The half-life of the active component was about 11 days for both *Bt* corn and the non-*Bt* sibling. The passive component had a half-life of 1070 days for *Bt* corn and 1370

days for the non-*Bt* corn. These results showed the *Bt* isoline decomposing slightly faster than the non-*Bt* isoline.

The decomposition data did not support the hypothesis that *Bt* corn would decompose slower than non-*Bt* corn. The concentrations of structural components (lignin, cellulose and hemicellulose) were similar between the non-*Bt* and *Bt* corn, which would be consistent with the observed patterns of decomposition (Table 1 and Fig. 2). It is possible that in the field *Bt* corn may break down more slowly compared to non-*Bt* corn. The modified corn could have less structural damage from corn borer, which may leave the corn stalks more intact. Decomposition would be accelerated by structural damage, which increases surface area. We ground the plant material to a uniform size, which would eliminate any difference in residue size. Another hypothesis that could be tested is the palatability of the material. Is there a difference in the mesofaunal feeding on *Bt* corn residue compared to non-*Bt* residue or other residues in the soil?

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Table 1. Nonstructural and structural component concentrations extracted from corn stover (leaves and stalks) from *Bt* and non-*Bt* corn isolines at harvest. N = 4.

Corn	Sucrose	Glucose	Fructose	Soluble Sugar	Starch
			mg g^{-1} plant material		
Bt	143	34	36	213	2.14
non *Bt*	106	21	21	148	2.73
p	ns	ns	ns	ns	ns

	Hemi-cellulose	Cellulose	Acid Insoluble Lignin	Acid Soluble Lignin	Ash
			mg g^{-1} plant material		
Bt	499	483	93	5.4	25
non *Bt*	536	517	99	5.7	23
p	ns	ns	ns	ns	ns

Figure 1. Rate of CO2 released from control (no residue added) or from soil amended with corn stover from either *Bt* or non-*Bt* isolines. Soil and residue mixtures were incubated at 60% water-filled pore space and at 25° C. Symbols represent mean of 4 replications, bars are ± one standard error, when bars not shown, error bars are smaller than the symbol.

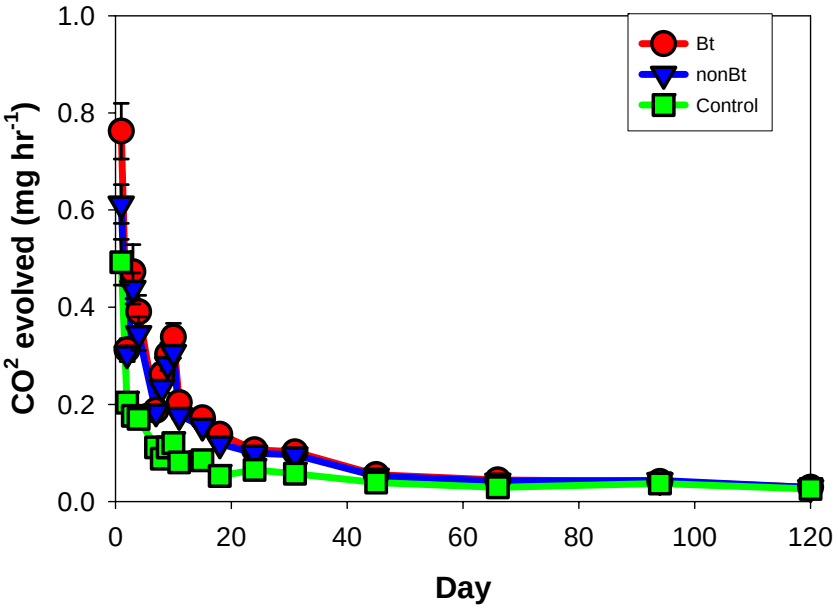

Figure 2. Cumulative net C evolved, assuming that the C evolved originating from the soil is the same after adding corn residue.

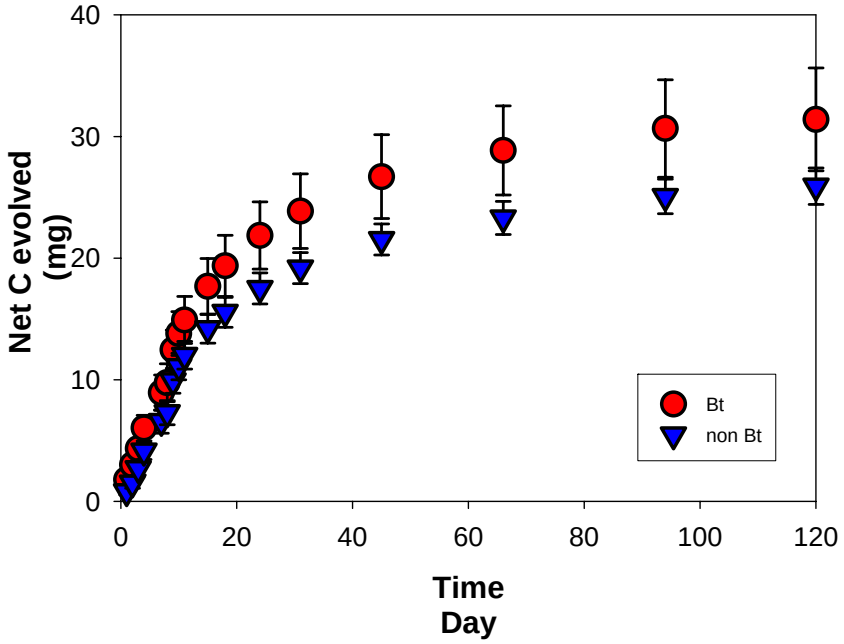

Figure 3. The net C remaining described using a double exponential function.

% C remaining = (Initial C added – net C evolved)/initial C added * 100

Net C evolved = C evolved from amended soil - C evolved from control

$$C_t = C_a \times \exp(-k_a \times t) + (100 - C_a) \times \exp(-k_p \times t)$$

C_t. Percent C remaining at time (t); C_a, percent C in the active fraction; k_a rate of decay for the active component, (100-Ca), percent C in the passive fraction; k_p rate of decay for the passive component.