

Effect of Cry3Bb Transgenic Corn and Tefluthrin on the Soil Microbial Community: Biomass, Activity, and Diversity

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ABSTRACT

Transgenic Bt corn expressing the Cry3Bb insecticidal protein active against corn rootworm (CRW) (*Diabrotica* spp.; Coleoptera: Chrysomelidae) was released for commercial use in 2003 and is expected to be widely adopted. Yet, the direct and indirect risks to soil microorganisms of growing this CRW-resistant Bt corn versus applying insecticides to control the rootworm have not been assessed under field conditions. The effects of CRW Bt corn and the insecticide tefluthrin [2,3,5,6-tetrafluoro-4-methylbenzyl (Z)-(1RS)-cis-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate] on soil microbial biomass, activity (N mineralization potential, short-term nitrification rate, and soil respiration), and bacterial community structure as determined by terminal restriction fragment length polymorphism (T-RFLP) analysis were assessed over two seasons in a field experiment. Bt corn had no deleterious effects on microbial activity or bacterial community measures compared with the non-transgenic isolate. The T-RFLP analysis indicated that amplifiable bacterial species composition and relative abundance differed substantially between years, but did not differ between rhizosphere and bulk soils. The application of tefluthrin also had no effect on any microbial measure except decreased soil respiration observed in tefluthrin-treated plots compared with Bt and non-transgenic isolate (NoBt) plots in 2002. Our results indicate that the release of CRW Bt corn poses little threat to the ecology of the soil microbial community based on parameters measured in this study.

THE USEPA has recently approved the commercial release of transgenic Bt corn resistant to corn rootworm. This CRW corn, which expresses the Cry3Bb toxin derived from *Bacillus thuringiensis* subsp. *kumamotoensis*, has tremendous potential for the management of a pest that is currently responsible for widespread insecticide use and yield losses of more than \$200 million per annum (Gray, 2000). The enormous progress made in developing and disseminating such insect-resistant Bt corn varieties is exciting from the perspective of increasing productivity and decreasing environmental and human health hazards posed by insecticides normally used to contain pest damage. A survey conducted by Pilcher and Rice (1998) demonstrated that Iowa farmers were as interested in planting corn resistant to the corn rootworm as they were in planting corn resistant to the corn borer. Thus, it is likely that the acreage planted to transgenic corn in the USA will continue to increase in the near future. In fact, Frederici (1998) estimated that by 2003, area planted to Bt corn would reach almost 30 million acres (12.15 million ha), about one-third of the total U.S. corn acreage. It should be

noted that CRW Bt corn expresses the Cry3Bb toxin in all plant parts throughout its life cycle, thus potentially creating routes of exposure for soil organisms that include root exudation, trophic level interactions, plant decomposition after harvest, and toxin persistence in the soil. Thus, concerns regarding the dissemination of this technology have been expressed in public and scientific debate, but have not yet been adequately addressed.

Data from laboratory and greenhouse studies on Bt corn producing Cry1 toxin to control the European corn borer have drawn attention to the persistence and activity of such toxins and their potential effects on soil microorganisms. The toxin is thought to resist microbial degradation by binding to clay and humic acid fractions in soil, but to retain insecticidal activity despite being bound (Venkateswerlu and Stotzky, 1992; Tapp and Stotzky, 1995, 1998; Koskella and Stotzky, 1997; Crecchio and Stotzky, 1998). Saxena et al. (1999) showed that Cry1Ab present in Bt corn root exudates extracted from rhizosphere soil retained insecticidal activity against tobacco hornworm larvae for weeks, causing 100% mortality even after 25 d of plant growth. Further, particles of rhizosphere soil in suspension placed directly on the bioassay medium caused mortality comparable with the extracts. Saxena and Stotzky (2000) also showed that larvicidal activity increased with the length of time Bt corn plants were grown, suggesting that microbial degradation may not have kept pace with the increased root exudation of the toxin into soil with time. Stotzky (1999) reported that CO₂ evolved from soil amended with ground Bt (Cry1Ab) corn biomass was significantly lower than that from soil amended with unmodified, isogenic corn biomass. While the mechanism by which the presence of the Cry1Ab toxin might depress microbial activity is unclear, it is unlikely to be directly toxic to microorganisms. It is more likely that the insertion of the Cry1Ab gene into corn may alter either the amount or character of one or more root exudates important to microorganisms or water availability in the rhizosphere, thereby reducing respiratory activity.

The literature contains several reports on the effects of other transgenic crops on nontarget soil microorganisms as well as persistence and activity of the toxins associated with them. Donegan et al. (1995) reported a transient stimulation of soil bacterial and fungal populations by two of three Bt cotton lines. These lines also produced changes in bacterial communities, as measured by BIOLOG analysis and DNA fingerprinting. Recent field studies with other transgenic crops have

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Abbreviations: Bt corn, transgenic corn engineered to express *Bacillus thuringiensis* toxin; CRW, corn rootworm; NoBt, non-transgenic isolate; NoBt + I, non-transgenic isolate with insecticide applied; PCR, polymerase chain reaction; T-RF, terminal restriction fragment; T-RFLP, terminal restriction fragment length polymorphism.

also consistently demonstrated changes in the diversity of microbial communities in soil (Di Giovanni et al., 1999; Donegan et al., 1999; Griffiths et al., 2000; Lukow et al., 2000). In experiments to evaluate the persistence of Cry1 toxins from Bt cotton leaves incorporated into soil microcosms, Palm et al. (1996) found that degradation of the toxin was microbially mediated as suggested by various reports from the Stotzky group (Koskella and Stotzky, 1997; Crecchio and Stotzky, 1998). However, it is difficult to make conclusions regarding persistence of the toxin from this study, as the rate of degradation varied greatly between experiments, with 0.1 to 85% of original extractable toxin remaining after 28 d.

Reports on the effects of pyrethroid insecticides such as tefluthrin on soil microbiota are scarce; however, data on persistence of the insecticide indicate that it has a half-life of 17 d at 30°C (24 d at 20°C), and is not expected to affect soil microflora at normal application rates (Tomlin, 1997). In their work with two other pyrethroid insecticides, Taiwo and Oso (1997) reported significant reductions in microbial populations after an initial rise. Carbon (C), nitrogen (N), potassium (K), and phosphorus (P) solubilization and pH were also reduced in insecticide-treated soil compared with controls. In contrast, a laboratory study demonstrated that soil bacterial and fungal populations depressed by tefluthrin recovered after 2 wk, as did nitrification activity (Tu, 1989). Sulfur oxidation and soil respiration evaluated in an organic soil were also not affected. To date, there have been no reports that have used field data to compare the effects of transgenic CRW Bt corn against those of insecticides commonly used against the corn rootworm. Such a comparative risk assessment is particularly important when one considers that farmers in the USA will generally rely on insecticides to control the corn rootworm in the absence of Bt corn (Gray et al., 1993; Gray, 2000).

Microbial activity measures such as N mineralization potential, nitrification, and soil respiration rate can indicate if there have been gross changes within the microbial community in response to use of Bt corn or insecticides, but these measures cannot provide information on which species or groups are affected. Coleman and Crossley (1996) stated that “we are past the time when measurements of the ‘soil biomass’ alone, by whatever method, are considered adequate.” Such measures need to be combined with phenotypic and genotypic assays to provide a broader perspective for assessing microbial ecology in soils. Two community fingerprinting techniques, terminal-restriction fragment length polymorphism (T-RFLP) analysis (Liu et al., 1997; Lukow et al., 2000; LaMontagne et al., 2002; Blackwood et al., 2003) and denaturing gradient gel electrophoresis (DGGE) (Muyzer and Smalla, 1998; Piceno et al., 1999), have both been used successfully to compare bacterial populations in differentially managed agricultural soils, with several studies reporting higher sensitivity with T-RFLP (Lukow et al., 2000; Osborn et al., 2000). In this study, we employed a polyphasic approach in which microbial biomass, activity, and T-RFLP analyses were combined to assess microbial ecology in soils planted to CRW Bt

corn, its non-transgenic isolate, and the non-transgenic isolate treated with the insecticide tefluthrin.

MATERIALS AND METHODS

Study Site

A field trial was established in May 2001 and May 2002 at Cornell University's Homer C. Thompson Vegetable Farm in Freeville, New York, on a Howard gravelly loam soil (loamy-skeletal, mixed, active, mesic Glossic Hapludalfs; 45.4% sand, 42.2% silt, 12.4% clay, 5.4% organic matter, pH 6.9, 10 mg kg⁻¹ available nitrate). Sampled treatments consisted of four replicated plots of transgenic Bt corn resistant to the corn rootworm (Bt), and three replicated plots each of the non-transgenic isolate (NoBt) and the non-transgenic isolate with the insecticide tefluthrin (Force G; Dow Elanco, St. Louis, MO) applied to the soil at planting (NoBt + I). All plots were sown with seed treated with the fungicide Captan (Drexel Chemical Co., Memphis, TN) and the insecticide Gaucho (Bayer AG, Leverkusen, Germany). Gaucho protects seeds from a variety of insect pests including wireworms, seedcorn maggot, and fire ants, and seedlings from damage by flea beetles and white grubs. All seed was supplied by Monsanto (St. Louis, MO). Planting was at 76-cm spacing into 50- × 46-m plots. Soil samples from each plot were taken three times during each of the two growing seasons, at planting (mid-May), anthesis (mid-August), and harvest (mid-November). Bulk and rhizosphere soil samples to a 15-cm depth were taken along two transects across each plot and were composites of 10 samples each. Bulb planters were used to collect samples in 2001; soil was immediately sieved (2 mm) and roots with adhering soil were removed from this bulk sample and sieved again (1 mm) to separate rhizosphere soil from the roots. This method did not yield sufficient rhizosphere soil for all measurements, and soil samples collected at anthesis in 2002 were too dry to permit separation of roots from bulk soil, so an alternative method was employed to collect rhizosphere soil in 2002. Whole plants were uprooted and soil that fell off the root mass when shaken was considered to be bulk soil. Rhizosphere soil was that which adhered tightly to the root mass and could be dislodged only by knocking the root mass several times against a collection bucket. A portion of bulk and rhizosphere soil samples was separated after sieving and immediately frozen at -20°C for subsequent molecular analyses or stored at 4°C for microbial biomass and activity measurements, which were begun within a day of sampling. Rainfall data were obtained from the on-farm weather station.

Microbial Biomass

Microbial biomass N was determined by the chloroform fumigation-extraction method using 10 g of soil (Brookes et al., 1985; Vance et al., 1987) followed by spectrophotometric measurement of ninhydrin-positive compounds in the extracts and conversion of biomass N to biomass C (Joergensen and Brookes, 1990). Biomass C was calculated using the empirical relationship: Biomass C = B_{nin} × 20.6, where B_{nin} = N_{nin} extracted from fumigated soil - N_{nin} extracted from unfumigated soil.

Microbial Activity

Three measurements of microbial activity were used to assess the effect of CRW Bt corn and tefluthrin on soil microorganisms in this study: N mineralization potential, short-term nitrification rate, and soil respiration rate.

Nitrogen Mineralization Potential

The method of Waring and Bremner (1964) was used to incubate soil for N mineralization potential. Five grams of soil was weighed into 20-mL screw-cap test tubes and 12.5 mL distilled water was added so that the soil would be waterlogged and most of the N would be mineralized as NH_4^+ . Test tubes were incubated at 40°C for 7 d, while replicate test tubes were incubated at 4°C. After incubation, steam distillation was employed followed by titration with 0.5 M HCl to estimate NH_4^+ -N in the samples (Keeney and Nelson, 1982).

Short-Term Nitrification Rate

Short-term nitrification rate was determined by the method of Berg and Rosswall (1985) using 5 g of each soil sample and an incubation time of 5 h at 25°C. Controls were incubated in the freezer at -20°C. This method involves the use of NaOCl_3 to inhibit the oxidation of nitrite to nitrate; the resulting NO_2^- -N produced in our samples was determined spectrophotometrically.

Soil Respiration Rate

The rate of soil respiration was measured using 20 g of soil weighed into 250-mL Ball canning jars (Isermeyer, 1952; Anderson, 1982). Carbon dioxide evolved from the soil was trapped in vials containing 10 mL of 0.5 M NaOH placed in the jars; these vials were sampled every 7 to 10 d and 10 mL of 0.5 M NaOH was replaced after each measurement. The CO_2 in the sampled NaOH was precipitated using 8 mL of 0.5 M BaCl_2 and the amount of remaining NaOH determined by titration against 0.5 M standard HCl.

Data Analysis

Analysis of variance was conducted on biomass and activity data using the general linear model in MINITAB Statistical Software (Minitab, 2002). All results are reported at $p = 0.05$.

Bacterial Community Analysis

DNA was extracted from soil samples stored at -20°C with the Ultraclean Soil DNA Extraction Kit (MoBio Laboratories, Solana Beach, CA). Polymerase chain reaction (PCR) was performed in triplicate per soil sample. The carboxyfluorescein (FAM)-labeled forward primers 27f (5'-/6-FAM/AGA GTT TGA TCC TGG CTC AG-3', *E. coli* numbering) and 1492r (5'-GGT TAC CTT GTT ACG ACT T-3'; both manufactured by Integrated DNA Technologies, Coralville, IA) were used to amplify 16S rRNA eubacterial genes, resulting in products of approximately 1500 bp. Final reactant concentrations in each 100 μL reaction were: 0.05 U μL^{-1} *Taq* polymerase, 1x PCR buffer and 2.0 mM MgCl_2 (Applied Biosystems, Foster City, CA), 0.2 mM deoxy-nucleotide triphosphates (dNTPs), 0.1 $\mu\text{g} \mu\text{L}^{-1}$ bovine serum albumin, and nuclease-free water (all from Promega, Madison, WI). Final template concentrations ranged between 10 and 30 $\text{ng} \mu\text{L}^{-1}$. Polymerase chain reaction was performed in a Hybaid PCR Express thermal cycler (Ashford, Middlesex, UK) using an initial denaturation step of 94°C for 3 min followed by 35 cycles of the following program: denaturation at 94°C for 30 s, primer annealing at 59°C for 15 s, and extension at 72°C. A final extension at 72°C for 15 min was performed after the cycling was complete. The three replicate PCR reactions were then pooled and purified using the Qiaquick PCR purification kit (Qiagen, Chatsworth, CA). Ten microliters of purified PCR product was mixed with 5 μL of restriction digest containing 1.5 μL of the appropriate 10x buffer, 2.75 μL of nuclease-free water,

and 0.75 μL of restriction enzyme. Three separate restriction digests with the enzymes *HhaI*, *MspI*, and *RsaI* (Promega, Madison, WI) were set up per purified PCR reaction. Restriction digests were performed in a Hybaid PCR Express thermal cycler using a 3-h incubation at 37°C followed by 15 min at 68°C to denature the enzyme and stop the reaction. One microliter of carboxytetramethylrhodamine (TAMRA) 2500 size standard (Applied Biosystems) was mixed with 1.5 μL of the digested PCR product. DNA fragments of different sizes were separated by electrophoresis at 3000 V for 6 h on a 5% polyacrylamide gel using an ABI 377 automated DNA sequencer at Cornell University's Biotechnology Resource Center.

Data Analysis

The gel image was analyzed with GeneScan Analysis Software (Applied Biosystems, 2000) using a peak height threshold of 20 fluorescence units. The Local Southern size-calling algorithm provided the best estimate of terminal restriction fragment (T-RF) sizes for the standard, and was therefore applied for data analysis. The Ribosomal Database Project II (RDPII; Cole et al., 2003) was used to generate similarity matrices; principal components analyses were conducted using CANOCO v. 4.5 (Microcomputer Power, 2002).

RESULTS AND DISCUSSION

Rainfall data were collected at the study site and are presented in Fig. 1 to illustrate that rainfall distribution varied greatly between 2001 and 2002 for the period of Day of Year 182 (1 July) to Day of Year 232 (20 August; anthesis sampling). There were fewer rain events and less cumulative rainfall during this period in 2002 (49 mm) than in 2001 (147 mm). Soil at the study site is a Howard gravelly loam, which consists of very deep, well-drained soils formed in glacial outwash deposits. These soils are classified as being "somewhat excessively drained to well-drained" (USDA Natural Resources Conservation Service, 2004), and are therefore unable to retain water for long periods of time. Thus, the small amount of

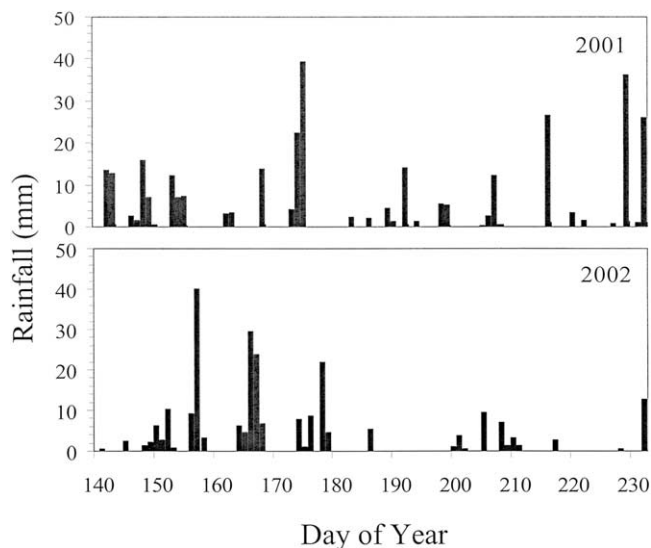


Fig. 1. Rainfall distribution at the study site from planting (Day of Year 140; 20 May) to anthesis sampling (Day of Year 232; 20 August) in 2001 and 2002.

Table 1. Effect of corn rootworm (CRW)-resistant transgenic corn engineered to express *Bacillus thuringiensis* toxin (Bt corn) and tefluthrin on microbial biomass C, N mineralization potential, and short-term nitrification rate in rhizosphere and bulk soil sampled from field trials at anthesis in 2001 and 2002.†

Year	Treatment‡	Microbial biomass C		N mineralization potential		Short-term nitrification rate	
		Rhizosphere	Bulk	Rhizosphere	Bulk	Rhizosphere	Bulk
		μg C g ⁻¹ soil		NH ₄ ⁺ -N g ⁻¹ soil		NO ₂ ⁻ -N g ⁻¹ soil h ⁻¹	
2001	Bt	423 (91) a§	499 (108) a	39 (10) a	34 (13) a	ND¶	ND
	NoBt	415 (105) ab	474 (76) a	28 (15) a	24 (8) a	ND	ND
	NoBt + I	499 (121) a	439 (95) ab	34 (7) a	32 (9) a	ND	ND
2002	Bt	313 (62) b	352 (49) b	37 (6) a	30 (4) a	0.173 (0.05) a	0.174 (0.04) a
	NoBt	356 (75) ab	353 (54) b	27 (3) a	27 (2) a	0.156 (0.03) a	0.170 (0.02) a
	NoBt + I	346 (113) b	388 (45) ab	30 (3) a	29 (2) a	0.149 (0.08) a	0.172 (0.03) a

† Values in parentheses are standard deviations.

‡ Bt, transgenic Bt corn; NoBt, non-transgenic isolate; NoBt + I, non-transgenic isolate + insecticide (tefluthrin).

§ Different letters within a column represent significant differences at $p = 0.05$.

¶ Not determined.

poorly distributed rainfall during this period in 2002 resulted in very dry soil and corn plants exhibiting severely rolled leaves indicating drought-stress for about 3 wk before the anthesis sampling. We believe the rainfall pattern may have influenced many of our results, as will be discussed in the following section.

Microbial Biomass

Neither Bt corn nor the application of insecticide had any significant effect on microbial biomass C (Table 1). Microbial biomass in the rhizospheres of Bt and tefluthrin-applied plots was significantly lower in 2002 compared to 2001. Similarly, microbial biomass in bulk soil from Bt and NoBt plots was lower in 2002 than in 2001. We believe these differences to be a result of the poorly distributed rainfall in 2002.

Microbial Activity

Nitrogen Mineralization Potential

Nitrogen mineralization potential was lowest in the NoBt plots in bulk and rhizosphere soils for both seasons of this study (Table 1); however, these results were not statistically significant, suggesting that there was no significant effect of Bt corn or insecticide on N-mineralization potential.

Short-Term Nitrification Rate

Tu (1989) reported a transient depression of nitrification in response to tefluthrin applied in a sandy loam and an organic soil. However, we observed that the nitrification rate was statistically similar for all treatments, indicating that neither Bt nor tefluthrin affected short-term nitrification in this study (Table 1). Further, no differences in NO₂⁻-N produced were observed between bulk and rhizosphere soil.

Soil Respiration Rate

Stotzky (1999) reported that CO₂ evolution from soil amended with ground Bt corn biomass expressing the Cry1Ab gene was significantly lower than that from soil containing unmodified, isogenic corn biomass. Although there are no published reports on the effect of CRW Bt corn on soil microbial respiration, tefluthrin has been reported to reduce soil respiration in laboratory studies of sandy loam, but not organic soil (Tu, 1989). As shown in Fig. 2, we also observed that the insecticide appeared to depress microbial respiration in rhizosphere soil from insecticide-treated plots compared with other rhizosphere treatments ($p = 0.05$), but there was no difference in soil respiration activity between Bt and NoBt plots. It is unclear why an effect of tefluthrin would persist almost 90 d after application when the insecticide

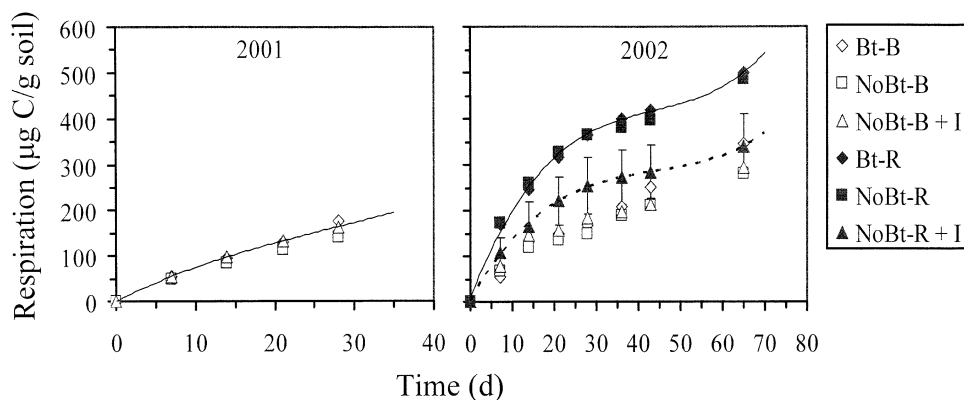


Fig. 2. Effect of corn rootworm (CRW)-resistant transgenic corn engineered to express *Bacillus thuringiensis* toxin (Bt) and the insecticide tefluthrin (I) on microbial respiration in bulk (B; open symbols) and rhizosphere (R; closed symbols) soil sampled at anthesis in 2001 and 2002. The 2002 rhizosphere respiration for Bt and non-transgenic isolate (NoBt) is estimated by a solid line; that for NoBt + I is estimated by a dotted line. Rhizosphere data were not available in 2001. Error bars represent one standard deviation from the mean.

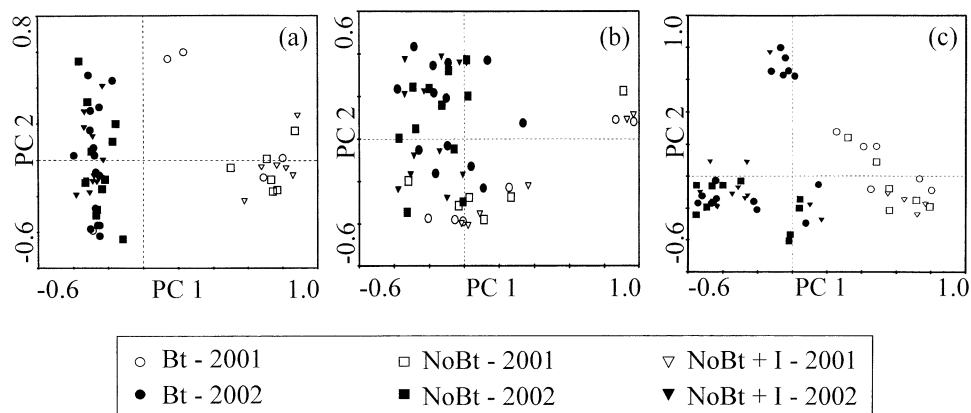


Fig. 3. Principal components (PC) analysis to compare presence of terminal restriction fragments (T-RFs) in corn rootworm (CRW) transgenic corn engineered to express *Bacillus thuringiensis* toxin (Bt), the non-transgenic isolate (NoBt), and the non-transgenic isolate with tefluthrin (NoBt + I) in 2001 and 2002 after digestion of polymerase chain reaction (PCR) products with (a) *HhaI*, (b) *MspI*, and (c) *RsaI*. Bulk and rhizosphere samples were pooled for this analysis.

has been reported to display a half-life of only 17 d at 30°C and 24 d at 20°C (Tomlin, 1997). Although rhizosphere soil respiration data are available only for 2002, we speculate that low soil moisture resulting from the poorly distributed rainfall before anthesis in this year may have negatively affected rates of biodegradation and leaching of tefluthrin. Thus, residual tefluthrin and its degradation products may have been present in soil sampled at anthesis, which may have reduced soil respiratory activity. We also observed that respiration in rhizosphere soil from Bt and NoBt plots was significantly higher than in bulk soil. The decreased microbial biomass C measured in the rhizosphere of these treatments appears to contradict soil respiration results. However, as anthesis sampling occurred 2 d after a rain event, it is possible that the higher rhizosphere respiration rate relative to biomass C reflects an increase in metabolic activity by the microbial community that survived the water stress before anthesis.

Bacterial Community Analysis

According to Marsh (1999), a restriction endonuclease that is capable of resolving a large number of known

sequences, that is, yielding a large number of terminal restriction fragments (T-RFs) upon virtual cutting, provides greater insight into estimates of biodiversity than one that yields fewer T-RFs. In silico digestion of all complete SSU rRNA gene sequences using Release VII of the Ribosomal Database Project with *HhaI*, *MspI*, and *RsaI* indicated that these enzymes yield a larger number of T-RFs than most other enzymes. Consequent T-RFLP analysis using these enzymes yielded approximately 90 to 110 distinct peaks per sample for *HhaI* and *MspI*, and about 30 to 50 peaks per sample for *RsaI*. We therefore concluded that these endonucleases, particularly used in combination, would allow us to use T-RF length to resolve a considerable portion of the eubacterial communities in our samples.

No clear separation of samples based on treatment was achieved by use of principal components analysis in conjunction with T-RFLP, indicating that there were no detectable differences in the composition of eubacterial communities in soil from Bt, NoBt, and NoBt + I plots at the resolution available by use of this method. However, samples separated clearly into two groups based on year for two of the three enzymes used, sug-

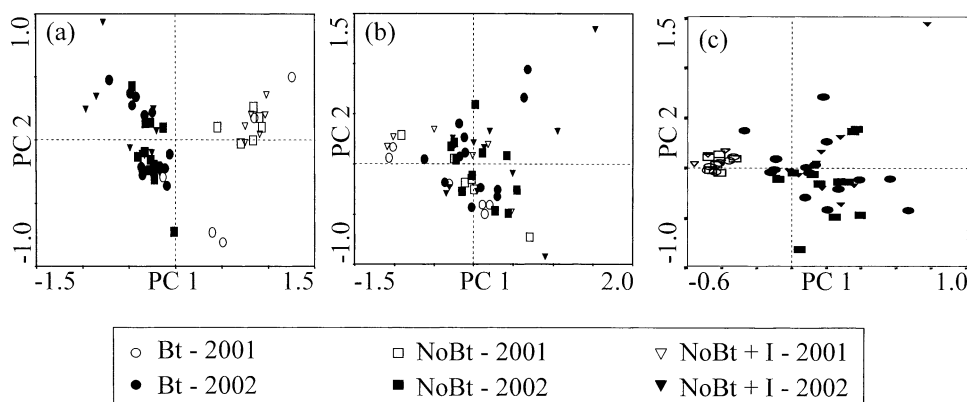


Fig. 4. Principal components (PC) analysis to compare relative abundance of terminal restriction fragments (T-RFs) in corn rootworm (CRW) transgenic corn engineered to express *Bacillus thuringiensis* toxin (Bt), the non-transgenic isolate (NoBt), and the non-transgenic isolate with tefluthrin (NoBt + I) in 2001 and 2002 after digestion of polymerase chain reaction (PCR) products with (a) *HhaI*, (b) *MspI*, and (c) *RsaI*. Bulk and rhizosphere samples were pooled for this analysis.

gesting that bacterial communities in these plots differed from one year of the study to the next (Fig. 3). Differences in the frequency and amount of rainfall for several weeks before the anthesis sampling in 2001 as compared with 2002 may have contributed to these observations. No clear trend was observed for bulk vs. rhizosphere samples in the T-RFs derived from the three restriction endonuclease digestions. Results of analyses to compare samples based on relative abundance of T-RFs (peak heights) were in agreement with the analyses based on presence or absence of T-RFs (Fig. 4). As before, no separation was obtained based on treatment, suggesting that the relative abundance of bacterial operational taxonomic units (OTUs) did not vary between Bt, NoBt, and NoBt + I plots. Bulk and rhizosphere samples could not be separated either, but *Hha*I and *Rsa*I samples could, once again, be grouped clearly by year.

CONCLUSIONS

We detected no deleterious effects of growing CRW Bt corn on microbial biomass, activity, or bacterial community structure as measured in this study. The only negative impact of insecticide application was the decreased soil respiration observed in the rhizospheres of tefluthrin plots compared with Bt and NoBt treatments in 2002, possibly a result of poor rainfall distribution and consequently low soil moisture, as has already been discussed. The T-RFLP analysis indicated significant differences in bacterial community structure between years in both bulk and rhizosphere soils; however, no effect of Bt corn or tefluthrin on bacterial community comparisons or relative abundance of T-RFs was evident. These results lead us to conclude that CRW Bt corn and tefluthrin did not adversely affect microbial biomass, activity, and bacterial diversity or relative abundance within the scope of this study. Molecular analyses to assess bacterial and fungal diversity at planting and harvest are ongoing and will be presented elsewhere.

It is well known that the activities of soil microorganisms are indispensable in nutrient cycling and organic matter decomposition. They also influence soil structure and water infiltration through the production of bacterial polymers and fungal hyphae that hold soil particles together to form aggregates (Tisdall and Oades, 1982; Lee, 1991). Rhizosphere microorganisms can benefit plants by suppressing pathogens, as has been documented for various crop-pathogen combinations (Beauchamp et al., 1991; Hebbbar et al., 1991; Kloepper et al., 1992). The current results are reassuring, for large changes in the activity or diversity of soil microorganisms may have the potential to adversely affect nutrient cycling, soil structure, and the natural control of pathogens or parasites by predatory organisms. Understanding the comparative risks of CRW corn and tefluthrin is important for the optimal management of physical, chemical, and biological aspects of soil ecology, which in turn will optimize returns and the long-term sustainability of the agroecosystem.

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