Transgenic *Bt* cotton tissues have no apparent impact on soil microorganisms

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ABSTRACT

The impact of transgenic *Bacillus thuringiensis* (*Bt*) cotton residues on soil microorganism communities was investigated. Leaves of three different varieties of transgenic *Bt* cotton and their near-isogenic lines were placed in soil and the numbers of indigenous soil microorganisms were measured with cultivation-dependent approaches under laboratory conditions. The soil samples were collected after 7, 14, 21, 28, 56 and 84 days of incubation. Numbers of bacteria, actinomycetes and fungi in the soil were measured by counting colony forming units after incubation on appropriate medium. Overall, although there were differences in bacteria, actinomycetes and fungi population between soil amended with *Bt* and non-*Bt* cotton throughout the whole incubation in three experiments, these differences were transient and not persistent from one sampling stage to the next. These results suggest that *Bt*-transgenic cotton tissues have no apparent impact on soil microorganism population.

Keywords: risk assessment; *Bacillus thuringiensis* (*Bt*) toxin; culturable microorganisms; microorganism population; residue decomposition

Some strains of cotton were genetically modified to express the *Cry* proteins from the bacterium *Bacillus thuringiensis* (*Bt*) to produce a protein that is toxic to the larvae of a number of lepidoptera species, particularly the cotton bollworm *Helicoverpa armigera* (Hübner) (Zhao et al. 1998, Shelton et al. 2002). This reduces the requirement for specific insecticide treatments (Mascarenhas and Luttrell 1997) and the risk of pollution from chemical insecticide applications. However, the large-scale commercial release of *Bt* crops is of public concern because of the potential threat to natural and agricultural ecosystems (Hails 2000, Stotzky 2000, 2004, Hu 2009, Velmourougane 2013). When assessing the ecological risks of transgenic plants, their impact on soil microbes should be considered, because the structure of the soil microbial community is an important component of soil quality and health, and soil microbiological properties are early and sensitive indicators of anthropogenic effects on soil ecology in both natural and agricultural ecosystems (Visser and Parkinson 1992).

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Inevitably, the *Bt* toxin will be introduced to soil in root exudates throughout the growth of the transgenic plant (Saxena et al. 1999, 2002, Saxena and Stotzky 2000, Icoz and Stotzky 2008), through pollen deposition during tasseling (Losey et al. 1999, Hansen-Jesse and Obrycki 2000), and by incorporation of plant residues after harvest (Zwahlen et al. 2003, Stotzky 2004). The toxin could accumulate to a concentration that might affect the composition and activity of soil microbial communities. Previous studies also suggested that the random insertion of a foreign gene into the plant genome may cause changes in the amount and composition of crop residues (Saxena and Stotzky 2001, Poerschmann et al. 2005, 2008, 2009) and may also affect organic matter decomposition and nutrient cycling in soil (Castaldini et al. 2005, Flores et al. 2005). These changes may affect soil microbial communities that are responsible for crop residue decomposition (Castaldini et al. 2005, Flores et al. 2005) and nutrient release in soil ecosystems. Large quantities of *Bt* crop residue are produced when the crop is harvested. It is therefore necessary to evaluate the effects of transgenic *Bt* cotton residue on soil microorganisms. In this study, the effects of *Bt*-transgenic cotton on the culturable soil microorganisms is investigated by comparing the incorporation of three hybrids of *Bt*-transgenic and non-transgenic cotton residue in soil under laboratory conditions.

MATERIAL AND METHODS

Material and treatments. Three varieties of transgenic *Bt* cotton [Guo-Kang 12 (*Bt*-GK), carrying *cry1A* gene, Zhong-Kang 30 (*Bt*-ZK), carrying *cry1A* gene, and SGK321 (*Bt*-SGK), carrying both *cry1A* and *CpTI* (Cowpea trypsin inhibitor) genes)] and their near-isogenic lines [Si-Mian 3 (non *Bt*-SM), Zhong-Mian 16 (non *Bt*-ZM) and Shi-Yuan 321 (non *Bt*-SY), respectively], were used in this study. Parental and transgenic cotton seeds were provided by the Cotton Research Institute of the Chinese Academy of Agricultural Sciences. Only cotton leaves were used in this experiment because they have the highest expression level of the B.t.k. endotoxin. Cotton leaves were frozen for a minimum of 12 h at –20°C to facilitate breakage into small units for uniform distribution in the soil and to promote decomposition. For treatments containing plant material, parental or transgenic cotton leaves were incorporated at a ratio of 1:3 by weight (leaves:soil) into sterile 125 mL flasks. Sterile water was added to all treatments to bring the soil to 45% water holding capacity. Soil was mixed with a sterile spatula and placed at 5°C for ~4 h to allow dispersal of the added water. Three replicates were prepared for each treatment. A control of soil without cotton leaves was also set up. Flasks were sealed with parafilm wrap, stored in the dark at 22°C and sampled after 7, 14, 21, 28, 56 and 84 days of incubation (Donegan et al. 1995). The soil (0–15 cm depth) used in this experiment was collected from the cotton area at Baoding in the Hebei Province, northern China. The soil was sieved to 2 mm and mixed thoroughly. It had an organic matter content of 21.5 mg/kg, available N of 47.4 mg/kg, total P of 9.08 mg/kg and total K of 160 mg/kg.

Quantification of bacteria, actinomycetes and fungi. Total bacteria, actinomycetes, and fungi were enumerated using a 10-fold dilution plate technique. Soil (10 g) from the various treatments was suspended in 100 mL sterile water, shaken for 20 min at 250 rpm, and 10-fold serially diluted. The colony forming units (CFU) of bacteria, actinomycetes, and fungi in each sample were determined by spreading 100 µL of the diluted sample on appropriate culture media in Petri plates, with three replicate plates per dilution. For bacteria, the medium was LB agar. Actinomycetes were counted on 20 g of Gauze No. 1 agar containing 20.0 g soluble starch, 1.0 g KNO_3, 0.5 g K_2HPO_4, 0.5 g $MgSO_4 \cdot 7\ H_2O$, 0.5 g NaCL and 0.01 g $FeSO_4 \cdot 7\ H_2O$, and a solution of 3.3 mL 3% potassium dichromate was added immediately before use. The number of CFU of fungi was estimated on 18 g of Martin's Rose Bengal streptomycin agar with 10.0 g glucose, 5.0 g peptone, 1.0 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7\ H_2O$ and 33 mg Rose Bengal, and a solution of 3.0 mL 1% streptomycin was added immediately before use. Plates were incubated at 28°C for 1–2 days for the assay of bacteria, 7–10 days for actinomycetes, and 2–4 days for fungi. Colonies were counted visually and expressed as CFU/g dry soil. All the results are expressed on the basis of oven-dried soil.

Statistical analysis. The number of bacteria, actinomycetes, and fungi ($\log_{10}$ CFU) were expressed as the means ± standard deviation. The statistical differences among the data were determined by analysis of variance (ANOVA) and the Tukey's honestly significant difference (*HSD*) test at the 5% significance level with SPSS 13.0 (SPSS Inc., Chicago, USA).

RESULTS AND DISCUSSION

The numbers of bacteria in soil amended with *Bt* and non-*Bt* cotton or not amended with cotton showed similar increasing trends over sample time in the three experiments. The CFU of bacteria in the soil with *Bt* or non-*Bt* cotton amendment were significantly lower than in the soil with no cotton amendment from 7 to 28 days of incubation (except non *Bt*-SY in 28 days), but were significantly higher from day 56 to 84 in the three experiments. There were no significant differences in the numbers of bacteria between soil amended with *Bt* and non-*Bt* cotton after 7, 14, 56 and 84 days in experiment 1, after 21, 28 and 84 days in experiment 2, and after 7, 28, 56 and 84 days in experiment 3 (Figure 1). Actinomycetes population followed the similar trend as that of bacterial population. The CFU of actinomycetes in the soil amended either with *Bt* or non-*Bt* cotton were significantly lower than in the soil with no cotton amendment in the initial incubation. Both soils with cotton amendments showed considerable increases in the numbers of actinomycetes compared with the unamended soil after 56 and 84 days of incubation. There were no significant differences in the numbers of actinomycetes between soil amended with *Bt* and non-*Bt* cotton from day 56 to 84 in the three experiments (Figure 2). The numbers of fungi in the soil amended with either *Bt* or non-*Bt* cotton were significantly higher than in the soil with no cotton amendment throughout the whole incubation in the three experiments (except non *Bt*-SM in 56 days). There were no significant differences in the population of fungi between soil amended with *Bt* and non-*Bt* cotton after 7, 28 and 56 days in experiment 1, after 28 days in experiment 2, and after 7, 21, 28, 56 and 84 days in experiment 3 (Figure 3).

The present study showed no consistent statistically significant differences between the soil amended with *Bt* and non-*Bt* cotton in the numbers of culturable bacteria, actinomycetes and fungi throughout the whole incubation in three experiments. Some differences between the soil amended with *Bt* and non-*Bt* cotton in the numbers of the various groups of microorganisms were observed in 26 instances (the total number of instances was 3 treatments × 6 sampling dates × 3 types = 54), but these differences were not consistent from one sampling stage to the next. These results were consistent with studies carried out by Donegan et al. (1995), who reported that in a microcosm experiment with *Bt*-transgenic cotton leaves, two of the three transgenic cotton lines produced a significant but transient increase in the number of CFU of culturable bacteria and fungi. Similarly, Saxena and Stotzky (2001) found that there were

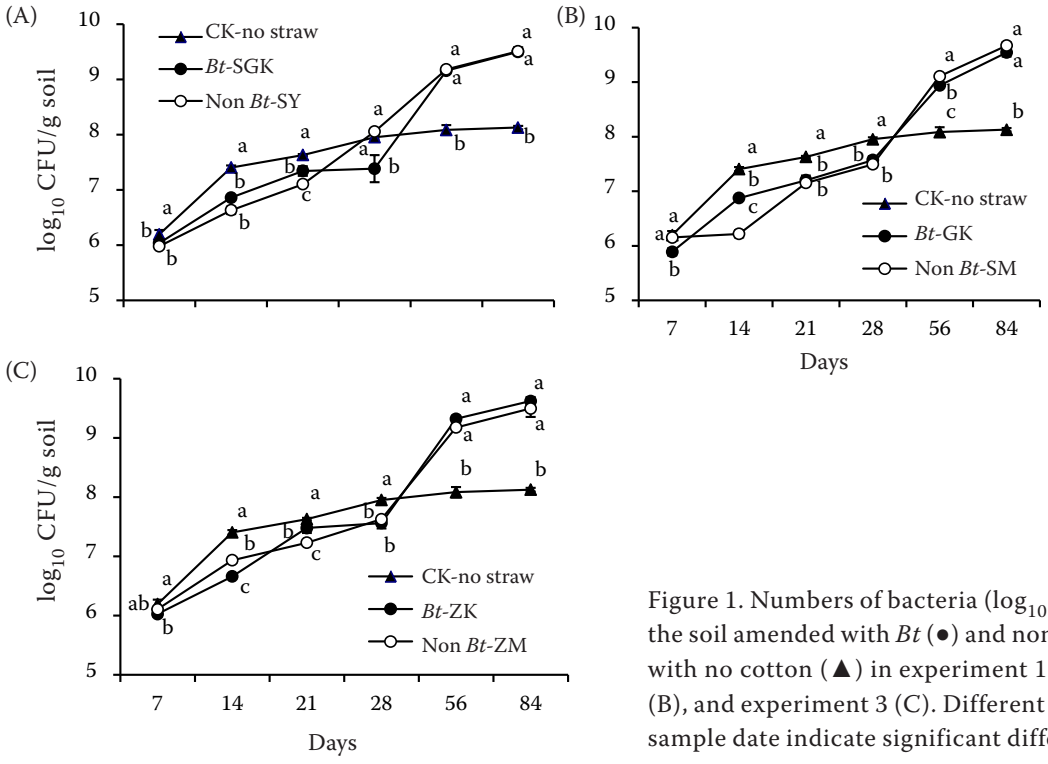

Figure 1. Numbers of bacteria ($\log_{10}$ CFU/g dry soil) in the soil amended with *Bt* (●) and non-*Bt* (○) cotton and with no cotton (▲) in experiment 1 (A); experiment 2 (B), and experiment 3 (C). Different letters at the same sample date indicate significant differences at $P = 0.05$

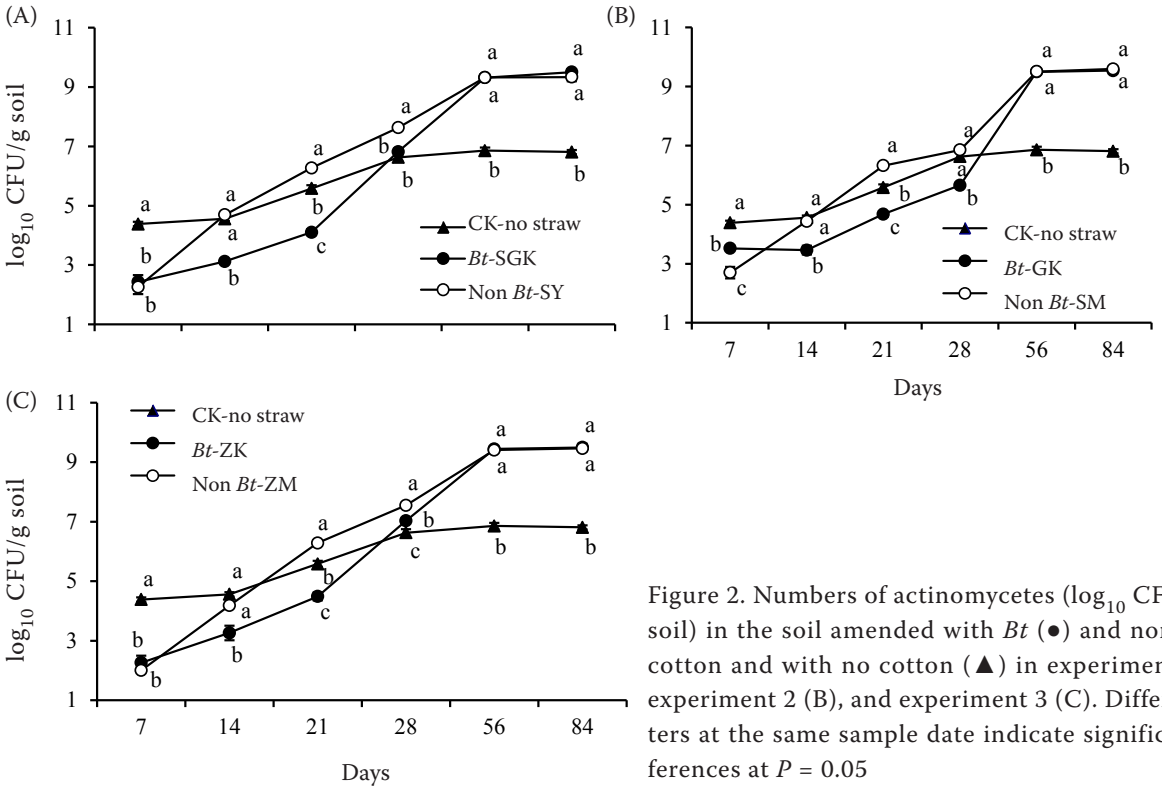

Figure 2. Numbers of actinomycetes ($\log_{10}$ CFU/g dry soil) in the soil amended with *Bt* (●) and non-*Bt* (○) cotton and with no cotton (▲) in experiment 1 (A); experiment 2 (B), and experiment 3 (C). Different letters at the same sample date indicate significant differences at $P = 0.05$

no significant differences in the CFU of culturable bacteria, actinomycetes and fungi between soil amended with biomass of *Bt* and non-*Bt* corn after 45 days of incubation. In addition, Wu et al. (2004) reported that there were only some occasional significant differences ($P < 0.05$) in the number of aerobic bacteria, actinomycetes and fungi and in the number of anaerobic fermentative bacteria, denitrifying bacteria, hydrogen-producing acetogenic bacteria, and methanogenic bacteria

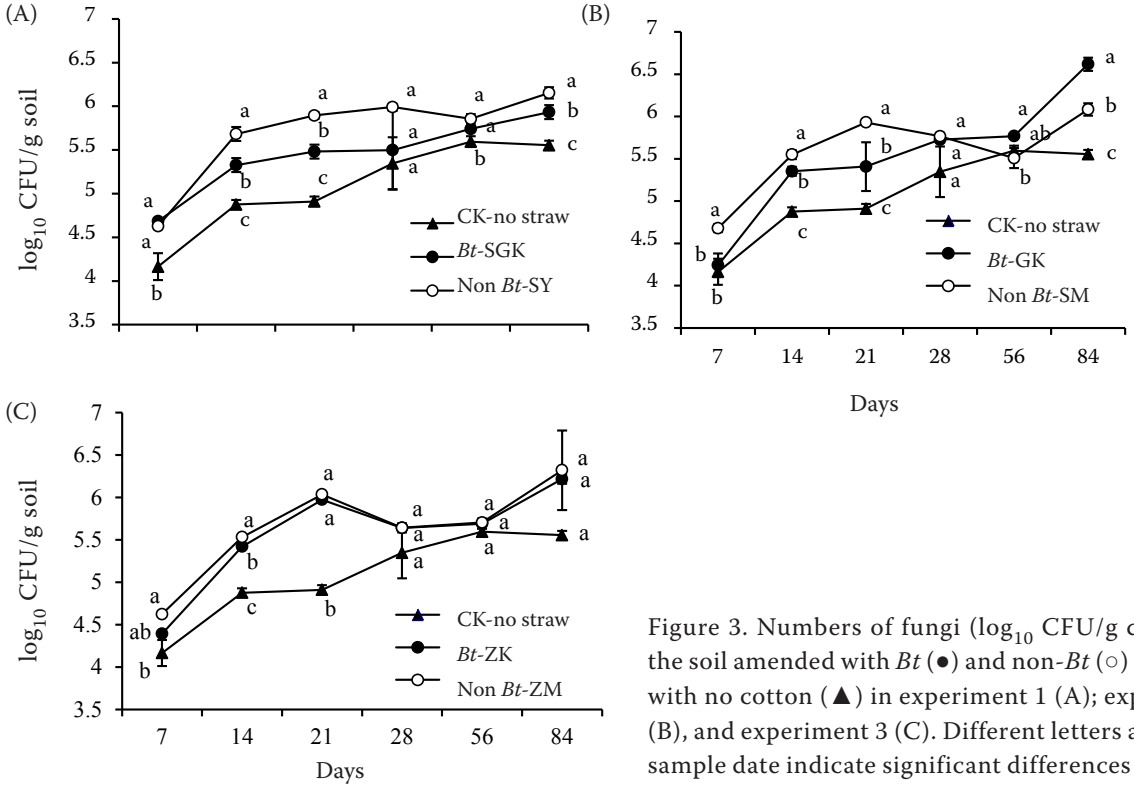

Figure 3. Numbers of fungi ($\log_{10}$ CFU/g dry soil) in the soil amended with *Bt* (●) and non-*Bt* (○) cotton and with no cotton (▲) in experiment 1 (A); experiment 2 (B), and experiment 3 (C). Different letters at the same sample date indicate significant differences at $P = 0.05$

between paddy soil amended with *Bt*-transgenic rice straw and non-*Bt* rice straw during the early stages of incubation. Tan et al. (2010) detected no apparent lasting effect on soil bacterial and fungal communities of *Bt* corn plants and their straw by polymerase chain reaction-denatured gradient gel electrophoresis (PCR-DGGE) and sequences of 16S rRNA and 18S rRNA genes. Lu et al. (2010a,b) also indicated that compared with the non-*Bt* rice residue treatment, *Bt* rice straw had no significant effects on the soil bacterial and fungal community composition in the rapeseed-rice cropping system and in a paddy field. The reason for the initial lower population of bacteria and actinomycetes in the soil amended with either *Bt* or non-*Bt* cotton relative to the unamended counterpart requires further investigation.

In conclusion, differences in the microorganism population between soil amended with *Bt* and non-*Bt* cotton were either transient or absent. These results suggest that *Bt*-transgenic cotton had no significant effects on the soil bacteria, actinomycetes and fungi population associated with residue decomposition. However, the results presented here should be considered preliminary because a culture-based technique (which detects only a small portion of the microbial community) was used and further work is required to investigate the impacts of other soil microorganisms.

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