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Analysis of Non-Target Impacts of Foray 48B on Soil Micro-Organisms

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The effect of Foray 48B (*Bacillus thuringiensis* subsp. *kurstaki*, *Btk*) on indigenous soil micro-organisms was assessed in a pot trial in which four rates of Foray were applied. Foray had no impact on the genetic diversity of the indigenous soil eubacterial community, as measured by PCR-DGGE. Using *Bacillus*-specific PCR primers, bands corresponding to *Btk* were detected within the natural soil populations of bacilli only at 100 and 1000× field rate (where field rate = 5 L/ha of Foray 48B). After 2 weeks, bacterial functional diversity (estimated by BIOLOG™ ecoplates) was similar in all treatments and total fungal and bacterial populations were greater in the 1000× FR treatment only.

Btk products such as Foray 48B are typically applied to foliage for controlling leaf-feeding insect pests. When repeated applications of biopesticide are made, for example to control exotic pests, high numbers of spores and crystals can reach the soil, leading to concerns about potential non-target effects of *Btk* products on soil microflora. The impact of *Bt* on other micro-organisms is largely unknown. *In vitro* antibiotic activity of *Bt* species other than *Btk* has been reported (9), but no effect of Dipel (*Btk*) application was found on soil microbial respiration and biomass when used at the recommended field rate (8). Similarly, more recent studies have not detected any effects of *Bt* toxins on culturable soil microorganisms (1, 4, 7).

In a greenhouse trial, pots containing perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) grown in field collected soil were treated with Foray 48B (Abbott Laboratories) at four rates (0 – water only, 1x, 100x, and 1000x field rate), where field rate was 5 L/ha^{-1} (equivalent to 83.5 BIU ha^{-1}) and the effects on non-target soil micro-organisms were monitored using a polyphasic approach. Four replicate pots of each treatment were sampled at 1, 2 and 4 weeks after treatment application. Bacterial community DNA extracted from the soil and bacterial 16S rDNA fragments were amplified by PCR using previously described primers and methodology (3, 5, 6). PCR products were separated by denaturing gradient gel electrophoresis (DGGE). The functional diversity of the soil bacterial community was estimated using BIOLOG ecoplates (10). Bacterial and fungal populations were enumerated by dilution plating on tryptic soy agar (TSA) and potato dextrose agar (PDA) containing 1µg/ml chlortetracycline.

DNA fingerprinting patterns showed that Foray 48B application had no impact on the diversity of the indigenous soil bacterial community (Fig. 1). Community analysis of the soil eubacteria revealed highly complex fingerprints in all treatments. The four replicate samples showed almost identical fingerprints, demonstrating low variability between pots and a high reproducibility of DNA extraction, by PCR and DGGE procedures. Using *Bacillus*-specific PCR primers (2), bands corresponding to *Btk* were detected within the natural soil populations of bacilli only at 100x and 1000 × FR (data not shown).

The soil bacterial functional diversity in pots treated with 1000x FR was significantly different from the other treatments at 1 week after treatments, but after 2 weeks functional diversity was similar in all treatments (results not shown). Total culturable bacterial numbers did not differ significantly among treatments, with the exception of 1000x FR, where bacterial numbers were significantly higher than in control soils (results not shown). Similarly the total culturable fungal populations were significantly higher only in the 1000× FR treatment at 1 and 2 weeks post treatments.

In conclusion, application of very high amounts of Foray 48B (1000x FR) caused only transient effects on bacterial functional diversity and the total numbers of culturable bacteria and fungi. The addition of Foray 48B even at very high rates (1000x FR) had no effect on diversity of predominant eubacterial populations present in soil, as determined by PCR-DGGE. When *Bacillus*-specific

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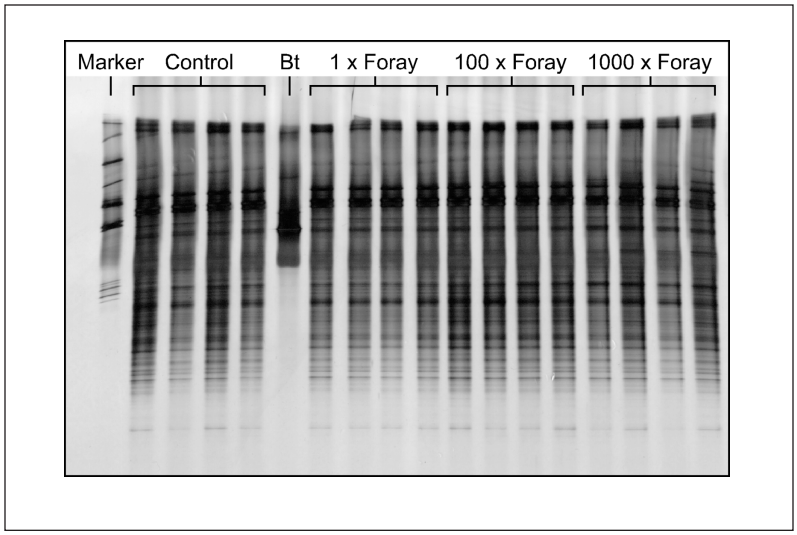

FIG. 1: DNA banding patterns of soil bacteria obtained by DGGE analysis of eubacterial-primer based amplicons from soil inoculated with Foray 48B at various rates. Bt = *Bt kurstaki*.

primers were used, bands corresponding to *Btk* were visible at 100x and 1000x FR 1 week after application; no corresponding bands were detected in the control or in the 1x FR treatment.

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