NON-TARGET MICROORGANISMS AFFECTED IN THE RHIZOSPHERE OF THE TRANSGENIC Bt CORN

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Introduction

Genetically modified *Bt* corn expressing the *cry1ab* gene from *B. thuringiensis* produces a larvicidal toxin that kills lepidopteran pests, especially the European corn borer (*Ostrinia nubilalis*) which is a major pest in Europe and North America (Höfte and Whiteley 1989).
The presence of the toxin released in root exudates and from damaged and degraded root cells was detected in the rhizosphere soil of 12 studied hybrids of *Bt* corn, representing three transformation events (Saxena et al. 2002; Saxena and Stotzky 2000; Saxena et al., 1999). Although the free toxin can be easily utilized as a carbon and energy source by soil microorganisms for growth, when released into soil it rapidly and strongly adsorbs and binds to surface-active particles, which renders it resistant to degradation by microorganisms (Koskella and Stotzky 1997; Tapp, Stotzky 1995). As a result the toxin accumulates in soil, meanwhile it retains its anti-lepidopteran activity for at least 180 days, the longest time studied (Crecchio and Stotzky 2001).
The toxin released in root exudates and from biomass of *Bt* corn had no apparent effects on earthworms, nematodes, protozoa, bacteria and fungi in soil (Saxena and Stotzky, 2001.a), and the purified free and clay-bound toxin from *Bacillus thuringiensis* subspp. *kurstaki* did not affect the growth of a variety of bacteria and algae in pure and mixed cultures (Koskella, Stotzky, 2002). Still it would be important to evaluate the impact of root exudates of *Bt* corn on other representatives of beneficial resident soil microbiota. Partly because any effect of the toxin on the microbiota in soil is probably more complex than simply a microbicidal or microbiostatic effect, and also because the transformation may have lead to pleiotropic effects, that could have further ecological implications.
The environmental effect of genetically modified corn (*Zea mays* L.) expressing the *cry1ab* gene from *Bacillus thuringiensis* ssp. *kurstaki* was studied on some non-target soil and endorhiza microbes.
The colonisation properties of the microsymbiont arbuscular mycorrhizal (AMF) fungi and the total enzymatic activity of rhizosphere soil microbes measured by FDA hydrolysis were tested during the seasonal development of the host-plants.

Methods

Isogenic (DK440) and transgenic corn line (DK440BTY) was provided by the Monsanto Company. Corn seeds were planted at the experimental station of the Plant Protection Institute of the Hungarian Academy of Sciences. Rhizosphere soil and roots of corn plants were sampled seasonally during the vegetation period of 2001.
Mycorrhizal infection development was evaluated by the method of Trouvelot et al. (1986). Presence of arbuscules and internal hyphae was expressed as intensity of infection (M%) and arbusculum richness (A%).
FDA hydrolysis was measured according to the method of Schnürer and Rosswall (1982) as modified by Adam and Duncan (2001).
All experiments were done in replicates. In order to correct any heterogeneity of the variances AM infection data were arcsine transformed. To compare the means of several samples and the interactions between treatments, two-way analysis of variance (ANOVA) were undertaken.

Results and discussion

Mycorrhizal colonization
At the beginning of the vegetation period intensity of mycorrhizal infection and arbuscular frequency were much lower in the root segments of *Bt* corn. Later these differences disappeared as symbiosis was reconstructed, resulting in similar colonisation values, although two-way analysis of variance (ANOVA) showed significant differences considering the entire sampling period (Figure 1 and 2).
The reason of this early handicap in mycorrhizal infection might be the smaller number of entry points in the root system of *Bt* corn (Takács et al. 2005).

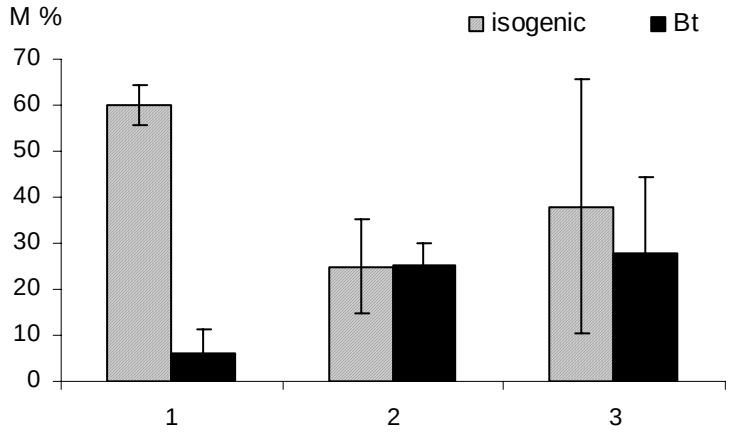

Fig. 1: Intensity of mycorrhizal infection in the roots of the transgenic *Bt* corn and the isogenic, non-*Bt* corn sampled seasonally (1=July, 2=August, 3=October) during the vegetation period of 2001.

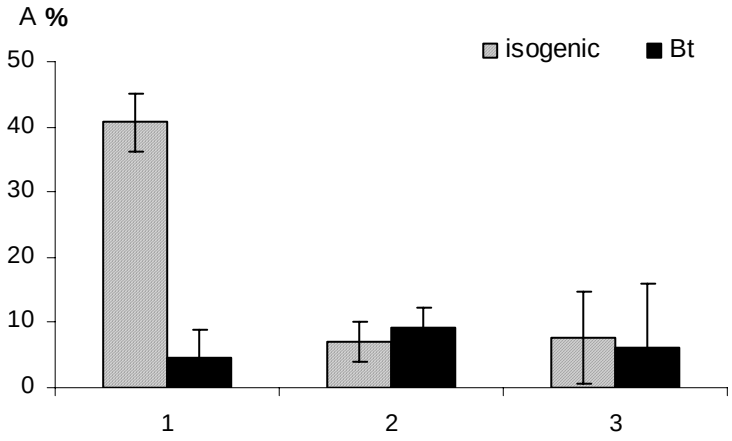

Fig. 2: Arbusculum richness in the roots of the transgenic *Bt* corn and the isogenic, non-*Bt* corn sampled seasonally (1=July, 2=August, 3=October) during the vegetation period of 2001.

Enzyme assay – fluorescein diacetate hydrolysis

Significant differences in hydrolytic enzym activity were observed: total microbial activity in the rhizosphere soil of transgenic *Bt* corn measured by fluorescein diacetate hydrolysis was significantly higher than that of the isogenic non-*Bt* corn considering the whole vegetation period (Figure 3).

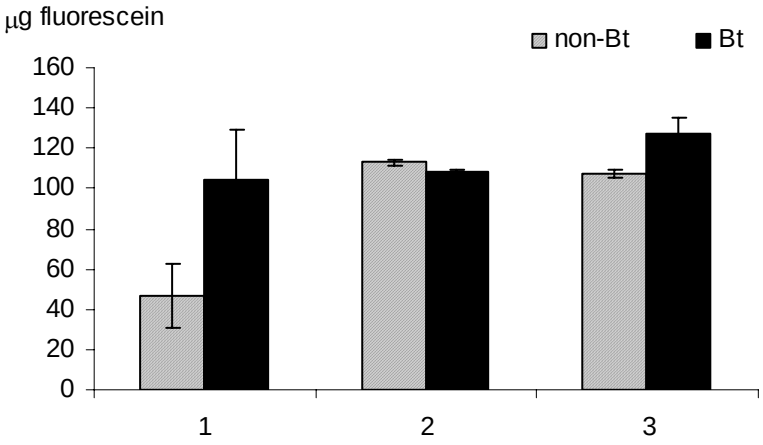

Fig. 3: Total microbial activity (μg fluorescein.g^{-1}dry soil.hour^{-1}) in the rhizosphere of the transgenic *Bt* corn and the isogenic non-*Bt* corn sampled seasonally (1=July, 2=August, 3=October) during the vegetation period of 2001.

Conclusions

To develop appropriate risk-assessment methodologies for the safe distribution of genetically modified organisms is an increasing demand in the world. Among the used assessment techniques there are only few, which are concentrating on non-target effects in the rhizosphere (Biró et al. 2005).
Our results suggest that the transgenic *Bt* corn and the isogenic non-*Bt* corn may differ in certain physiological properties beside toxin content. The genetic transformation may have lead to pleiotropic effects. Saxena and Stotzky showed that the content of lignin of the stems was significantly higher in 10 hybrids of *Bt* corn studied than in their respective non-*Bt* isolines (Saxena, Stotzky, 2001. b). Villányi et al. measured significant differences of transgenic and isogenic corn plants considering C:N ratio of leaf tissue.
Likewise, genetic manipulation might have lead to changes in plant physiology, root-exudate composition, etc., affecting rhizosphere microorganisms.

Acknowledgement

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