

## Experimental Systems to Monitor the Impact of Transgenic Corn on Keystone Soil Microorganisms

Turrini, A.<sup>1</sup>, Sbrana, C.<sup>2</sup>, & Giovannetti, M.<sup>3</sup>

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### Abstract

*Risks and benefits of transgenic crop plants should be evaluated not only by assessing pollen flow, but also by considering soil persistence of transgenic products, such as Bt toxins, which can accumulate in the soil and remain active for a long time. Moreover, transgenic plants are often plowed under as crop residues, representing a potential hazard for nontarget arbuscular mycorrhizal (AM) fungi, a group of beneficial plant symbionts fundamental for soil fertility. In this study, we monitored the effects of transgenic corn plants (Bt 11 and Bt 176) and their residues on AM fungal growth and root colonization ability. Both transgenic plants decreased mycorrhizal colonization and Bt 11 plant residues negatively affected mycorrhizal establishment by indigenous endophytes four months after their incorporation into soil.*

### Introduction

After the approval of the European Community Directive 2001/18, a debate began in Europe about the coexistence, in space and time, of genetically modified organisms (GMO) and organic or conventional agriculture. So far, poor knowledge exists on the interactions among the different components of agroecosystems and on the potential hazards posed by unintended modifications occurring during genetic manipulation. The increasing number of reports on the ecological risks of GM plants stress the need for experimental works aimed at evaluating the environmental impact of GM crops, not only assessing pollen flow, but also considering soil persistence of transgenic products (Stotzky 2004). Major environmental risks associated with GM crops include their potential impact on nontarget soil microorganisms, such as arbuscular mycorrhizal (AM) fungi, fundamental for sustainable and organic agriculture, given their important role in soil fertility, plant nutrition, and ecosystems functioning. AM fungi are strongly affected by agricultural practices, including treatments with chemical fertilizers and pesticides, and by changes in soil characteristics, thus representing potential key nontarget microorganisms to be monitored in studies on environmental impact of GM plants. In this work, we describe an experimental system to investigate the potential effects of two *Bt* corn lines and their plant residues on AM fungi.

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<sup>1</sup> Department of Crop Plant Biology, University of Pisa, Via del Borghetto 80, 56124 Pisa, Italy, e-mail: [turrini@agr.unipi.it](mailto:turrini@agr.unipi.it)

<sup>2</sup> Institute of Biology and Agricultural Biotechnology, CNR, UO Pisa, Via del Borghetto 80, Pisa, Italy, e-mail: [sbrana@ibba.cnr.it](mailto:sbrana@ibba.cnr.it), Internet: [www.ibba.cnr.it](http://www.ibba.cnr.it)

<sup>3</sup> Department of Crop Plant Biology, University of Pisa, Via del Borghetto 80, 56124 Pisa, Italy, e-mail: [mgiova@agr.unipi.it](mailto:mgiova@agr.unipi.it), Internet: [www.agr.unipi.it/dbpa/giovannetti](http://www.agr.unipi.it/dbpa/giovannetti)

## Materials and methods

Transgenic *Bt* corn lines (transformation events *Bt* 11—isogenic to NK4640—and *Bt* 176) genetically modified to express the *cry1Ab* gene from *Bacillus thuringiensis* and the nontransgenic maize NK4640 (wt) were used to study their impact on the AM fungal species *Glomus mosseae*. The experimental system (microcosm) used to study mycorrhizal establishment was described by Turrini et al. (2004). Ten replicates were set up for each trial. After 5 weeks' growth in the microcosm, the plants were transferred into pots filled with soil from conventional agriculture. Corn plants were cultivated and maintained in a greenhouse for 10 weeks. After 5, 8, and 10 weeks' growth, plant root systems were sampled and the percentage of mycorrhizal colonization was assessed. In a second experiment, corn plants were grown in pots for 12 weeks and then plowed under: leaves and stems of *Bt* 176, *Bt* 11, and Wt plants were cut into 2–3 cm pieces and mixed with the soil originating from the same pot where they were grown. Levels of colonizations by indigenous AM propagules were assessed on *Medicago sativa* plants grown in residues-amended soil. In order to test the effects of *Bt* plant residues on hyphal growth of *G. mosseae*, 15 sporocarps were placed on membranes in a sandwich system (Giovannetti et al. 2006). Sandwiches were placed onto petri dishes and covered with soil containing residues. After 21 days, membranes were opened and stained with 0.05% Trypan blue. Data on root colonization were arcsin(sqrt(x)) transformed and submitted to two-way ANOVA and to Test for the Equality of regression slopes.

## Results

The impact of *Bt* plants on AM fungal symbionts was monitored both on the collection isolate and on indigenous endophytes from corn experimental soil. Colonization in *Bt* corn plants (both *Bt* 11 and *Bt* 176) by the symbiont *G. mosseae* was significantly lower than in wt plants, by slopes equality test ( $F=8.59$ ,  $P<0.001$ ) (Figure 1).

The impact of transgenic plant residues on AM fungi was assessed by monitoring both presymbiotic mycelial growth of *G. mosseae* in the experimental soil and *Medicago sativa* root colonization by AM fungal propagules living in the experimental soil. Mycelial length of *G. mosseae* grown in soil samples containing *Bt* and non-*Bt* corn residues was monitored up to four months and did not show significant differences among lines. Two ways ANOVA showed that indigenous AM fungi were significantly affected in their ability to colonize *M. sativa* roots grown in soils containing different plant residues at different times after plowing under ( $F=45.97$ ,  $P<0.001$ ). Moreover, regression slopes of root colonization percentages of *M. sativa* grown in soil containing corn residues were different by the slopes equality test ( $F=27.13$ ,  $P<0.001$ ). Data obtained suggested that indigenous AM fungal colonization ability was affected, by GM corn cultivation, and that it was particularly reduced in *Bt* 11-amended soil (Figure 2).

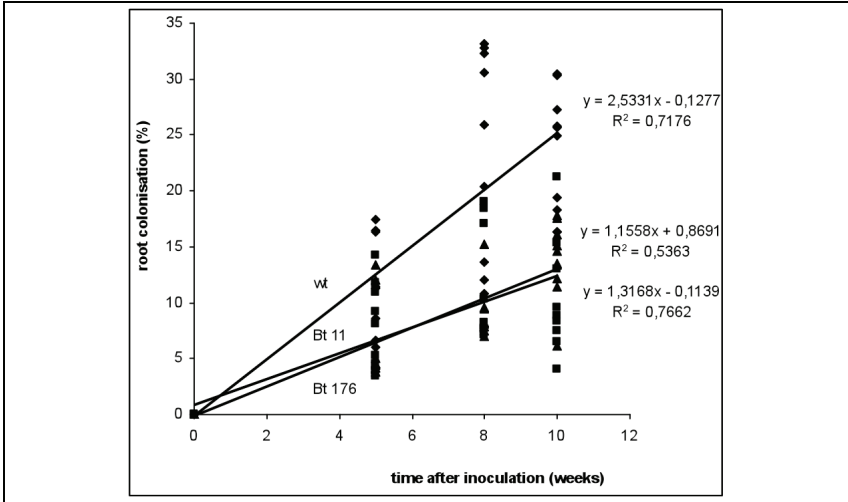


Figure 1: Distribution of data and regression lines of root colonization by the AM fungus *Glomus mosseae* on Bt and wild type corn plants, from inoculation to 10 weeks of culture.

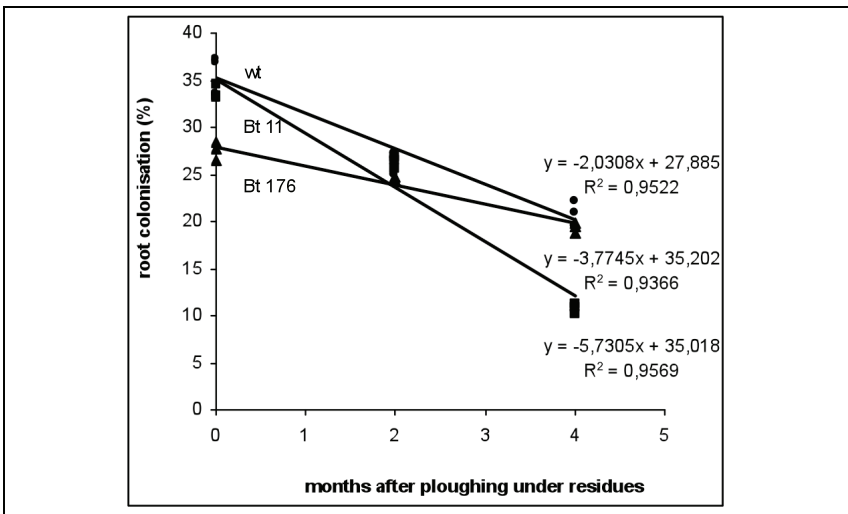


Figure 2: Distribution and regression lines of root colonization data by indigenous arbuscular mycorrhizal fungi on *M. sativa* during culture in soil samples containing Bt and wild type corn plant residues ploughed under.

## Discussion

Our experimental systems allowed us to monitor the impact of two *Bt* corn plants and their residues on AM fungi. Both transgenic plants decreased mycorrhizal colonization by *G. mosseae* and *Bt* 11 plant residues negatively affected mycorrhizal establishment by indigenous endophytes after their incorporation into soil. Mycelial growth in the presence of transgenic residues was not affected. Transgenic root exudates and residues incorporated into soil may produce long-term effects on soil microbes (Castaldini et al. 2005). Studies on *Bt* toxin persistence have shown that this protein maintains its activity after absorption to clays or binding to humic acids (Saxena & Stotzky 2001) and retains its activity for 234 days (Saxena et al. 1999; Stotzky 2004). Other authors have demonstrated slower litter decomposition for *Bt* compared with non-*Bt* lines (Flores et al. 2005). It remains to be established whether mycorrhizal colonization is reduced directly by the *Bt* toxin present in corn litter or indirectly by soil microbial population alterations or by other factors. Moreover, it is possible that prolonged permanence of litter in the soil could significantly affect inoculum potential of mycorrhizal fungi.

## Conclusions

Further long-term studies in the field are necessary to evaluate the impact of GM plants on microbial communities fundamental for soil fertility and quality. In particular, the risk posed by GM plant residues to nontarget beneficial soil microbes should be thoroughly investigated, since any reduction in their biodiversity might produce long-term effects, in space and time, on crops sequentially cultivated in the same soil in years to come.

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