

### 3.1. The Decomposition of *Bt*-Corn on the Fields and its Impact on Earthworms and on other Macroorganisms in the Soil

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Several transgenic maize (corn) lines were analyzed during a complete growing season in a field trial. The aim of the study was to evaluate potential risks resulting from the decomposition of litter from transgenic *Bt*-corn.

This study is based on the results obtained during the doctoral dissertations of Linda Hönemann and Corinne Zurbrügg and on those of the master-thesis of Simon Knecht. Together with the project leader Wolfgang Nentwig, it was observed between 2005 and end of 2007, how transgenic *Bt*-Corn decomposes on the ground and how non-target soil organisms react to this decomposition.

#### Background and Need of the Study

Two to six tons of dry plant materials per hectare remain on the fields after corn has been harvested (Zwahlen et al., 2003). In agricultural practice, this material gets incorporated into the upper soil layers during tillage. If *Bt*-corn has been planted, the litter still contains the *Bt*-toxin, which is even detectable in the soil after 200 days of exposure (Zwahlen et al., 2003). *Bt*-Toxins act very selectively. Nevertheless, one cannot absolutely exclude that non-target organisms would be affected. Would this be the case, the soil ecosystem could be damaged. If decomposers, that are essential for nutrient recycling, are affected, it might impair the maintenance of soil fertility.

So far, most of the available studies were carried out in the laboratory over short periods and with isolated species. There is also only one “litter bag” study that lasted several months. The field trial described here, with a total of 9 corn varieties (3 *Bt*-lines with each their corresponding near-isogenic control line as well as 3 conventional cultivars) is to date the most comprehensive analysis.

#### Field trials: Litter Decomposition of Corn and the Soil Animal Community

##### *Experimental design for the litter decomposition of corn*

The central field trial was a “litter bag” analysis that was carried out from October 2005 to July 2006. Analyses were made on the degradation of the different *Bt*-toxins, as well as on the components of the plant material relevant for the decomposition (such as the C to N ratio, cellulose, hemicelluloses, and lignin). In addition, the communities of soil organisms taking part in the decomposition process were analyzed.

Maize plants were grown in climate chambers. Leaves that dried on the plants were removed, and kept frozen until the start of the field trial. This plant material was then distributed in “litter bags” (a polyethylene net with 4 mm mesh width; Fig. 1) onto 10 fields. Every month, litter bags of all 9 corn varieties were harvested. An aliquot of the leaf material was taken for laboratory analysis, while the remaining material was used to determine the soil organisms after extraction according to the method of Macfadyen.



Figure 1. Litter bag filled with dried maize leaves.

#### Results of the litter decomposition

The Cry3Bb1-toxin of Mon88017 is expressed at higher levels than the Cry1Ab-toxin of the lines Mon810 and N4640Bt. But it is also degraded much faster (Fig. 2). In addition, there is hardly any break-down of the Cry1Ab-Toxin during the cold season. The soil organisms are therefore exposed for a shorter time period to Cry3Bb1-toxin than to Cry1Ab. Differences in the decomposition of the biomass could be explained by differences in the C to N ratio, while the lignin, cellulose and hemicellulose seemed to play a secondary role. Transgenic lines differed from their respective near-isogenic lines only in the C/N ratio but the values were within the expected variance between conventional cultivars.

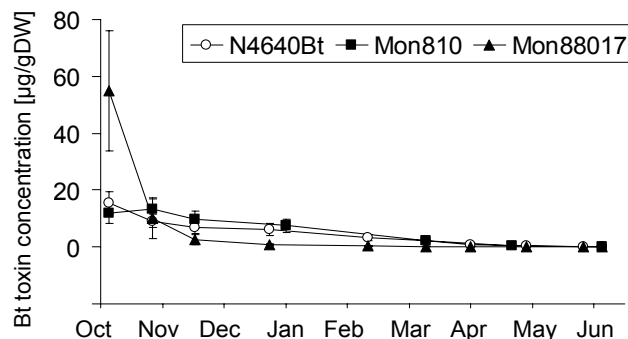


Figure 2. Evolution of the *Bt*-toxin concentration in senescent corn leaves of N4640Bt, Mon810 (Cry1Ab) and Mon88017 (Cry3Bb1) during nine months. Mean values  $\pm$  SD are indicated in  $\mu\text{g}$  per g dry weight (DW) for  $N = 10$  for each cultivar.

#### Experimental design for the analysis of Soil Animal Community

Using the method developed by Macfayden in 1961, the monthly samples from the litterbags were extracted for mesofauna and macrofauna. The animals were determined and counted under the binocular. A total of 41'342 animals from 21 taxa were determined.

#### Results of the analysis on the Soil Animal Community

Statistically significant differences between the colonization of transgenic and non-transgenic litter could *not* be found. Less than 1% of the variance of the abundances could be attributed to the transgenic vs. non-transgenic nature of the litter. In contrast, the location of the fields and the season had significant effects on the composition of the animal soil community (as described earlier by e.g. Dunger & Fiedler, 1997; Cassagne, 2002; Robertson & Grandy, 2005). These factors also influenced the overall abundances.

### *Conclusion of the field trials*

During the 9 months of the field trial *no* significant impact of Bt-containing corn litter could be detected on the composition and abundance of the soil animal community. The *Bt*-toxins were degraded by 99% during this time. The expected line-specific effect due to different contents of plant components was therefore not confirmed.

### **Experiments in the Laboratory: The Reaction of snails, fly larvae and segmented worms to Bt-corn leaves**

Laboratory experiments were carried out to complement the field trials. In selected species that occur in the agricultural environment and that participate, either directly or indirectly, to the decomposition of litter, following parameters were analyzed: The quantitative ingestion of transgenic leaf material; the fate (the degradation) of *Bt*-toxin in the gastrointestinal tracts and the impact of *Bt*-toxin uptake on reproduction.

In plastic containers, spanish slugs (*Arion vulgaris*) and reticulated slugs (grey field slugs, *Deroceras reticulatum*) were fed with *Bt*-corn leaves for three days. Both Cry-toxins were detected after three days in the snail guts as well as in their excrements. Three days after ceasing the *Bt*-containing diet, the concentrations were strongly diminished, particularly in the case of Cry3Bb1. Corn borer larvae (the target organism) were fed with Cry1Ab-containing feces. Their weight was significantly reduced as compared to the control group, indicating a sublethal effect. The possible consequences resulting from this finding need to be further clarified.

Enchytraeids ((pot worms) that colonize the upper 5-10 cm of the soil) were fed with ground pieces of *Bt*-leaves. The values for mortality and reproduction of the animals revealed differences, but not related to the presence or absence of the *Bt*-toxins. The differences reflected rather an influence of the maize cultivars. Hence, the consumption of transgenic Bt-corn does not represent a risk for the pot worms.

Saprophagous larvae of two-winged insects play an important role in decomposition. Flies of the species *Megaselia scalaris* and *Drosophila melanogaster* were analyzed in feeding assays over 3-4 generations. Offspring number per couple varied in both species and above all between the generations but only occasionally between the different treatments. No negative trend was observed. Neither the maize diet, nor the *Bt*-toxin had an effect on fertility or on the development of the flies. It is rather the variable nutritional quality of the different maize cultivars that can account for the variations observed.

### **Conclusions**

*No side effects* as a consequence of *Bt*-corn could be detected: a) in the composition of the soil fauna, b) in tropic reactions of individual species, and c) in their reproductive behavior and in their offspring. In the domains analyzed, the effect of *Bt*-containing lines was equivalent to their non-transgenic, near-isogenic lines or to conventional maize cultivars.

The experiments presented were performed with a large number of maize lines over a prolonged time. A large spectrum of soil organisms was analyzed and the fertility and development of flies observed over several generations.

For these Bt-corn lines, one can therefore *not* assume that they represent an ecological risk.

## Literature

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