

## 2.4. The fate of Bt toxin in the soil

2.4. THE FATE OF BT TOXIN IN THE SOIL .....	1
2.4.1. Soil fertility.....	1
2.4.2. The initial publication of Saxena et al. in Nature .....	2
2.4.3. Followup field studies: Bt proteins released by transgenic crops does not harm soil organisms .....	2
Literature cited .....	38

### 2.4.1. Soil fertility

Soil fertility is crucial to agriculture and the conservation of biodiversity, as summarized by (Hagvar, 1998). Interestingly enough, there is a wide variety of agricultural management methods which lead to improved soil fertility, from organic farming (Mäder et al., 2002) to conservation tillage related to herbicide tolerant crops (Cerdeira & Duke, 2006; Fawcett & Towery, 2002; Fracchia et al., 2006; Gomes et al., 2001; Miethling et al., 2003; Singer et al., 2004). The influence of various plant species growing and rooting in the soil should not be underestimated (Grayston et al., 1998; Hodge et al., 1998). It is also clear that different kinds of crop rotation and tillage have decisive influence on soil fertility and mineral content (Meek et al., 1994) – and – as we shall see below, differences in management methods usually have a far greater impact on soil fertility than anything else, including the impact of insect resistant transgenic crops and their possible addition of toxic proteins to the soil via litter, rotting roots or root exudates. A review article on proteins in soils has been recently published: (Quiquampoix & Burns, 2007)

**Quiquampoix, H. and R. G. Burns (2007).** "Interactions between Proteins and Soil Mineral Surfaces: Environmental and Health Consequences." ELEMENTS %R 10.2113/GSELEMENTS.3.6.401 3(6): 401-406. <http://elements.geoscienceworld.org/cgi/content/abstract/3/6/401> AND <http://www.botanischergarten.ch/Bt/Quiquampoix-Interactions-Proteins-2007.pdf>

Proteins have long been recognized as important compounds in the biogeochemical cycles of terrestrial ecosystems. They can, for example, provide a source of nitrogen for plants and soil microorganisms following proteolysis and ammonification. Extracellular enzymes liberated in soil are essential catalysts in the mobilization of carbon, nitrogen, phosphorus and sulphur from macromolecular organic matter. Proteins are also implicated in new environmental topics, such as soil carbon storage, horizontal transmission of spongiform encephalopathies **and potential negative effects of insecticidal toxins released from transgenic plants.** (Quiquampoix & Burns, 2007)

## 2.4.2. The initial publication of Saxena et al. in Nature

The story about possible detrimental effects of Bt exuding from maize hair roots was launched with a letter to Nature by (Saxena et al., 1999) in the same way as the story on the monarch butterflies by ( Losey, 1999). And again, the news was published without stressing the authors considerations that the case might not fundamentally challenge the new Bt strategy to fight pest insects with transgenic Bt crops, that more studies need to be done in order to assess possible damage to the agricultural ecosystems under natural conditions and on the long run. Despite the fact, that the authors of this first letter to Nature stated:

“We have no indication of how soil communities might be affected by *Bt* toxin in root exudates in the field.” it caused immediate and inflammatory comments of opponents, which persisted even in a time when followup studies demonstrated that the issue was not so dramatic as Greenpeace and other NGOs advocated: (Greenpeace, 2002).

## 2.4.3. Followup field studies: Bt proteins released by transgenic crops does not harm soil organisms

It triggered more research in the following years, and – as in the case of Losey, the followup studies were also done with the collaboration of the original team of Saxena, Flores and Gunter Stotzky. The team of Stotzky did field research with the original Bt crop traits of the companies such as Monsanto, and found that the alarm was not justified:

Soil organisms tested were not harmed by the Bt toxins, tested with bioassays. (Saxena & Stotzky, 2001) Their conclusions:

“There were no significant differences in the percent mortality and weight of earthworms (*Lumbricus terrestris*) after 40 days in soil planted with Bt (NK4640Bt) or non-Bt corn or after 45 days in soil amended with biomass of Bt or non-Bt corn. The toxin was present in the guts and casts of earthworms in soil planted with Bt corn or amended with biomass of Bt corn, but it was cleared within 2-3 days from the guts after placing in fresh soil. There were no significant differences in the colony-forming units of culturable bacteria (including actinomycetes) and fungi and in the numbers of protozoa and nematodes between rhizosphere soil of Bt and non-Bt corn or between soil amended with biomass of Bt and non-Bt corn. The CryIAb protein in root exudates and biomass of Bt corn appears not to be toxic to earthworms, nematodes, protozoa, bacteria, and fungi. The presence of the toxin in the guts and casts of earthworms confirmed that the toxin released in root exudates and from transgenic biomass was bound on surface-active particles in soil, which protected the toxin from biodegradation, as has been observed in this laboratory with purified toxin.”

There are many more papers published on the subject: They all tell more or less the same story: Bt exudates in the soil pose no long term problems, here a list of selected papers with the abstracts, the author analyzed up to March 2008 some 35 papers published in peer reviewed literature, they all present field data on the basis of a comparison between Bt and non-Bt crops: The results also demonstrate some regional differences – depending on climate and soil properties. It is interesting to note that experiments done with bio-assays under controlled glasshouse conditions revealed

shorter persistence of Bt proteins than experiments in the field (Muchaonyerwa & Waladde, 2007), they comment:

“However, extracts obtained from Bt maize plant materials incubated in the field showed decreased larval mortality, from 60% to 30% in two weeks and remained >25% after 12 weeks of incubation in the Shortlands soil and from 55% to 15% within four weeks and *eventually down to 10% in 23 weeks* of incubation in the Oakleaf soil. The findings suggest that Bt maize plant parts contribute comparable amounts of Bt protein toxin to the soil, and toxin persistence in the soil appears to depend on soil type, temperature and moisture conditions.”

These findings are confirmed by two papers (Pagel-Wieder et al., 2004; Pagel-Wieder et al., 2007) on experiments demonstrating the important influence of soil characteristics on the adsorption and desorption of Bt toxins in soil: The authors comment:

“Although their mineralogical composition was nearly identical, the soil clay fractions showed different *k* values. The different *k* values were correlated with the physical and chemical properties of the soil clay fractions, such as the organic carbon content, the specific external surface area, and the electrokinetic charge of the external surfaces of the clays, as well as with the external surface charge density. An increase in the amount of soil organic matter, as well as an increase in the electrokinetic external surface charge of the soil clays, decreased the distribution coefficient *k*. An increase of the specific external surface areas of the soil clays resulted in a higher distribution coefficient *k*. Less than 10% of adsorbed Cry1Ab protein was reversibly adsorbed on the soil clays and, thus, desorbed.”

Some dynamics influencing the fate of Bt toxins in soil need still to be studied in more detail – but let's discuss below on whether this is really necessary:

Theoretically, one could ask in addition, that the experiments should relate more precisely to life in soil as a whole community of microorganisms, bioassays with arbitrarily chosen soil-living organisms help, but a more holistic assay would be interesting to apply: But this will not be an easy task, as shown by (Weisse, 2007): It is by far not clear what kind of species concept and thus definition of biodiversity and its sensitivity on all kinds of agricultural influences (pesticides, biopesticides, exudates of endotoxins, tillage etc. etc). should be applied. This is also emphasized by (Vilas-Boas et al., 2007; Vilas-Boas et al., 2000), who recommend (as Weisse does p.p.) to work with an ecological species concept of (Vanvalen, 1976).

Asking all those questions is certainly interesting for science, but it is doubtful whether they are really important for the present day agriculture for the following reasons: There are dozens of pesticides, biopesticides and other management methods with greater impact on soil quality which should be studied in detail, there is no reason to concentrate on the fate of Bt endotoxin in such detail.

After all, here we ask for scientific clarifications, which could have been undertaken for decades already with impact factors of conventional agriculture, if these then were really vital for a sustainable production.

So here again we are asking ‘nice-to-knows’ which are highly interesting for science, but we forget the true baseline comparison with a conventional agriculture using chemical pesticides and spraying in high concentrations biopesticides.

So, instead of asking for unprecedented precision and intensive and costly field research we should learn from relatively simple experiments done under strictly natural conditions but extended over multiple years in large areas, such as undertaken by (Head et al., 2005; Head et al., 2002): the results show no alarming levels of Bt toxicity in soils, see the summaries of many scientific papers below. This opinion is now finally also confirmed by G. Stotzky, the senior author of the following review, in all the most comprehensive one published until today (although, compared to this study, numerous papers are not cited).

**Icoz, I. and G. Stotzky (2008).** "Fate and effects of insect-resistant Bt crops in soil ecosystems." *Soil Biology & Biochemistry* **40**: 559-586.<Go to ISI>://WOS:000253000800001 AND <http://www.botanischergarten.ch/Bt/Icoz-Fate-Effects-Review-2008.pdf>

Recent applications of biotechnology, especially genetic engineering, have revolutionized crop improvement and increased the availability of valuable new traits. A current example is the use of the insecticidal Cry proteins from the bacterium, *Bacillus thuringiensis* (Bt), to improve crops, known as Bt crops, by reducing injury from various crop pests. The adoption of genetically modified (GM) crops has increased dramatically in the last 11 years. However, the introduction of GM plants into agricultural ecosystems has raised a number of questions, including the ecological impact of these plants on soil ecosystems. Crop residues are the primary source of carbon in soil, and root exudates govern which organisms reside in the rhizosphere. Therefore, any change to the quality of crop residues and rhizosphere inputs could modify the dynamics of the composition and activity of organisms in soil. Insect-resistant Bt crops have the potential to change the microbial dynamics, biodiversity, and essential ecosystem functions in soil, because they usually produce insecticidal Cry proteins through all parts of the plant. It is crucial that risk assessment studies on the commercial use of Bt crops consider the impacts on organisms in soil. In general, few or no toxic effects of Cry proteins on woodlice, collembolans, mites, earthworms, nematodes, protozoa, and the activity of various enzymes in soil have been reported. Although some effects, ranging from no effect to minor and significant effects, of Bt plants on microbial communities in soil have been reported, using both culturing and molecular techniques, they were mostly the result of differences in geography, temperature, plant variety, and soil type and, in general, were transient and not related to the presence of the Cry proteins. The respiration (i.e., CO<sub>2</sub> evolution) of soils cultivated with Bt maize or amended with biomass of Bt maize and other Bt crops was generally lower than from soils cultivated with or amended with biomass of the respective non-Bt isolines, which may have been a result of differences in chemical composition (e.g., the content of starch, soluble N, proteins, carbohydrates, lignin) between Bt plants and their near-isogenic counterparts. Laboratory and field studies have shown differences in the persistence of the Cry proteins in soil, which appear to be the result primarily of differences in microbial activity, which, in turn, is dependent on soil type (e.g., pH, clay mineral composition, other physicochemical characteristics), season (e.g., temperature, water tension), crop species (e.g., chemical composition, C:N ratio, plant part), crop management practices (e.g., till vs. no-till), and other environmental factors that vary with location and climate zones. This review discusses the available data on the effects of Cry proteins on below-ground organisms, the fate of these proteins in soil, the techniques and indicators that are available to study these aspects, and future directions. (Icoz & Stotzky, 2008)

Here some more citations from the conclusions – the author of this study has nothing to add:

Biotechnology represents a means for enhancing genetic diversity in crop species through the introduction of novel genes. The use of GM crops can positively impact agriculture if the GM crops enable the management of weeds and insect pests more specifically than chemical herbicides and pesticides. In particular, the use of insect-resistant Bt crops, expressing highly specific Bt proteins, represents an opportunity to replace the use of broad-spectrum insecticides. Concerns related to the risks of GM crops, especially Bt crops, to the environment have been extensively assessed worldwide during the past 11 or so years of commercial cultivation of Bt crops. Consequently, considerable data on the environmental effects of these Bt crops are available. These data have provided no scientific evidence that cultivation of Bt crops has caused sustained environmental harm to below-ground microbial and invertebrate communities. Moreover, these data have suggested that the effects resulting from the cultivation of Bt crops fall within the normal variation expected in agricultural systems and that they are not as large as those resulting from growing different (conventional) maize cultivars and other crops or from natural differences between sites or times of sampling. However, the lack of evidence of negative effects of Bt crops does not mean that other GM plants are without risk. Moreover, the possibility of long-term effects of Bt crops cannot be excluded and must be examined on a case-by-case basis, especially as a number of issues related to the interpretation of the scientific data on the effects of Bt crops on the environment are still controversially debated.

Among the questions that still need to be addressed is whether the cultivation of Bt crops affects the yield of subsequent crops, especially non-Bt crops, grown on the same soils on which Bt crops have been grown. If effects on subsequent crops are observed, the duration (e.g., number of seasons) of the effects would be of practical interest. Although there appear to be no significant, long-term, detrimental effects of Bt plants on below-ground organisms, the potential impact of Bt plants on nontarget aboveground organisms (e.g., predators, parasitoids, pollinators, butterflies, herbivores) still remains controversial, as does gene flow from Bt crops to compatible local crops and wild relatives.

Because most studies have generally indicated few or no significant detrimental effects on microbes and other organisms in below-ground soil ecosystems, more studies on the risks associated with Bt plants, at least those currently available, to these organisms are probably not indicated. *The time and money would be better spent on studies of the potential risks associated with the release of transgenic plants genetically engineered to express pharmaceutical and industrial products that, in contrast to Cry proteins, are targeted primarily to human beings and other higher eukaryotic organisms* (e.g., (Marvier et al., 2007); Fiorito et al., 2007; Sabharwal et al., 2007; Stotzky and Saxena, 2007).

More reviews:

**Bruinsma, M., G. A. Kowalchuk and J. A. van Veen (2003).** "Effects of genetically modified plants on microbial communities and processes in soil." *Biology and Fertility of Soils* 37(6): 329-337. <Go to ISI>://WOS:000183871900001 AND <http://www.botanischergarten.ch/Bt/Bruinsma-Effect-Microbial-2003.pdf>

The development and use of genetically modified plants (GMPs) has been a topic of considerable public debate in recent years. GMPs hold great promise for improving agricultural output, but the potential for unwanted effects of GMP use is still not fully understood. The majority of studies addressing potential risks of GMP cultivation have addressed only aboveground effects. However, recent methodological advances in soil microbial ecology have allowed research focus to move underground to try to gain knowledge of GMP-driven effects on the microbial communities and processes in soil that are essential to key terrestrial ecosystem functions. This review gives an overview of the research performed to date on this timely topic, highlighting a number of case studies. Although such research has advanced our understanding of this topic, a number of knowledge gaps still prevent full interpretation of results, as highlighted by the failure of most studies to assign a definitively negative, positive or neutral effect to GMP introduction. Based upon our accumulating, yet incomplete, understanding of soil microbes and processes, we propose a synthesis for the case-by-case study of GMP effects, incorporating assessment of the potential plant/ecosystem interactions, accessible and relevant indicators, and tests for unforeseen effects. (Bruinsma et al., 2003)

**Lang, A., M. Arndt, R. Beck, J. Bauchhenss and G. Pommer (2006).** "Monitoring of the Environmental Effects of the Bt Gene; Research Project Sponsored by the Bavarian State Ministry for Environment, Health, and Consumer Protection (StMUGV) " LfL-Schriftenreihe aus dem Institut für Pflanzenschutz 10/2006: 113.<http://www.lfl.bayern.de> AND [http://www.lfl.bayern.de/publikationen/daten/schriftenreihe\\_url\\_1\\_43.pdf](http://www.lfl.bayern.de/publikationen/daten/schriftenreihe_url_1_43.pdf) AND <http://www.botanischergarten.ch/Bt/Lang-Monitoring-2006.pdf>

Genetic engineering makes it possible to transfer genes for insecticidal toxins from the bacterium *Bacillus thuringiensis* (Bt) into corn. This genetically modified corn expresses Bt endotoxins (CryA1b) in a highly specific fashion protecting it from infestation by corn borers. The research at LfL dealt mainly with the question whether non-target organisms may be potentially affected by the use of the Bt gene. With a focus on a sustainable and environment-friendly agriculture, special attention was directed towards 'species protection', 'soil fertility', and 'biological control'. Die deutsche Version ist als Schriftenreihe 7/2005 erschienen.

In this monitoring report, for the first time we have conducted a comparative study at several sites on all the essential soil microbial cumulative parameters, comparing Bt corn and isogenic comparison varieties within a long study timeframe and for the entire growing season. After eleven sampling dates over a four year period, we cannot establish any change in the studied soil microbial characteristics at the four evaluated sites under continuous Bt corn cultivation. The occasional significant differences appearing in

the pairwise comparisons were both positive and negative; and according to our numerical data, no dependence on transgenic corn plants can be identified. Additional support for this result also comes from the analytical values for the selected LSO fields. According to our numerical data, we can rule out any adverse effect on soil fertility and microbial soil activity from cultivation of the Bt corn varieties Navares and Novelis. From a soil microbial standpoint, we have no reservations concerning cultivation of the two studied transgenic corn varieties. (Lang et al., 2006)

**Widmer, F. (2007).** Assessing effects of Transgenic crops on soil microbial communities. Green Gene Technology: Research in an Area of Social Conflict. BERLIN, SPRINGER-VERLAG BERLIN: pp 207-234 <Go to ISI>://000248483300011 AND <http://www.botanischergarten.ch/Bt/Widmer-Assessing-Effects-2007.pdf>

Deleterious effects of transgenic plants on soils represent an often expressed concern, which has catalyzed numerous studies in the recent past. In this literature review, studies addressing this question have been compiled. A total of 60 studies has been found, and their findings as well as their analytical approaches are summarized. These studies analyzed the effects of seven different types of genetically engineered traits, i.e., herbicide tolerance, insect resistance, virus resistance, proteinase inhibitors, antimicrobial activity, environmental application, and biomolecule production. Sixteen genetically engineered plant species were investigated in these studies including corn, canola, soybean, cotton, potato, tobacco, alfalfa, wheat, rice, tomato, papaya, aubergine, and silver birch. Many of these plants and traits have not been commercialized and represent experimental model systems. Effects on soil microbial characteristics have been described in various studies, indicating the sensitivity and feasibility of the analytical approaches applied. However, classification of the observed effects into acceptable and unacceptable ones has not been possible so far. Establishment of validated indicators for adverse effects represents a scientific challenge for the near future, and will assist risk assessment and regulation of transgenic plants commercially released to the field. (Widmer, 2007)

Specifically added here to the abstract the full paragraph on Bt corn from (Widmer, 2007)

#### "4.2.2

##### Insect Resistant Bt Corn

Extractable lipids in Bt and conventional corn shoots and soil were analyzed at harvest (Dinel et al., 2003) Concentrations of total alkenes, n-alkanes, and n-fatty acids were increased in soils planted with Bt corn, while unsaturated fatty acid contents were higher in soil planted with non-Bt corn. Cumulative CO<sub>2</sub> released from soils was lower under Bt corn, indicating that cultivation of Bt corn may reduce microbial activity. In a growth chamber experiment (Blackwood & Buyer, 2004), two lines of Bt corn expressing either Cry1Ab or Cry1F were compared with nontransgenic isolines in three soil types. PLFA profiles of bulk soil and CLPP profiles of rhizosphere soils revealed only for one soil significant Bt corn effects in the rhizosphere. Expression of Bt toxin also significantly reduced the presence of eukaryotic PLFA biomarkers in bulk soils; however, it remained unclear which eukaryotes they represented. From this data, the authors concluded that potential effects of Bt corn on soil and rhizosphere microbial communities may be small. In a glasshouse study (Brusetti et al., 2004), the effects of Bt-176 corn on the rhizosphere bacterial community have been analyzed. Bacterial plate counts and CLSU revealed no significant differences between plant genotypes. On the other hand, differences between the rhizosphere and bulk soil bacterial communities could be detected. Bacterial RISA revealed differences in the rhizosphere communities at different plant growth stages, as well as between Bt-176 and control corn. The authors attributed the different bacterial communities in the rhizospheres to altered root exudates of the transgenic corn. In soil samples from field trials with Bt corn expressing Cry1Ab (Griffiths et al., 2005), microbial communities were analyzed based on CLSU and PLFA profiling, as well as based on protozoa analyses. Two occasions were reported when soil protozoa populations under Bt corn were reduced as compared to non-Bt corn. CLSU profiling revealed one occurrence of differences between Bt and control corn cultivars. The effects of Bt corn were classified by the authors as small and comparable to the variation expected in these agricultural systems. Finally, PLFA profiling was used to analyze the microbial communities in soil samples collected from fields with Bt corn (Xue et al., 2005) (full text not seen). Analyses revealed a

reduction in fungal abundance and ratios of Gram-positive to Gram-negative bacteria in soils from Bt corn; however, the authors have stated that the causes of these observed effects remained unknown and require more detailed investigations. Several studies focused on mycorrhizal fungi for the assessment of effects of Bt corn. An experimental model system was used to study the effects of root exudates of Bt corn on different stages of the life cycle of the arbuscular mycorrhizal fungal species *Glomus mosseae* (Turrini et al., 2004). Root exudates of Bt-176 corn significantly affected presymbiotic hyphal growth and development of appressoria, as compared to Bt-11 and control corn. Differential hyphal morphogenesis occurred irrespective of Bt or control corn, suggesting that Bt toxin did not interfere with fungal host recognition mechanisms. In microcosm experiments (Castaldini et al., 2005), the impact of genetically modified Bt-11 and Bt-176 corn on soil respiration, rhizosphere, and bulk soil bacterial communities, and the mycorrhizal symbiont *G. mosseae*, were further assessed. DGGE profiling of bacterial 16S rRNA gene fragments showed differences in rhizosphere bacterial communities associated with all corn lines, while mycorrhizal colonization was significantly reduced for Bt-176 corn only. Additional glasshouse experiments confirmed the differences between Bt and non-Bt corn, and addition of Bt corn residues to soil affected soil respiration, bacterial communities, and mycorrhizal establishment. In another study [132], colonization with arbuscular mycorrhizal fungi and activity of rhizosphere soil microbiota were determined during growth of Cry1Ab-expressing Bt corn in the field. The results suggested that Bt corn and conventional corn may differ in their C/N ratios. In addition, reduced colonization with arbuscular mycorrhizal fungi and increased microbial activity were found during early Bt corn development. The authors conclude that genetic transformation might have led to changes in plant physiology and root-exudate composition, which in turn may have affected symbiotic and rhizosphere microorganisms. There are also a number of studies which revealed no effects of Bt corn on microbiological soil characteristics. For instance, soils were planted with Cry1Ab-expressing Bt corn or amended with Bt corn biomass and compared to controls (Saxena & Stotzky, 2001). Analysis was based on a cultivation-dependent approach and revealed no significant differences in the plate counts for bacteria and fungi, as well as in the numbers of protozoa between rhizosphere soil of Bt and control corn. Also, amendment with plant biomass of these plants revealed no different effects. The authors concluded that the Bt protein in corn-root exudates and plant biomass appeared not to be toxic to protozoa, bacteria, and fungi. In a field study (Devare et al., 2004), effects of corn rootworm (*Diabrotica* spp.) resistant Bt corn expressing Cry3Bb and application of the insecticide tefluthrin were assessed. Analyses included soil microbial biomass, N-mineralization potential, short-term nitrification rate, basal respiration, and bacterial community structures based on T-RFLP analysis. The data showed no effects of Bt corn on microbial measures or bacterial community structures when compared to the near isoline. T-RFLP analysis revealed substantial temporal differences and tefluthrin application reduced soil respiration. The authors concluded that *Diabrotica* resistant Bt corn may pose little or no threat to soil microbiology. In other field studies with Cry1Ab-expressing Bt corn (Baumgarte & Tebbe, 2005), the persistence of Bt toxin in soil and the effects on rhizosphere bacterial communities were assessed. An improved ELISA method for Bt toxin quantification and SSCP analysis of PCR-amplified bacterial 16S rRNA gene fragments were used. Despite the presence of Cry1Ab protein in the rhizosphere of Bt corn, effects on bacterial community structures were small when compared to other factors, such as plant age or field heterogeneities. In glasshouse and field studies (Fang et al., 2005) bacterial diversity in Bt and conventional corn rhizospheres was determined. CLSU profiling and DGGE of PCR-amplified 16S rRNA gene fragments allowed differentiation of bacterial communities among different soil textures but not among corn varieties. From these results the authors concluded that cultivation of transgenic varieties may not affect rhizosphere bacterial communities. These results have been supported by a recent glasshouse experiment (Griffiths et al., 2006) on the effects of Bt corn (Cry1Ab) and the insecticide deltamethrin on soil microbiota. The Bt trait induced an increase of protozoa, but significant effects on soil microbial community structure, as determined with CLSU and PLFA analyses, were caused only by soil type and plant growth stages. Results from this glasshouse experiment were in broad agreement with those of a field experiment using the same plant material grown in the same soils (Griffiths et al., 2005).”

Missed papers in the above review paragraph on Bt maize impact on soil microbial systems by (Widmer, 2007)

(Devare et al., 2007; Donegan et al., 1995; Donegan et al., 1996; Donegan et al., 1999; Donegan et al., 1997; Flores et al., 2005; Griffiths et al., 2007a; Griffiths et al., 2007b; Icoz & Stotzky, 2007, 2008; Koskella & Stotzky, 2002; Lang et al., 2006)

**Accinelli, C., C. Screpanti, A. Vicari and P. Catizone (2004).** "Influence of insecticidal toxins from *Bacillus thuringiensis* subsp. *kurstaki* on the degradation of glyphosate and glufosinate-ammonium in soil samples." *Agriculture, Ecosystems & Environment* **103**(3): 497-507. <http://www.sciencedirect.com/science/article/B6T3Y-4BFPF04-1/2/87df895c770f86200d2c90a0aa3063a0> and <http://www.botanischergarten.ch/Bt/Accinelli-Herbicide-Persistence-Btk-2004.pdf>

Investigations dealing with the persistence in soil of glyphosate [N-(phosphonomethyl)glycine] (GLYP) and glufosinate ammonium [the ammonium salt of dl-homoalanin-4-yl(methyl)phosphinic acid] (GLUF) herbicides and of insecticidal toxins produced by *Bacillus thuringiensis* subsp. *kurstaki* (Berliner) are largely reported in the literature. However, no information on the influence of these insecticidal toxins on the persistence in soil of herbicides is available. Preliminary results regarding the influence of insecticidal toxins extracted from a commercial formulation of *B. thuringiensis* subsp. *kurstaki* (Berliner) (Btk) on the degradation of the herbicides glyphosate and glufosinate-ammonium in a loam and a sandy loam soil, under laboratory conditions, were obtained. Soil microbial carbon (SMC) and insecticidal activity of incubated soil samples were also estimated. In both soil types, persistence of GLYP was significantly higher with respect to GLUF. Average GLYP and GLUF half-life was 14.4 and 8.0 days, respectively. Addition of Btk toxins lead to a significant increase of GLYP and GLUF persistence in both soil types. More specifically, average GLYP and GLUF half-life in soil samples receiving the Btk treatment was 24.3 and 14.2 days, respectively. In contrast to herbicide persistence in soil, Btk toxins did not influence microbial carbon content of incubated soil samples. The insecticidal activity of Btk toxins in soil rapidly decreased during the 28-day incubation time. Considering that degradation of GLYP and GLUF was mainly a microbial process, the absence of effects of Btk toxins on the soil microbial carbon and the rapid decrease of insecticidal activity of Btk toxins in the soil suggest a possible effect of the Btk toxins on other soil properties and/or mechanisms influencing herbicide degradation. The present preliminary investigation permitted to highlight the possibility of the Btk toxins to enhance the persistence of GLYP and GLUF in soil, under laboratory conditions. However, further studies are necessary to investigate whether or not the effects observed in this study under artificial and controlled conditions can be extrapolated to field conditions. (Accinelli et al., 2004)

**Ahmad, A., G. E. Wilde and K. Y. Zhu (2005).** "Detectability of coleopteran-specific Cry3Bb1 protein in soil and its effect on nontarget surface and below-ground arthropods." *Environmental Entomology* **34**(2): 385-394. <Go to ISI>://000228262000017 AND <http://www.botanischergarten.ch/Bt/Ahmad-Detectability-Bt-Soil-2005.pdf>

Corn engineered to produce the Cry3Bb1 protein from *Bacillus thuringiensis* (Bt) *kumamotoensis* has provided unprecedented control for corn rootworm (*Diabrotica* spp.). However, the Bt protein may be released in soil by root exudates or decaying plant residues that may affect soil organisms. Field studies were conducted to determine the abundance of surface and below-ground nontarget arthropods in fields planted with Bt or non-Bt corn for the first year or planted over 3 consecutive yr. Results of these studies showed that there were no significant differences in numbers of surface and below-ground arthropods in soil planted with Bt and non-Bt corn at any of the studied locations. Enzyme-linked immunosorbent assay (ELISA) showed no detectable Cry3Bb1 protein in any of the soil samples collected in a field planted with a Bt corn hybrid and its non-Bt isogenic hybrid for the first year or planted over 3 consecutive yr near Manhattan, KS. However, a small amount of Cry3Bb1 protein (3.38 -6.89 ng/g dry soil) was detected in the soil samples collected from all area near plants in a Bt corn field that was planted for the first year near Scandia, KS. These findings indicate that the Cry3Bb1 protein released from root exudates or decaying plant residues does not persist and is rapidly broken down in the soil. The rapid degradation of Cry3Bb1 in soil results in none or trace amounts of protein being detected by ELISA. (Ahmad et al., 2005)

**Bakonyi, G., F. Szira, I. Kiss, I. Villanyi, A. Seres and A. Szekacs (2006).** "Preference tests with collembolas on isogenic and Bt-maize." *European Journal of Soil Biology* **42**: S132-S135.<Go to ISI>://WOS:000243263800017 AND <http://www.botanischergarten.ch/Bt/Bakonyi-Preference-Collembolas-2006.pdf>

Collembolas are important members of belowground food webs. There is little information available on the effects of the plant residues of transgenic maize expressing *Bacillus thuringiensis* (Bt) toxin on soil animals, including collembola. This is why two questions were addressed in laboratory feeding experiments with three collembolan species: (i) Are collembola equally distributed on residues of isogenic and Bt-maize? and (ii) Do collembola show feeding preference to either of the maize types? Bt-maize (producing Cry1Ab toxin) proved to be a less preferred food source for *Folsomia candida* than the isogenic one. No similar phenomenon was found in the case of *Heteromurus nitidus* and *Sinella coeca*. *F. candida* reacted to as low as 3.45 (+/- 0.8 mg g<sup>-1</sup>) Bt-toxin content of the maize. Our results show that the effect of the Bt-toxin producing maize on the collembolan is species specific. (Bakonyi et al., 2006)

**Baumgarte, S. and C. C. Tebbe (2005).** "Field studies on the environmental fate of the Cry1Ab Bt-toxin produced by transgenic maize (MON810) and its effect on bacterial communities in the maize rhizosphere." *Molecular Ecology* **14**(8): 2539-2551.<Go to ISI>://000229961500023 AND <http://www.botanischergarten.ch/Bt/Baumgartner-SoilBacteria-2005.pdf>

Field studies were done to assess how much of the transgenic, insecticidal protein, Cry1Ab, encoded by a truncated cry1Ab gene from *Bacillus thuringiensis* (Bt), was released from Bt-maize MON810 into soil and whether bacterial communities inhabiting the rhizosphere of MON810 maize were different from those of the rhizosphere of nontransgenic maize cultivars. Bacterial community structure was investigated by SSCP (single-strand conformation polymorphism) of PCR-amplified 16S rRNA genes from community DNA. Using an improved extraction and detection protocol based on a commercially available ELISA, it was possible to detect Cry1Ab protein extracted from soils to a threshold concentration of 0.07 ng/g soil. From 100 ng of purified Cry1Ab protein added per gram of soil, only an average of 37% was extractable. At both field sites investigated, the amount of Cry1Ab protein in bulk soil of MON810 field plots was always lower than in the rhizosphere, the latter ranging from 0.1 to 10 ng/g soil. Immunoreactive Cry1Ab protein was also detected at 0.21 ng/g bulk soil 7 months after harvesting, i.e. in April of the following year. At this time, however, higher values were found in residues of leaves (21 ng/g) and of roots (183 ng/g), the latter corresponding to 12% of the Cry1Ab protein present in intact roots. A sampling 2 months later indicated further degradation of the protein. Despite the detection of Cry1Ab protein in the rhizosphere of MON810 maize, the bacterial community structure was less affected by the Cry1Ab protein than by other environmental factors, i.e. the age of the plants or field heterogeneities. The persistence of Cry1Ab protein emphasizes the importance of considering post-harvest effects on nontarget organisms. (Baumgarte & Tebbe, 2005)

**Blackwood, C. B. and J. S. Buyer (2004).** "Soil microbial communities associated with Bt and non-Bt corn in three soils." *Journal of Environmental Quality* **33**(3): 832-836.<Go to ISI>://WOS:000221509200005 AND <http://www.botanischergarten.ch/Bt/Blackwood-Microbial-Communities-2004.pdf>

The effects of expression of Cry endotoxin by Bt corn (transgenic corn engineered to express *Bacillus thuringiensis* toxin) on soil microbial community structure were assessed in a growth chamber experiment. Two lines of transgenic corn expressing different Cry endotoxins were compared with their respective non-transgenic isolines in three soil types with differing textures. Phospholipid fatty acid (PLFA) profiles from bulk soil and community-level physiological profiles (CLPP) from the rhizosphere community were used to assess community structure. Differences in PLFA profiles due to soil type were significant, accounting for 73% of the total variability in the dataset. Differences in bacterial and fungal CLPP profiles due to soil type were statistically significant, but probably not biologically important, accounting for 6.3 and 3.8% of the total variability, respectively. Neither expression of Cry endotoxin nor corn line had a significant effect on microbial profiles, except in the high-clay soil where both factors significantly affected bacterial CLPP profiles (accounting for 6.6 and 6.1% of the variability in that soil, respectively). Expression of Cry endotoxin also significantly reduced the presence of eukaryotic PLFA biomarker in bulk soils, although it is unclear which groups of eukaryotes were affected. We conclude that the effects of

transgenic Bt corn in this short-term experiment are small, and longer-term investigations are necessary. (Blackwood & Buyer, 2004)

**Bruseti, L., P. Francia, C. Bertolini, A. Pagliuca, S. Borin, C. Sorlini, A. Abruzzese, G. Sacchi, C. Viti, L. Giovannetti, E. Giuntini, M. Bazzicalupo and D. Daffonchio (2004).** "Bacterial communities associated with the rhizosphere of transgenic Bt 176 maize (*Zea mays*) and its non transgenic counterpart." *Plant and Soil* 266(1-2): 11-21.<Go to ISI>://000226385500003 AND <http://www.botanischergarten.ch/Bt/Bruseti-Bacterial-communities-2004.pdf>

The effect of transgenic Bt 176 maize on the rhizosphere bacterial community has been studied with a polyphasic approach by comparing the rhizosphere of Bt maize cultivated in greenhouse with that of its non transgenic counterpart grown in the same conditions. In the two plants the bacterial counts of the copiotrophic, oligotrophic and sporeforming bacteria, and the community level catabolic profiling, showed no significant differences; differences between the rhizosphere and bulk soil bacterial communities were evidenced. Automated ribosomal intergenic spacer analysis (ARISA) showed differences also in the rhizosphere communities at different plant ages, as well as between the two plant types. ARISA fingerprinting patterns of soil bacterial communities exposed to root growth solutions, collected from transgenic and non transgenic plants grown in hydroponic conditions, were grouped separately by principal component analysis suggesting that root exudates could determine the selection of different bacterial communities. (Bruseti et al., 2004)

**Castaldini, M., A. Turrini, C. Sbrana, A. Benedetti, M. Marchionni, S. Mocali, A. Fabiani, S. Landi, F. Santomassimo, B. Pietrangeli, M. P. Nuti, N. Miclaus and M. Giovannetti (2005).** "Impact of Bt corn on rhizospheric and on beneficial mycorrhizal symbiosis and soil eubacterial communities in experimental microcosms." *Applied and Environmental Microbiology* 71(11): 6719-6729.<Go to ISI>://WOS:000233225000033 AND <http://www.botanischergarten.ch/Bt/Castaldini-Impact-Bt-Soil-2005.pdf>

A polyphasic approach has been developed to gain knowledge of suitable key indicators for the evaluation of environmental impact of genetically modified Bt 11 and Bt 176 corn lines on soil ecosystems. We assessed the effects of Bt corn (which constitutively expresses the insecticidal toxin from *Bacillus thuringiensis*, encoded by the truncated Cry1Ab gene) and non-Bt corn plants and their residues on rhizospheric and bulk soil eubacterial communities by means of denaturing gradient gel electrophoresis analyses of 16S rRNA genes, on the nontarget mycorrhizal symbiont *Glomus mosseae*, and on soil respiration. Microcosm experiments showed differences in rhizospheric eubacterial communities associated with the three corn lines and a significantly lower level of mycorrhizal colonization in Bt 176 corn roots. In greenhouse experiments, differences between Bt and non-Bt corn plants were detected in rhizospheric eubacterial communities (both total and active), in culturable rhizospheric heterotrophic bacteria, and in mycorrhizal colonization. Plant residues of transgenic plants, plowed under at harvest and kept mixed with soil for up to 4 months, affected soil respiration, bacterial communities, and mycorrhizal establishment by indigenous endophytes. The multimodal approach utilized in our work may be applied in long-term field studies aimed at monitoring the real hazard of genetically modified crops and their residues on nontarget soil microbial communities. (Castaldini et al., 2005)

**Clark, B. W. and J. R. Coats (2006).** "Subacute effects of Cry1Ab Bt corn litter on the earthworm *Eisenia fetida* and the springtail *Folsomia candida*." *Environmental Entomology* 35(4): 1121-1129.<Go to ISI>://WOS:000239524600035 AND <http://www.botanischergarten.ch/Bt/Clark-Subacute-Eisenia-2006.pdf>

Laboratory toxicity studies were conducted to determine the subacute effects of Bt Cry1Ab corn leaf material on nontarget soil organisms. Survival and growth were measured for an earthworm, *Eisenia fetida* Savigny, and survival and reproduction were measured for a springtail, *Folsomia candida* Willem. The organisms were provided leaf material of two Bt11 corn varieties, two Mon810 corn varieties, and the isolines of each, in a soil system and monitored for 28 d. An assay control treatment of an optimal food and a reference control treatment, using the herbicide pendimethalin, were used to provide a context for the observed results. Basic nutritional data of protein, fat, and sugar content were analyzed for each food

type. Greater growth was observed for *E. fetida* in two Bt varieties, Bt11 90-d and Mon810 108-d, compared with their isolines. *F. candida* receiving Bt11 90-d isolate material had more offspring compared with those in the corresponding Bt line, but no other pairs were different. Time to reproduction of *E. candida* was only affected by the reference control treatment. Both protein and sugar content were found to correlate significantly with growth for *E. fetida*, but the nutritional parameters were not found to correlate with the effects observed for *E. candida*. These results indicate that there is little direct hazard from Bt corn leaf material to *E. fetida* and *F. candida* but that differences in nutritional parameters of the Bt lines and the isolines may lead to differences in the effects on nontarget organisms. (Clark & Coats, 2006)

**Clark, B. W., K. R. Prihoda and J. R. Coats (2006).** "Subacute Effects of Transgenic Cry1Ab *Bacillus thuringiensis* Corn-Litter on the Isopods *Trachelipus rathkii* and *armadillidium nasatum*." *Environmental Toxicology and Chemistry* 25(10): 2653–2661 DOI: 10.1897/05-471R.1 AND <http://www.botanischergarten.ch/Bt/Clark-Subacute-Effects-Isopods-2006.pdf>

Laboratory studies were conducted to investigate the subacute effects of transgenic Cry1Ab corn leaf material containing *Bacillus thuringiensis* (Bt) protein on the terrestrial isopods *Trachelipus rathkii* and *Armadillidium nasatum*. Survival and growth were measured for eight weeks in isopods fed leaf material of two Bt11 corn varieties, two Monsanto 810 (Mon810) corn varieties, and the isolines of each. Total lipid and protein content of the organisms was measured to examine effects on energetic reserves. *Armadillidium nasatum* individuals in all treatments responded similarly. For *T. rathkii*, no statistically significant effect of Bt was observed, but statistical differences were observed in growth between hybrids. Protein and sugar content of the food were found to be correlated with the differences in growth for *T. rathkii*. Total protein content was higher in *T. rathkii* and *A. nasatum* fed material with higher protein and sugar content. A trend toward less growth in *T. rathkii* on Bt corn varieties versus their isolines triggered a concentration–response assay with purified Cry1Ab protein. No adverse effects of purified Bt protein were observed. These results indicate that little hazard to *T. rathkii* and *A. nasatum* from Bt corn leaf material from these hybrids exists. However, nutritional differences in corn hybrids contributed to differences in isopod growth. (Clark et al., 2006)X1

**Cortet, J., M. N. Andersen, S. Caul, B. Griffiths, R. Joffre, B. Lacroix, C. Sausse, J. Thompson and P. H. Krogh (2006).** "Decomposition processes under Bt (*Bacillus thuringiensis*) maize: Results of a multi-site experiment." *Soil Biology & Biochemistry* 38(1): 195-199.<Go to ISI>://000234815200023 AND <http://www.botanischergarten.ch/Bt/Cortet-Decomposition-Multisite-2006.pdf>

The effects of maize expressing the *Bacillus thuringiensis* Cry1Ab protein (Bt maize) on decomposition processes under three different European climatic conditions were assessed in the field. Farming practices using Bt maize were compared with conventional farming practices using near-isogenic non-Bt maize lines under realistic agricultural practices. The litter-bag method was used to study litter decomposition and nitrogen mineralization dynamics of wheat straw. After 4 months incubation in the field, decomposition and mineralization were mainly influenced by climatic conditions with no negative effect of the Bt toxin on decomposition processes. (c) 2005 Elsevier Ltd. All rights reserved. (Cortet et al., 2006)

**Crecchio, C. and G. Stotzky (1998).** "Insecticidal activity and biodegradation of the toxin from *Bacillus thuringiensis* subsp. *kurstaki* bound to humic acids from soil." *Soil Biology & Biochemistry* 30(4): 463-470.<Go to ISI>://WOS:000073488200005 AND <http://www.botanischergarten.ch/Bt/Crecchio-Insecticidal-Activity-1998.pdf>

The equilibrium adsorption and binding of the active toxin from *Bacillus thuringiensis* subsp. *kurstaki*, toxic to lepidopteran larvae, to humic acids extracted from two forest and two cultivated soils, as well as the insecticidal activity and the biodegradation of the bound toxin, were studied. From 75 to 85% of the toxin added was rapidly adsorbed to the humic acids at equilibrium, and adsorption to a constant amount of humic acids increased with the concentration of the toxin until a plateau was reached. Differences in total acidity and in the content of phenolic groups of the humic acids appeared to be primarily responsible for differences in the amounts of toxin bound (45-80% of the adsorbed toxin) after extensive washing with distilled water. The content of carboxyl groups and the degree of polymerization (E4/E6) did not appear to influence significantly the differential binding. Bound humic acid-toxin complexes were toxic to larvae of the tobacco hornworm (*Manduca sexta*). The lethal concentration necessary to kill 50% of the larvae (LC50) of the bound toxin was comparable with that of the free toxin, indicating that the binding of the toxin to humic acids did not affect its insecticidal activity. The bound toxin did not support the growth of a mixed microbial culture from soil, although the free toxin was rapidly utilized as a carbon and energy source for growth, indicating that binding of the toxin to humic acids reduced its biodegradability. The result of these studies indicate that the toxins from *B. thuringiensis* introduced in transgenic plants and microbes could persist, accumulate, and remain insecticidal in soil as a result of binding to humic acids, as well as on clays, as previously described. This persistence could pose a hazard to non-target organisms and enhance the selection of toxin-resistant target species. (Crecchio & Stotzky, 1998b)

**Crecchio, C. and G. Stotzky (1998).** "Binding of DNA on humic acids: Effect on transformation of *Bacillus subtilis* and resistance to DNase." *Soil Biology & Biochemistry* **30**(8-9): 1061- 1067  
<http://www.botanischergarten.ch/Bt/Crecchio-Binding-DNA-1998.pdf>

Equilibrium adsorption and binding of DNA from *Bacillus subtilis* on humic acids (HA) extracted from a forest soil, the capacity of the bound DNA to transform *B. subtilis*, and the resistance of the bound DNA to degradation by DNase I are reported. Adsorption of DNA on the HA was maximal after 2 and 4 h at pH 3.0 and 4.0, respectively. The adsorption of a constant amount of DNA (50pg) on increasing concentrations of the HA reached a plateau with 2 and 3 mg of HA at pH 3.0 and 4.0, respectively. No plateau was observed when increasing amounts of DNA were adsorbed on a constant amount of the HA (2 mg). 70 to 80% of the adsorbed DNA was tightly bound after two washes with 0.1 M NaCl (pH 6.41, double distilled water (pH 5.5), or DNA buffer (pH 4.0). Bound DNA was capable of transforming auxotrophic and chloramphenicol-sensitive cells of *B. subtilis*, although at a lower frequency than free DNA. DNA bound on the HA was protected more against degradation by DNase I than free DNA: the concentration of DNase required to inhibit transformation by bound DNA was approximately 100 times higher than that required to inhibit transformation by comparable amounts of free DNA. (Crecchio & Stotzky, 1998a)

**Crecchio, C. and G. Stotzky (2001).** "Biodegradation and insecticidal activity of the toxin from *Bacillus thuringiensis* subsp. *kurstaki* bound on complexes of montmorillonite-humic acids-Al hydroxypolymers." *Soil Biology & Biochemistry* **33**(4-5): 573-581. <Go to ISI>://WOS:000167756300016 AND  
<http://www.botanischergarten.ch/Bt/Crecchio-Biodegradation-Activity-Bt-2001.pdf>

The equilibrium adsorption and binding of the active toxin from *Bacillus thuringiensis* subsp. *kurstaki* on complexes of montmorillonite-humic acids-Al hydroxypolymers, as well as the biodegradation and the insecticidal activity of the bound toxin, were studied. Seventy percent of the total adsorption occurred within the first hour, and maximal adsorption occurred in <8 h. Adsorption of the toxin on a constant amount of the complexes increased as the amount of the toxin added increased, and equilibrium adsorption isotherms of the L-type were obtained. There was essentially no desorption of the toxin after extensive washing of the toxin-organomineral complexes with double distilled H<sub>2</sub>O and 1 M NaCl. The bound toxin was resistant to utilization by mixed microbial cultures from soil and to enzymatic degradation by Pronase E. Free and bound toxin were active against the larvae of *Manduca sexta*; the bound toxin retained the same activity after exposure to microbes or Pronase, whereas the toxicity of the free toxin decreased significantly. The results of these studies indicate that the release of transgenic plants and microorganisms expressing truncated genes that encode active insecticidal toxins from *B. thuringiensis* could result in the accumulation of these toxins in soil as a consequence of binding on surface-active soil

particles. This persistence could pose a hazard to nontarget organisms, enhance the selection of toxin-resistant target species, and increase the control of target insect pests. (Crecchio & Stotzky, 2001)

**Crecchio, C., M. Curci, M. D. R. Pizzigallo, P. Ricciuti and P. Ruggiero (2001).** "Molecular approaches to investigate herbicide-induced bacterial community changes in soil microcosms." Biology and Fertility of Soils **33**(6): 460-466.<Go to ISI>://WOS:000170021900003

Since biochemical and microbiological methods used to study microbial community changes induced by anthropogenic activities can be biased, the impact of two herbicides on soil microorganisms was investigated by culture-independent molecular techniques. The effect of three different amounts (the recommended field dose, tenfold, and 100-fold the dose) of propanil or prometryne on the bacterial community of a clay soil, two modalities of incubation (soil moisture at 70% of the field capacity and a soil-herbicide suspension, 1:10, w:v), and time of incubation were investigated by denaturing gradient gel electrophoresis (DGGE) and amplified rDNA restriction analysis (ARDRA). Two sets of primers for 16S rDNA were used to amplify total soil DNA. Sterile and non-sterile samples were used to determine, by HPLC, the amounts of herbicides adsorbed on soil and transformed by soil microorganisms. Prometryne persisted in soil longer than propanil. Propanil was removed significantly more by non-sterile than by sterile samples, while for prometryne, slight differences were observed. 3,4-Dichloroaniline, a product of propanil hydrolysis, was detected in non-sterile samples and increased with incubation time. Propanil did not affect soil bacteria significantly as indicated by DGGE and ARDRA, with the only exception being the soil-herbicide suspension. Despite a lower utilization of prometryne by soil microorganisms, DGGE analysis showed a more diverse banding than with propanil. Some bands were also detected in the DNA sample extracted from the soil-prometryne suspension, and could be representative of bacterial species utilizing the herbicide as a carbon source, in two very different soil microcosms. (Crecchio et al., 2001)

**Crecchio, C., A. Gelsomino, R. Ambrosoli, J. L. Minati and P. Ruggiero (2004).** "Functional and molecular responses of soil microbial communities under differing soil management practices." Soil Biology & Biochemistry **36**(11): 1873-1883.<Go to ISI>://WOS:000224476600020 AND <http://www.botanischergarten.ch/Bt/Crecchio-Functional-Responses-2004.pdf>

The effects of soil management on some microbiological properties and soil bacterial community structure were evaluated. Two field sites with the same soil type, located on the same geographic area adjacent to one other, have received different soil management practices and Cultivation. One site has been subjected for 20 years to intensive horticulture under conventional tillage and irrigation with low quality salt-rich water, the second field site has been uncultivated for a long period and was turned to organic farming practices over the last 5 years and is currently cultivated with fruit orchard. Total bacterial counts, microbial ATP, microbial community metabolic (BILOG(R)) profiles, and DNA fingerprinting by PCR-DGGE were determined. Two-way ANOVA revealed that total bacterial counts were not significantly ( $P > 0.3$ ) affected by the two different management practices; ATP content was consistently and significantly ( $P < 0.001$ ) lower in salt-water irrigated soil than in organic soil at the three sampling times. The cluster analysis of community level physiological profiles indicated that microbial communities were much more uniform in organic soil than in irrigated one, suggesting that salt-water irrigation could have affected the size of the microbial population, its metabolic activities, as well as its composition. Molecular patterns fitted the BILOG(R) profile diversity. In particular, at any sampling time, PCR-DGGE patterns of bacterial DNA, extracted by an indirect method, significantly discriminated irrigated from organic soil samples. The PCR-DGGE patterns of total soil DNA, extracted by a direct method, showed a moderate to significant variation among irrigated and organic soil samples. Biochemical, microbiological and molecular data contributed to evidence a significantly different response of indigenous microflora to soil management by using saline water or organic farming. (Crecchio et al., 2004)

**Crecchio, C., P. Ruggiero, M. Curci, C. Colombo, G. Palumbo and G. Stotzky (2005).** "Binding of DNA from *Bacillus subtilis* on montmorillonite-humic acids-aluminum or iron hydroxypolymers: Effects on transformation and protection against DNase." Soil Science Society of America Journal **69**(3): 834-841.<Go to ISI>://000229009800031 AND <http://www.botanischergarten.ch/Bt/Crecchio-Binding-DNA-2005.pdf>

The equilibrium adsorption and binding of DNA from *Bacillus subtilis* on complexes of montmorillonite-humic acids Al or Fe hydroxypolymers (Al-M-HA or Fe-M-HA) at different M/HA ratios, the desorption of DNA, the capacity of bound DNA to transform competent cells of *B. subtilis* in vitro, and the protection of bound DNA from degradation by free and organomineral-bound DNase I are reported. Adsorption was rapid (maximal after 2 h), occurred from pH 3 to 10, and was higher on Al-M-HA than on Fe-M-HA. Saturation of the sites on the surface or between the layers of Al- or Fe-M-HA occurred with only some complexes, depending on how the complexes were prepared. Essentially no desorption under stringent conditions was observed. Bound DNA transformed auxotrophic competent cells of *B. subtilis*, although at a lower frequency than free DNA. Bound DNA was protected more than free DNA against degradation by DNase I, and differences in resistance to degradation between free and bound DNA were more evident when DNase was also bound on the organomineral complexes. (Crecchio et al., 2005)

**Crecchio, C., M. Curci, A. Pellegrino, P. Ricciuti, N. Tursi and P. Ruggiero (2007).** "Soil microbial dynamics and genetic diversity in soil under monoculture wheat grown in different long-term management systems." *Soil Biology & Biochemistry* **39**(6): 1391-1400.<Go to ISI>://WOS:000245999800017 AND <http://www.botanischergarten.ch/Bt/Crecchio-Soil-Microbial-Dynamics-2007.pdf>

Organic matter incorporation into soil can increase nutrient availability to plants but it can affect soil microbial communities. These in turn influence soil fertility and plant growth. Soil biochemical and microbiological properties are indicators of soil quality, but there is still no consensus as to how these should be used. Recent developments in molecular biology have provided new tools to obtain a view of the whole microbial community. The long-term impact of crop residue management on the microbial biomass, and on the activity and community structure of soil bacteria was evaluated in a clay soil of Southern Italy, where a monoculture of durum wheat (*Triticum durum* Desf.) was grown in, semiarid conditions, and burning or incorporation of post harvest plant residues were typical practices. The role of N-mineral fertilization, Simultaneously with the ploughing in of crop residues and during the plant growth cycle was also investigated. Total bacterial counts of viable cells, biomass C, ATP content of soil microorganisms, genetic fingerprinting of the total eubacterial community and of ammonia oxidizers were evaluated. Burning and incorporation did not affect microbial biomass C, ATP content, and total bacterial counts of viable cells although statistically relevant changes were detected among rhizosphere and bulk soil samples regardless of the crop residue management used. Molecular fingerprinting confirmed that: no significant change in the composition and diversity of total bacteria, as well as of ammonia oxidizers was induced by the crop residue managements; that soil bacteria were more sensitive to N fertilizer application during the plant growth cycle; and that rhizosphere soil samples were significantly different from those of the bulk soil. As microbiological and genetic factors related to soil fertility were not affected significantly, the long-term incorporation of crop residues, under the field conditions investigated, is a sustainable practice to manage post-harvest residues. (Crecchio et al., 2007)

**Devare, M. H., C. M. Jones and J. E. Thies (2004).** "Effect of Cry3Bb transgenic corn and tefluthrin on the soil microbial community: Biomass, activity, and diversity." *Journal of Environmental Quality* **33**(3): 837-843.<Go to ISI>://WOS:000221509200006

Transgenic Bt corn expressing the Cry3Bb insecticidal protein active against corn rootworm(CRW)(*Diabrotica* spp.; Coleoptera:Chrysomelidae) was released for commercial use in 2003 and is expected to be widely adopted. Yet, the direct and indirect risks to soil microorganisms of growing this CRW-resistant Bt corn versus applying insecticides to control the rootworm have not been assessed under field conditions. The effects of CRW Bt corn and the insecticide tefluthrin. [2,3,5,6-tetrafluoro-4-methylbenzyl (Z)-(1RS)-cis-3-(2-chloro-3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate] on soil microbial biomass, activity (N mineralization potential, short-term nitrification rate, and soil respiration), and bacterial community structure as determined by terminal restriction fragment length polymorphism (T-RFLP) analysis were assessed over two seasons in a field experiment. Bt corn had no deleterious effects on microbial activity or bacterial community measures compared with the non-transgenic isolate. The T-RFLP analysis indicated that amplifiable bacterial species composition and relative abundance differed substantially between years, but did not differ between rhizosphere and bulk

soils. The application of tefluthrin also had no effect on any microbial measure except decreased soil respiration observed in tefluthrin-treated plots compared with Bt and non-transgenic isoline (NoBt) plots in 2002. Our results indicate that the release of CRWBt corn poses little threat to the ecology of the soil microbial community based on parameters measured in this study. (Devare et al., 2004)

**Devare, M., L. M. Londono-R and J. E. Thies (2007).** "Neither transgenic Bt maize (MON863) nor tefluthrin insecticide adversely affect soil microbial activity or biomass: A 3-year field analysis." Soil Biology & Biochemistry **39**(8): 2038-2047. <Go to ISI>://WOS:000247295800020 AND <http://www.botanischergarten.ch/Bt/Devare-Neither-transgenic-2007.pdf>

Laboratory and greenhouse studies on transgenic *Bacillus thuringiensis* (Bt) maize have drawn attention to the persistence and activity of the Cry proteins in soil and their potential effects on soil microorganisms, but there have been few field assessments that evaluate the effects of Bt maize with those of insecticides on soil microbial populations. This study was conducted to determine the effects of Cry3Bb Bt maize with those of the insecticide tefluthrin on soil microbial biomass and activity in the field over a 3-year cropping cycle. The recently commercialized maize variety YieldGard (R) Rootworm (MON863), which produces the Cry3Bb protein, was grown along with a non-Bt isoline with and without tefluthrin applied at planting. Microbial biomass, nitrogen (N) mineralization potential, short-term nitrification rate, and respiration rate were measured in rhizosphere and bulk soil samples collected from three replicate field plots just before planting, at anthesis, and at harvest in each year. There were clear seasonal effects on microbial biomass and activity in the field soils—as represented by the consistent changes in all measured variables across years and sampling times. Differences in the measured variables were also sometimes observed between bulk and rhizosphere soil. However, there were no adverse effects of either the Bt or non-Bt maize with insecticide applied compared to the non-Bt controls; on the contrary, microbial biomass and soil respiration data suggested a stimulatory effect of the Bt genotype, particularly in comparison to the non-Bt isoline. Although 'higher' does not necessarily mean 'better', the higher microbial biomass and respiration rates observed in the Bt and insecticide-applied soils compared to non-Bt soils does allay concerns that either the Bt protein or the tefluthrin typically used to control the corn rootworm reduce microbial biomass or its respiratory activity in field soils. Similarly, the higher N mineralization potential and nitrification rates observed in some soil samples from the Bt and tefluthrin-treated plots indicate higher activity of N-mineralizing microorganisms, a potentially positive consequence as both ammonium and nitrate are effective N sources for maize during grain filling. Our data suggest that cropping MON863 Bt maize is unlikely to adversely affect soil ecology in the short term. Longer-term monitoring of transgenic cropping systems should assure that the biotic functioning of the soil is maintained as a part of studies on overall ecosystem integrity. (Devare et al., 2007)

**Dinel, H., M. Schnitzer, M. Saharinen, F. Meloche, T. Pare, S. Dumontet, L. Lemee and A. Ambles (2003).** "Extractable soil lipids and microbial activity as affected by Bt and non Bt maize grown on a silty clay loam soil." Journal of Environmental Science and Health Part B-Pesticides Food Contaminants and Agricultural Wastes **38**(2): 211-219. <Go to ISI>://000181171600009 NEBIS TO ORDER 20080325

Pyrolysis-gas (Py-GC) chromatography was used to characterize extractable lipids from Bt and non-Bt maize shoots and soils collected at time of harvesting. Py-GC-MS (mass spectrometry) showed that the concentrations of total alkenes identified in nonBt shoots and soils were 47.9 and 21.3% higher than in Bt maize shoots and soils, respectively. N-alkanes identified were of similar orders of magnitude in Bt and nonBt maize shoots, but were 28.6% higher in Bt than in non-Bt soils. Bt maize shoots contained 29.7% more n-fatty acids than non-Bt maize shoots, whereas the concentrations of n-fatty acids in Bt soils were twice as high as those in non-Bt soils. Concentrations of unsaturated fatty acids in Bt maize shoots were 22.1 % higher than those in non-Bt maize shoots, while concentrations of unsaturated fatty acids were 22.5% higher in non-Bt than in Bt soils. The cumulative CO<sub>2</sub>-C evolved from soils under Bt and non-Bt crops was 30.5% lower under Bt as compared to non-Bt crops, whereas when maize shoots were added to Bt and non-Bt soils, the decrease in CO<sub>2</sub>-C evolved were 16.5 and 23.6%, respectively. Our data showed that the cultivation of Bt maize significantly increased the saturated to unsaturated lipid ratios in soils which appeared to negatively affect microbial activity. (Dinel et al., 2003)

**Donegan, K. K., C. J. Palm, V. J. Fieland, L. A. Porteous, L. M. Ganio, D. L. Schaller, L. Q. Bucuo and R. J. Seidler (1995).** "Changes in levels, species and DNA fingerprints of soil microorganisms associated with cotton expressing the *Bacillus thuringiensis* var. *kurstaki* endotoxin." *Applied Soil Ecology* 2(2): 111-124. <http://www.sciencedirect.com/science/article/B6T4B-3YCDPPV-P/2/d08cbd3b6688aba8069d3586132395d1> AND <http://www.botanischergarten.ch/Bt/Doohan-Influence-Climatic-2004.pdf>

An important aspect of the risk assessment of pesticidal transgenic plants is the potential for detrimental effects on the soil ecosystem from residual plant material following harvesting and tillage. We evaluated this concern by placing leaves of three different lines of cotton genetically engineered to produce the *Bacillus thuringiensis* var. *kurstaki* (B.t.k.) endotoxin in soil and monitoring numbers and species of indigenous soil bacteria and fungi. Four experiments, lasting 28 or 56 days, were performed using combinations of the following treatments: (1) soil only; (2) soil +purified B.t.k. toxin; (3) soil +parental cotton; (4) soil +purified B.t.k. toxin +parental cotton; (5) soil + B.t.k. toxin-producing cotton.

Two of the three transgenic cotton lines caused a transient increase in total bacterial and fungal population levels that was significantly higher on several sample days in the experiments than the levels in the other treatments. In contrast, neither the third transgenic cotton line nor the purified B.t.k. toxins had any significant effects on the total numbers of bacteria and fungi.

Transient changes in bacterial species composition, measured by biochemical tests of individual cultures, community substrate utilization and DNA fingerprinting, were also observed in treatments with the two transgenic plant lines.

The plant line specificity of the response, and the lack of effects from the purified B.t.k. toxins, suggest that the observed effects of the two transgenic plant lines on soil microorganisms may not have resulted from the plants' production of B.t.k. toxin. We suggest that genetic manipulation or tissue culturing of the plants may have produced a change in plant characteristics, aside from B.t.k. toxin production, that can influence growth and species composition of soil microorganisms. (Donegan et al., 1995)

**Donegan, K. K., D. L. Schaller, J. K. Stone, L. M. Ganio, G. Reed, P. B. Hamm and R. J. Seidler (1996).** "Microbial populations, fungal species diversity and plant pathogen levels in field plots of potato plants expressing the *Bacillus thuringiensis* var. *tenebrionis* endotoxin." *Transgenic Research* 5(1): 25-35. <Go to ISI>://WOS:A1996TT63000004 NEBIS TO ORDER 20080325

The environmental release of genetically engineered (transgenic) plants may be accompanied by ecological effects including changes in the plant-associated microflora. A field release of transgenic potato plants that produce the insecticidal endotoxin of *Bacillus thuringiensis* var. *tenebrionis* (Bit) was monitored for changes in total bacterial and fungal populations, fungal species diversity and abundance, and plant pathogen levels. The microflora on three phenological stages of leaves (green, yellow and brown) were compared over the growing season (sample days 0, 21, 42, 63 and 98) for transgenic potato plants, commercial Russet Burbank potato plants treated with systemic insecticide (Di-Syston) and commercial Russet Burbank potato plants treated with microbial Btt (M-Trak). In addition, plant and soil assays were performed to assess disease incidence of *Fusarium* spp., *Pythium* spp., *Verticillium dahliae*, potato leaf roll virus (PLRV) and potato virus Y (PVY). Few significant differences in phylloplane microflora among the plant types were observed and none of the differences were persistent. Total bacterial populations on brown leaves on sample day 21 and on green leaves on sample day 42 were significantly higher on the transgenic potato plants. Total fungal populations on green leaves on sample day 63 were significantly different among the three plant types; lowest levels were on the commercial potato plants treated with systemic insecticide and highest levels were on the commercial potato plants treated with microbial Btt. Differences in fungal species assemblages and diversity were correlated with sampling dates, but relatively consistent among treatments. *Alternaria alternata*, a common saprophyte on leaves and in soil and leaf litter, was the most commonly isolated fungus species for all the plant treatments. Rhizosphere populations of the soilborne pathogens *Pythium* spp., *Fusarium* spp. and *V. dahliae* did not differ between the transgenic potato plants and the commercial potato plants treated with systemic insecticide. The incidence of tuber infection at the end of the growing season by the plant pathogen *V. dahliae* was highest for the transgenic potato plants but this difference was related to longer viability of the transgenic potato plants. This difference in longevity between the transgenic potato plants and the commercial + systemic insecticide potato plants also made comparison of the incidence of PVY and PLRV problematic. Our

results indicate that under field conditions the microflora of transgenic Bt-producing potato plants differed minimally from that of chemically and microbially treated commercial potato plants. (Donegan et al., 1996)

**Douville, M., F. Gagne, L. Masson, J. McKay and C. Blaise (2005).** "Tracking the source of *Bacillus thuringiensis* Cry1Ab endotoxin in the environment." *Biochemical Systematics and Ecology* **33**(3): 219-232.<Go to ISI>://000227414300001 AND <http://www.botanischergarten.ch/Bt/Douville-Tracking-Source-2005.pdf>

The application of *Bacillus thuringiensis* (Bt) and the growing of genetically-modified crops are currently practised to control infestations of crop-eating insects. The increasing use of these biopesticides could lead to an increase in Cry1Ab endotoxin in both terrestrial and aquatic environments. The aim of this study was to quantify levels of Cry1Ab endotoxin and locate its source in the environment. Agricultural soils and surface waters were spiked with crystals (biopesticide-Dipel(R)) or with pure Bt-corn endotoxin. Cry1Ab concentrations were then determined with immunoassays. Additionally, surface water, soils and sediments were sampled in an area sprayed with Bt kurstaki and at a site where genetically-modified corn expressing Cry1Ab is grown. Isotopic analysis was performed on the endotoxin from Bt and Bt corn to characterize the proportions of C-13/C-12 and N-15/N-14. The results showed that Bt-corn endotoxin is degraded more rapidly in water than in Soils ( $t(1/2)$ : 4 and 9 days, respectively), while crystals appeared to be more resilient, as expected. The isotopic patterns of C-13 and N-15 in Bt-corn endotoxin differed markedly from Bt, making it possible to track the source of Cry1Ab in the environment. Preliminary field surveys indicate that Cry1Ab is fairly uncommon in aquatic environments, being found only at trace concentrations when it is detected. (Douville et al., 2005)

**Dubelman, S., B. R. Ayden, B. M. Bader, C. R. Brown, C. J. Jiang and D. Vlachos (2005).**

"Cry1Ab protein does not persist in soil after 3 years of sustained Bt corn use." *Environmental Entomology* **34**(4): 915-921.<Go to ISI>://000231161400023 AND <http://www.botanischergarten.ch/Bt/Dubelman-Not-Persist-Soil-2005.pdf>

The purpose of this study was to assess the persistence and accumulation of the Cry1Ab protein in soil as a result of sustained planting of genetically modified *Bacillus thuringiensis* (Bt) corn hybrids. Soil samples were collected from agricultural fields in five corn-growing regions of the United States where Bt corn hybrids (MON 810 or Bt11) had been planted for at least 3 consecutive yr. At each site, soil samples were collected during the corn-growing period (postanthesis) and again within 6 A after harvest. Multiple soil specimens from matched Bt cornfields and nearby, non-Bt control fields were analyzed by diet-incorporation insect bioassay, using growth inhibition (GI) of the European corn borer (*Ostrinia nubilalis*) as the toxicity endpoint. Positive control soil samples containing Cry1Ab protein at the GI(50) level (0.05  $\mu$ g/g soil) were analyzed in tandem with test and control samples to verify that the bioassay was able to detect low levels of Cry1Ab protein. The limit of detection for Cry1Ab protein in soil was 0.03  $\mu$ g/g soil. The presence of Cry1Ab protein in soil was assessed by statistical comparison of the insect toxicity (GI) of soils collected from Bt and non-Bt (control) cornfields. Only one soil sample, collected postanthesis in a Bt cornfield that had also been treated with carbofuran insecticide, showed insect toxicity. This toxicity was below the GI(50) level, and no toxicity was detected in the soil collected from the same plot shortly after harvest. Therefore, there is no evidence of persistence or accumulation of Cry1Ab protein in soils from fields planted for at least three consecutive growing seasons with Bt corn hybrids. (Dubelman et al., 2005)

**Dunfield, K. E. and J. J. Germida (2001).** "Diversity of bacterial communities in the rhizosphere and root interior of field-grown genetically modified *Brassica napus*." *Fems Microbiology Ecology* **38**(1): 1-9.<Go to ISI>://WOS:000172988200001 AND <http://www.botanischergarten.ch/Bt/Dunfield-Diversity-Communities-2001.pdf>

Plant roots significantly affect microbial diversity in soil, but little is known on how genetically modified plants influence soil microbial communities. We conducted a 2-year field study to assess the effects of herbicide-tolerant genetically modified canola (oilseed rape, *Brassica* sp.) on microbial biodiversity in the rhizosphere. During the 1998 and 1999 field seasons, four genetically modified and four conventional

canola varieties were grown at four different Field locations across Saskatchewan, Canada. The rhizosphere and root interior microbial communities were characterized through fatty acid methyl ester analysis and community level physiological profiles. Principal component analysis indicated that the root interior and rhizosphere bacterial community associated with the genetically modified variety Quest (*Brassica napus*) was different from conventional varieties Excel (*B. napus*) and Fairview (*Brassica rapa*), based on both fatty acid composition and carbon substrate utilization. In addition, all root-associated microbial communities associated with genetically modified canola varieties had significantly higher levels of 10:02OH, 12:02OH, 12:03OH, a15:0, 15:1 omega 5c, cy17:0, 18:3 omega6,9,12c, 19:0 omega 8c and Sum in Feature 3, suggesting alterations in the composition of the microbial community associated with plants. This Study indicates that the composition and functional diversity and the microbial community were influenced by plant variety. (Dunfield & Germida, 2001)

**Dunfield, K. E. and J. J. Germida (2003).** "Seasonal changes in the rhizosphere microbial communities associated with field-grown genetically modified canola (*Brassica napus*)." *Applied and Environmental Microbiology* **69**(12): 7310-7318.<Go to ISI>://WOS:000187234000044 AND <http://www.botanischergarten.ch/Bt/Dunfield-Seasonal-Change-2003.pdf>

The introduction of transgenic plants into agricultural ecosystems has raised the question of the ecological impact of these plants on nontarget organisms, such as soil bacteria. Although differences in both the genetic structure and the metabolic function of the microbial communities associated with some transgenic plant lines have been established, it remains to be seen whether these differences have an ecological impact on the soil microbial communities. We conducted a 2-year, multiple-site field study in which rhizosphere samples associated with a transgenic canola variety and a conventional canola variety were sampled at six times throughout the growing season. The objectives of this study were to identify differences between the rhizosphere microbial community associated with the transgenic plants and the rhizosphere microbial community associated with the conventional canola plants and to determine whether the differences were permanent or depended on the presence of the plant. Community-level physiological profiles, fatty acid methyl ester profiles, and terminal amplified ribosomal DNA restriction analysis profiles of rhizosphere microbial communities were compared to the profiles of the microbial community associated with an unplanted, fallow field plot. Principal-component analysis showed that there was variation in the microbial community associated with both canola variety and growth season. Importantly, while differences between the microbial communities associated with the transgenic plant variety were observed at several times throughout the growing season, all analyses indicated that when the microbial communities were assessed after winter, there were no differences between microbial communities from field plots that contained harvested transgenic canola plants and microbial communities from field plots that did not contain plants during the field season. Hence, the changes in the microbial community structure associated with genetically modified plants were temporary and did not persist into the next field season. (Dunfield & Germida, 2003)

**Dunfield, K. E. and J. J. Germida (2004).** "Impact of Genetically Modified Crops on Soil- and Plant-Associated Microbial Communities." *J Environ Qual* **33**(3): 806-815.<http://jeq.scijournals.org/cgi/content/abstract/joenq;33/3/806> AND <http://www.botanischergarten.ch/Bt/Dunfield-Impact-GM-Crops-Soil-2004.pdf>

Transgenic or genetically modified plants possess novel genes that impart beneficial characteristics such as herbicide resistance. One of the least understood areas in the environmental risk assessment of genetically modified crops is their impact on soil- and plant-associated microbial communities. The potential for interaction between transgenic plants and plant residues and the soil microbial community is not well understood. The recognition that these interactions could change microbial biodiversity and affect ecosystem functioning has initiated a limited number of studies in the area. At this time, studies have shown the possibility that transgenes can be transferred to native soil microorganisms through horizontal gene transfer, although there is not evidence of this occurring in the soil. Furthermore, novel proteins have been shown to be released from transgenic plants into the soil ecosystem, and their presence can influence the biodiversity of the microbial community by selectively stimulating the growth of organisms that can use them. Microbial diversity can be altered when associated with transgenic plants; however, these effects are both variable and transient. Soil- and plant-associated microbial communities are influenced not only by plant species and transgene insertion but also by environmental factors such as

field site and sampling date. Minor alterations in the diversity of the microbial community could affect soil health and ecosystem functioning, and therefore, the impact that plant variety may have on the dynamics of the rhizosphere microbial populations and in turn plant growth and health and ecosystem sustainability, requires further study. (Dunfield & Germida, 2004)

**Escher, N., B. Kaech and W. Nentwig (2000).** "Decomposition of transgenic *Bacillus thuringiensis* maize by microorganisms and woodlice *Porcellio scaber* (Crustacea: Isopoda)." *Basic and Applied Ecology* 1(2): 161-169. <http://www.sciencedirect.com/science/article/B7GVS-4DS332N-R/2/f258cf295a4bfead7df9199de26459e5> AND <http://www.botanischergarten.ch/Bt/Escher-Composition-Porcellio-2000.pdf>

Foliage of transgenic maize *Zea mays* L., expressing a Cry1Ab protein derived from *Bacillus thuringiensis* (Berliner) subsp. *kurstaki*, was compared with foliage of the corresponding non-transgenic maize variety in laboratory feeding and decomposition experiments to study the effects of the *B. thuringiensis* protein on the chemical composition of the maize leaves, on the decomposer *Porcellio scaber* (Crustacea: Isopoda), and on leaf-litter-colonising microorganisms. Initial contents of fructose and soluble carbohydrates were significantly higher in non-transgenic maize. Lignin was decomposed more quickly in transgenic maize. Starch, cellulose, hemicellulose and ash content did not differ. Bacterial growth on faeces of *P. scaber* fed on non-transgenic maize was up to 60% higher than on faeces of the transgenic-fed woodlice, but bacterial growth on leaves and fungal growth on faeces were equal on both maize varieties. *P. scaber* showed no significant difference in its consumption rate of transgenic and non-transgenic maize. The number of offspring did not differ between the two treatment groups, but the mortality of juveniles reared on non-transgenic maize leaves was significantly higher. During the first 131 days weight increase of the offspring was significantly higher in the non-transgenic group, but weight increase of adult *P. scaber* was higher in the transgenic group. Due to a slightly lower C:N ratio, a lower lignin content, and a higher content of soluble carbohydrates, the nutritional quality of transgenic maize leaves was better than that of the non-transgenic variety. This explains the lower mortality of *P. scaber* offspring and the faster weight gain of adult *P. scaber* on the transgenic diet. (Escher et al., 2000)

**Fang, M., R. J. Kremer, P. P. Motavalli and G. Davis (2005).** "Bacterial diversity in rhizospheres of nontransgenic and transgenic corn." *Applied and Environmental Microbiology* 71(7): 4132-4136. <Go to ISI>://WOS:000230445700094 AND <http://www.botanischergarten.ch/Bt/Fang-Bacterial-Diversity-2005.pdf>

Bacterial diversity in transgenic and nontransgenic corn rhizospheres was determined. In greenhouse and field studies, metabolic profiling and molecular analysis of 16S rRNAs differentiated bacterial communities among soil textures but not between corn varieties. We conclude that bacteria in corn rhizospheres are affected more by soil texture than by cultivation of transgenic varieties. (Fang et al., 2005)

**Fang, M., P. P. Motavalli, R. J. Kremer and K. A. Nelson (2007).** "Assessing changes in soil microbial communities and carbon mineralization in Bt and non-Bt corn residue-amended soils." *Applied Soil Ecology* 37: 150-160. <Go to ISI>://WOS:000249874700016 AND <http://www.botanischergarten.ch/Bt/Fang-Assessing-Changes-2007.pdf>

The effects of Bt corn (*Zea mays* L.) residue on soil microbial communities and rates of C mineralization were investigated. The Bt corn residue had a higher lignin content (12%) and lignin/N (9.9) ratio compared with its non-Bt near-isoline (10% lignin; lignin/N = 8.6). We examined the relationships among the Bt/non-Bt residue properties, residue component, soil texture, sampling time, and tillage management in microcosm and field studies. Bt corn residue incorporated in soils of different textural classes (silty clay, silt loam and sandy loam) in microcosms affected bacterial substrate metabolism. Substrate utilization profiles (Biolog) of soils amended with Bt residue differed from those with non-Bt residue based on principal component analysis (PCA). Denaturing gradient gel electrophoresis (DGGE) patterns revealed only slightly altered microbial communities in the soils amended with Bt residue compared with the non-Bt isolate. Soil texture significantly ( $P < 0.05$ ) affected C mineralization and substrate utilization profiles. Carbon dioxide evolution rate constants  $k$  of 0.085-0.087 for non-Bt and Bt corn leaf tissue added to silt loam indicated higher rates of Soil CO<sub>2</sub> evolution compared with addition of

roots and stems ( $k = 0.06-0.07$ ). However, cumulative CO<sub>2</sub> production after 73 days was similar regardless of residue component amendment. Significant ( $P < 0.05$ ) interactions between soil texture, residue type (Bt versus non-Bt) and residue component illustrated the influence of soil on decomposition. In the field study, sampling time significantly correlated with Biolog metabolic activity and DGGE profiles. The field study also confirmed the effects of Bt residue on total plate count and substrate utilization profiles. Based on the results of the microcosm and field studies, we concluded that incorporation of Bt residue with higher lignin content and lignin/N ratio in soil significantly affected the structure of microbial communities compared with the residue from its non-Bt isolate. Abiotic factors including soil texture and sampling time also influenced the soil microbial communities and the decomposition of corn residues. (Fang et al., 2007)

**Ferreira, L., J. C. Molina, C. Brasil and G. Andrade (2003).** "Evaluation of *Bacillus thuringiensis* bioinsecticidal protein effects on soil microorganisms." *Plant and Soil* 256(1): 161-168. <Go to ISI>://WOS:000186045900012 AND <http://www.botanischergarten.ch/Bt/Ferreira-Evaluation-2003.pdf>

The effect of *B. thuringiensis* and its crystal protein on plant growth and on functional groups of microorganisms is not well understood. Soybean (*Glycine max*) var. Br 322 was grown in non-sterile soil infested with three *B. thuringiensis* (Bt) inocula: insecticidal crystal protein producer (Cry+), a mutant non-producer (Cry-), or insecticidal crystal protein (ICP), at a rate of 10<sup>7</sup> cells g<sup>-1</sup> dry soil or 1.25 mg of protein g<sup>-1</sup> dry soil. Non-inoculated plants were maintained as control. Measurements were carried out on soil samples before sowing (time zero) and after sowing and inoculation (5, 15, 25, 35 and 45 d) on samples of rhizosphere soil. The effect of spore and crystal protein produced by *B. thuringiensis* on the populations of functional groups of microorganisms (bacteria including actinomycetes and fungi) involved in the biogeochemical cycling of carbon (cellulolytic, amylolytic and proteolytic), phosphorus (arbuscular mycorrhizal fungi), and nitrogen (number of nodules and proteolytic) were evaluated. Population sizes of culturable heterotrophic bacteria and saprophytic fungi were also evaluated. No difference was found in heterotrophic bacterial populations inoculated with *B. thuringiensis*. Difference was observed in functional groups of C-cycling microorganisms. Nodule formation and plant growth were increased by Cry+ strain and ICP when compared with uninoculated plants. Crystal protein did not show any effect on arbuscular mycorrhiza (AM) colonization. However, a deleterious effect was observed with Cry+ and Cry- strains that inhibited colonization of AM fungi when compared with uninoculated plants. (Ferreira et al., 2003)

**Fiorito, T. M., I. Icoza and G. Stotzky (2007).** "Adsorption and binding of the transgenic plant proteins, human serum albumin, [beta]-glucuronidase, and Cry3Bb1, on montmorillonite and kaolinite: Microbial utilization and enzymatic activity of free and clay-bound proteins." *Applied Clay Science In Press, Corrected Proof*(online): xxx.<http://www.sciencedirect.com/science/article/B6V8Y-4PB6VPW-1/2/487fcf6b24b8088c723b11f099509867> AND <http://www.botanischergarten.ch/Bt/Fiorito-Adsorption-Binding-2007.pdf>

Human serum albumin (HSA),  $\beta$ -glucuronidase (GUS), and the Cry3Bb1 protein from *Bacillus thuringiensis* subsp. *kumamotoensis* are expressed by genetically-modified plants. Commercial samples of these proteins adsorbed and bound rapidly on the clay minerals, kaolinite (K) and montmorillonite (M). Adsorption increased as the concentration of protein increased and then reached a plateau. The greatest amount of adsorption and binding occurred with the Cry3Bb1 protein, of which there was no desorption: 6.7 $\pm$ 0.21  $\mu$ g adsorbed and bound  $\mu$ g<sup>-1</sup> of M; 2.1 $\pm$ 0.39  $\mu$ g adsorbed and bound  $\mu$ g<sup>-1</sup> of K. With GUS, 2.2 $\pm$ 0.29  $\mu$ g adsorbed and 1.7 $\pm$ 0.21  $\mu$ g bound  $\mu$ g<sup>-1</sup> of M; 1.5 $\pm$ 0.28  $\mu$ g adsorbed and 1.0 $\pm$ 0.03  $\mu$ g bound  $\mu$ g<sup>-1</sup> of K. HSA was adsorbed and bound the least: 1.2 $\pm$ 0.04  $\mu$ g adsorbed and 0.8 $\pm$ 0.05  $\mu$ g bound  $\mu$ g<sup>-1</sup> of M; 0.4 $\pm$ 0.05  $\mu$ g adsorbed and 0.4 $\pm$ 0.03  $\mu$ g bound  $\mu$ g<sup>-1</sup> of K. However, X-ray diffraction analyses indicated that only HSA intercalated M, and none of the proteins intercalated K, a nonswelling clay. When bound, the proteins were not utilized for growth by mixed cultures of soil microorganisms, whereas the cultures readily utilized the free (i.e., not adsorbed or bound) proteins as sources of carbon and energy. The enzymatic activity of GUS was significantly enhanced when bound on the clay minerals. These results indicated that recombinant proteins expressed by transgenic plants could persist and function in soil after

release in root exudates and from decaying plant residues as the result of the protection provided against biodegradation by binding on clay minerals. (Fiorito et al., 2007)

**Flores, S., D. Saxena and G. Stotzky (2005).**

"Transgenic Bt plants decompose less in soil than non-Bt plants." *Soil Biology & Biochemistry* **37**(6): 1073-1082. <Go to ISI>://000228712700007 and <http://www.botanischergarten.ch/Bt/Flores-Stotzky-Bt-plants-2005.pdf>

Bt plants are plants that have been genetically modified to express the insecticidal proteins (e.g. Cry1Ab, Cry1Ac, Cry3A) from subspecies of the bacterium, *Bacillus thuringiensis* (Bt), to kill lepidopteran pests that feed on corn, rice, tobacco, canola, and cotton and coleopteran pests that feed on potato. The biomass of these transgenic Bt plants (Bt+) was decomposed less in soil than the biomass of their near-isogenic non-Bt plant counterparts (Bt-). Soil was amended with 0.5, 1, or 2% (wt wt(-1)) ground, dried (50 degrees C) leaves or stems of Bt corn plants; with 0.5% (wt wt(-1)) ground, dried biomass of Bt rice, tobacco, canola, cotton, and potato plants; with biomass of the near-isogenic plants without the respective cry genes; or not amended. The gross metabolic activity of the soil was determined by CO<sub>2</sub> evolution. The amounts of C evolved as CO<sub>2</sub> were significantly lower from soil microcosms amended with biomass of Bt plants than of non-Bt plants. This difference occurred with stems and leaves from two hybrids of Bt corn, one of which had a higher C:N ratio than its near-isogenic non-Bt counterpart and the other which had essentially the same C:N ratio, even when glucose, nitrogen (NH<sub>4</sub>NO<sub>3</sub>), or glucose plus nitrogen were added with the biomass. The C:N ratios of the other Bt plants (including two other hybrids of Bt corn) and their near-isogenic non-Bt counterparts were also not related to their relative biodegradation. Bt corn had a significantly higher lignin content than near-isogenic non-Bt corn. However, the lignin content of the other Bt plants, which was significantly lower than that of both Bt and non-Bt corn, was generally not statistically significantly different, although 10-66% higher, from that of their respective non-Bt near-isolines. The numbers of culturable bacteria and fungi and the activity of representative enzymes involved in the degradation of plant biomass were not significantly different between soil amended with biomass of Bt or non-Bt corn. The degradation of the biomass of all Bt plants in the absence of soil but inoculated with a microbial suspension from the same soil was also significantly less than that of their respective inoculated non-Bt plants. The addition of streptomycin, cycloheximide, or both to the soil suspension did not alter the relative degradation of Bt+ and Bt- biomass, suggesting that differences in the soil microbiota were not responsible for the differential decomposition of Bt+ and Bt- biomass. All samples of soil amended with biomass of Bt plants were immunologically positive for the respective Cry proteins and toxic to the larvae of the tobacco hornworm (*Manduca sexta*), which was used as a representative lepidopteran in insect bioassays (no insecticidal assay was done for the Cry3A protein from potato). The ecological and environmental relevance of these findings is not clear. (Flores et al., 2005)

**Fu, Q., Y. Donga and Q. Huang (2007).** "Adsorption of the insecticidal protein of *Bacillus thuringiensis* subsp. *kurstaki* by soil minerals: Effects of organic acid ligands." *Applied Clay Science* **37**(1-2): 201-206. <http://www.sciencedirect.com/science/article/B6V8Y-4MMPNDS-1/2/c1de30d56a46d45204e1ad4a0ff95a18> AND <http://www.botanischergarten.ch/Bt/Fu-Adsorption-Bt-soil-2007.pdf>

The introduction of the toxin of *Bacillus thuringiensis* (Bt) into soil, as a result of the rapid increase in the planting of commercial Bt-transformed crops worldwide, may constitute a hazard to the soil ecosystem. The present investigation is concerned with the effect of low molecular-weight organic acid ligands (acetate, oxalate and citrate) on the adsorption of the toxin of *B. thuringiensis* subsp. *kurstaki* by kaolinite, montmorillonite, goethite, and silicon dioxide (SiO<sub>2</sub>) and its desorption from the surface of these minerals. At the same time, we measured the desorption of bound toxin by NaCl and phosphate buffer. Low concentrations (< 10 mmol L<sup>-1</sup>) of organic acid anions inhibited toxin adsorption by kaolinite, goethite, and silicon dioxide, whereas high concentrations promoted adsorption. For montmorillonite, however, an increase in the concentration of oxalate or citrate inhibited the adsorption of toxin. Less than 15% of the adsorbed amount was desorbed by each of the three organic acid anions. A small proportion

(< 11%) of the toxin was adsorbed via electrostatic forces and ligand exchange, and this percentage increased when organic acid ligands were present. Our results indicate that low molecular-weight organic acid ligands can markedly influence the adsorption of Bt toxin by soil minerals, and result in the binding of the toxin on the minerals getting looser. (Fu et al., 2007)

**Guan, J. W., J. L. Spencer and B. L. Ma (2005).**

"The fate of the recombinant DNA in corn during composting." Journal of Environmental Science and Health Part B-Pesticides Food Contaminants and Agricultural Wastes **40(3)**: 463-473.<Go to ISI>://000229117200007 AND <http://www.botanischergarten.ch/Bt/Guan-Fate-Composting-2005.pdf>

In order to make regulations that safeguard food and the environment, an understanding of the fate of transgenes from genetically modified (GM) plants is of crucial importance. A compost experiment including mature transgenic corn plants and seeds of event Bt 176 (*Zea mays* L.) was conducted to trace the fate of the transgene cryIA(b) during the period of composting. In bin 1, shredded corn plants including seeds were composted above a layer of cow manure and samples from the corn layer were collected at intervals during a 12-month period. The samples were tested for the transgene persistence and microbial counts and also the compost was monitored for temperature. In bin 2, piles of corn seeds, surrounded by sheep manure and straw, were composted for 12 months. A method combining nested polymerase chain reaction (PCR) and southern hybridization was developed for detection of the transgene in compost. The detection sensitivity was 200 copies of the transgene per gram of dry composted corn material. Composting commenced on day 0, and the transgene was detected in specimens from bin 1 on days 0 and 7 but not on day 14 or thereafter. The transgene in corn seeds was not detectable after 12 months of composting in bin 2. Temperatures in both bins rose to about 50&DEG; C within 2 weeks and remained above that temperature for about 3 months, even when the ambient temperature dropped below - 20&DEG; C. Extracts from compost were inoculated onto culture plates and then were incubated at 23 to 55&DEG; C. Within the first 2 weeks of composting in bin 1, the counts of bacteria incubated at 55&DEG; C increased from 3.5 to 7.5 log(10), whereas those incubated at 23&DEG; C remained at about 7.5 log(10). The counts of fungi incubated at 45&DEG; C increased slightly from 2.5 to 3.1 log(10), but those incubated at 23&DEG; C decreased from 6.3 to 3.0 log(10). The rapid degradation of the transgene during composting of Bt corn plants suggested that the composting process could be used for safe disposal of transgenic plant wastes. (Guan et al., 2005)

**Haddad, M. D. L., R. A. Polanczyk, S. B. Alves and M. D. O. Garcia (2005).** "Field persistence of *Bacillus thuringiensis* on maize leaves (*Zea mays* L.)." Brazilian Journal of Microbiology **36(4)**: 309-314.<Go to ISI>://000238508900001

The persistence slope of *Bacillus thuringiensis* (Bt) based products in the field is an important parameter to evaluate their efficacy. The half-life, estimated based on persistence slope parameters, is one of the most effective tools to select microbial pesticides. The aim of this research was to study the relationship between viability loss of Bt spores on maize leaves and their concentration, comparing it with field persistence. The experimental design was split-plot on time, composed by maize plants, in which three concentrations (half, normal and double doses) of a Dipel commercial formulation were applied. In each plot three leaves in the upper part of three plants were randomly selected. Samples of these leaves were collected 3 to 72 hours after treatment, to count the number of viable spores in two foliar dishes with 1 cm in diameter. The field persistence was determined using an exponential model, linearized by a logarithmic transformation of viable spores number in time. Using the log linear method of confidence intervals, there were no significant differences ( $P = 0.05$ ) in half-lives: 18.2 hours for half-dose, 16.5 hours for normal dose and 13.6 hours for double dose. Assuming a fictitious index of insect consumption equal to one, the effective doses according to concentrations were calculated. It was verified that 77%, 78% and 80.5% of the effective doses (viable spores) remained on the leaf surface after the first day of treatment, respectively. (Haddad et al., 2005)

**Head, G., J. B. Surber, J. A. Watson, J. W. Martin and J. J. Duan (2002).** "No detection of Cry1Ac protein in soil after multiple years of transgenic Bt cotton (Bollgard) use." Environmental Entomology **31(1)**: 30-36.<http://www.botanischergarten.ch/Bt/Head-Monitoring-Bt-v31n1p30.pdf>

Soil samples were collected from within and outside six fields where insect-resistant transgenic cotton (Bollgard) encoding the *Bacillus thuringiensis* Berliner (Bt) subsp. *kurstaki* cryIAc gene had been grown and subsequently incorporated into soil by postharvest tillage for 3-6 consecutive years. The level of CryIAc protein in these samples (collected 3 mo after the last season's tillage) was evaluated using both enzyme-linked immunosorbent assays (ELISA) and bioassays with a susceptible insect species, *Heliothis virescens* (F.), the tobacco budworm. Both methods revealed that no detectable CryIAc protein was present in any of the soil samples collected from within or outside the Bollgard fields. Based on the results from reference standards, the limit of detection for the ELISA was 3.68 ng of extractable protein per gram of soil, and that of the bioassay (measured by EC50) was 8 ng of biologically active protein per gram of soil. Together, these findings demonstrate that the amount of CryIAc protein accumulated as a result of continuous use of transgenic Bt cotton, and subsequent incorporation of plant residues into the soil by postharvest tillage, is extremely low and does not result in detectable biological activity. (Head et al., 2002)

**Heckmann, L. H., B. S. Griffiths, S. Caul, J. Thompson, M. Puzsai-Carey, W. J. Moar, M. N. Andersen and P. H. Krogh (2006).** "Consequences for *Protaphorura armata* (Collembola : Onychiuridae) following exposure to genetically modified *Bacillus thuringiensis* (Bt) maize and non-Bt maize." *Environmental Pollution* 142(2): 212-216. <Go to ISI>://000237772200003 AND <http://www.botanischergarten.ch/Bt/Heckmann-Consequences-Protaphorura-2006.pdf>

Studies on the effect of genetically modified *Bacillus thuringiensis* (Bt) crops on true soil dwelling non-target arthropods are scarce. The objective of this study was to assess the influence of a 4-week exposure to two Bt maize varieties (Cry1Ab) Cascade and MEB307 on the collembolan *Protaphorura armata*. For comparison three non-Bt maize varieties, Rivaldo (isogenic to Cascade), Monumental (isogenic to MEB307) and DK242, and two control diets based on baker's yeast (uncontaminated and contaminated with Bt toxin Cry I Ab) were also tested. Due to a lower C:N ratio, individuals reared on yeast performed significantly better in all of the measured endpoints than those reared on maize. *P. armata* performed equally well when reared on two Bt and three non-Bt maize varieties. Although there were no negative effects of Bt maize in this experiment, we recommend future studies on Bt crops to focus on species interactions in long-term, multi-species experiments. (Heckmann et al., 2006)

**Herman, R. A., P. N. Scherer and J. D. Wolt (2002).** "Rapid degradation of a binary, PS149B1, delta-endotoxin of *Bacillus thuringiensis* in soil, and a novel mathematical model for fitting curve-linear decay." *Environmental Entomology* 31(2): 208-214. <Go to ISI>://000178325900003 AND <http://www.botanischergarten.ch/Bt/Herman-Rapid-Degradation-Environ-2002.pdf>

A novel, binary delta-endotoxin from *Bacillus thuringiensis* Berliner (Bt) strain PS149B1 has been identified, and the two genes that code for the peptides that make up the binary insecticidal crystal protein (bICP) have been inserted into maize plants, *Zea mays* L. Transformed maize plants that express the proteins are resistant to western corn rootworm, *Diabrotica virgifera virgifera* LeConte, a major pest of maize. A laboratory study was conducted to better understand the degradation of the bICP in soil. Insect bioassays using southern corn rootworm, *Diabrotica undecimpunctata howardi* Barber, were used to track degradation. A first-order kinetic model using a truncated data set predicts a half-life of <4 d, indicating a rapid rate of decay in soil. The degradation pattern for the complete data set exhibits systematic departures from a first-order kinetic model. A novel 3-parameter degradation model was developed and validated with 23 additional degradation data sets representing both Bt proteins and synthetic organic molecules. This new model often fits degradation patterns better than a first-order model and a 3-parameter, biexponential (biphasic) model. The new model also retains an additional degree of freedom in the analyses compared with the biexponential model, making it especially useful when modeling small data sets. The time until 50% dissipation of the bICP was estimated at <2 d based on this new model. (Herman et al., 2002)

**Herman, R. A., J. D. Wolt and W. R. Halliday (2002).** "Rapid degradation of the Cry1F insecticidal crystal protein in soil." *Journal of Agricultural and Food Chemistry* 50(24): 7076-7078. <Go to ISI>://000179266000023 AND <http://www.botanischergarten.ch/Bt/Herman-Rapid-Degradation-Environ-2002.pdf>

The gene for the core Cry1F insecticidal crystal protein (ICP) from *Bacillus thuringiensis* Berliner (Bt) has been incorporated into the genome of maize plants, *Zea mays* L. Plants expressing this ICP are protected from attack by various Lepidopteran pests including the European corn borer, *Ostrinia nubilalis* (Hubner). The stability of the Cry1F ICP in soil was assessed in a laboratory study designed to determine the persistence of the active protein residue in soil over time, using insect bioassay as the analytical quantification method. The GI(50) (concentration estimated to inhibit growth by 50%) rose at each consecutive incubation interval, indicating a consistent decline in Cry1F activity over time. The residue data were poorly described by a first-order model when fit to either the full data or a truncated data set where the last interval (28 days) was excluded. Data were well described by a shift-log model, and this model predicted DT50 (time until 50% decay) and DT90 (time until 90% decay) values of 0.6 and 6.9 days, respectively. This rapid degradation rate was consistent with other Bt proteins evaluated in our laboratory. (Herman et al., 2002)

**Hopkins, D. W. and E. G. Gregorich (2003).** "Detection and decay of the Bt endotoxin in soil from a field trial with genetically modified maize." *European Journal of Soil Science* **54**(4): 793-800.<Go to ISI>://000186563400016 AND <http://www.botanischergarten.ch/Bt/Hopkins-Detection-Decay-Soil-2003.pdf>

Genetically modified plants and their residues may have direct effects on ecosystem processes. We aimed to determine the amount in soil of the insecticidal delta-endotoxin, originally from the bacterium *Bacillus thuringiensis*, introduced into soil by root exudates and residues from genetically modified maize, to compare the short-term rates of decay of Bt-maize and non-Bt-maize, and to determine the rate at which the toxin in Bt-maize leaves decomposes in soil. Intact soil, size fractions of soil, soluble fractions from soil and fractions of organic residues from a field where Bt-maize had been cultivated for 4 years were analysed for the Bt delta-endotoxin. Traces of the delta-endotoxin were detected in the whole (unfractionated) soil, the water-soluble fractions, and some of the particle-size fractions, but it was sufficiently concentrated only in the > 2000- $\mu$ m size fraction to be quantified. The delta-endotoxin concentrations in this fraction ranged between 0.4 and 4.4 ng toxin  $g^{-1}$  fraction, which equated to 70, 6 and 50 mg toxin  $m^{-2}$  in the 0-15, 15-30 and 30-60 cm depths, respectively (or 126 mg toxin  $m^{-2}$  over the 0-60 cm depth) in the field in June (early summer). The > 2000- $\mu$ m size fraction was a mixture of light- and dark-coloured organic material and mineral material comprising sand grains and stable aggregates. For samples collected early in the growing season, most of the detected delta-endotoxin was present in the light-coloured organic material, which was comprised of primarily live roots. However, recognizable maize residues, probably from previous years' crops, also contained delta-endotoxin. In a laboratory incubation study, Bt- and non-Bt-maize residues were added to soil and incubated for 43 days. There was no detectable difference in the decomposition of plant material from the two lines of maize, as determined by CO<sub>2</sub> production. The quantity of delta-endotoxin in the decomposing plant material and soil mixtures declined rapidly with time during the incubation, with none being detectable after 14 days. The rapid disappearance of the delta-endotoxin occurred at a rate similar to that of the water-soluble components of the maize residues. The results suggested that much of the delta-endotoxin in crop residues is highly labile and quickly decomposes in soil, but that a small fraction may be protected from decay in relatively recalcitrant residues. (Hopkins & Gregorich, 2003)

**Hopkins, D. W. and E. G. Gregorich (2005).** "Decomposition of residues and loss of the delta-endotoxin from transgenic (Bt) corn (*Zea mays* L.) in soil." *Canadian Journal of Soil Science* **85**(1): 19-26.<Go to ISI>://000228417200002 AND <http://www.botanischergarten.ch/Bt/Hopkins-Decomposition-Resitues-soil-2005.pdf>

Corn and other crops genetically modified to express the insecticidal delta-endotoxin from *Bacillus thuringiensis* (Bt) are grown widely across north America. Studies have shown that the delta-endotoxin can be stabilised on soil colloids where its activity is retained, but reports of direct ecological effects of the delta-endotoxin on soil processes are limited. We have determined the concentrations of the delta-endotoxin in organic residues from Bt-corn plants at increasing stages of ageing and decay, and the subsequent decomposition in soil of these residues and the delta-endotoxin in them. The delta-endotoxin concentrations declined from 6.8  $\mu g g^{-1}$  in the fresh plant material, to 0.82  $\mu g g^{-1}$  in the post-harvest residues collected in the fall, and to 0.026  $\mu g g^{-1}$  in the residues collected from soil surface the following spring. The concentration of delta-endotoxin in buried residues collected in the spring was not significantly different from zero. When incubated in soil in the laboratory over 84 d, the delta-endotoxin decomposed more rapidly than bulk plant C by factors of 1.85 for the fresh plant materials and 3.21 for the

post-harvest residues. Within 14 d of incubation, the 5-endotoxin concentration in the residues collected at the soil surface was below the limit of detection. We contrasted the laboratory decomposition data with data from a field experiment to estimate the period that the delta-endotoxin in corn residues may survive in the field. Based on estimates derived from this comparison, we predict that following an October harvest in eastern Ontario the delta-endotoxin would fall below the detection threshold during November for post-harvest residues. Since stabilisation of the 5-endotoxin on soil colloids depends on it surviving (i.e., not being decomposed) for long enough to be released from the plant residue matrix and come into proximity with colloid surfaces, the rapid decay of the delta-endotoxin suggests that only a small fraction of the delta-endotoxin from post-harvest residues persists long enough to become stabilised in the field. (Hopkins & Gregorich, 2005)

**Icoz, I. and G. Stotzky (2007).** "Cry3Bb1 protein from *Bacillus thuringiensis* in root exudates and biomass of transgenic corn does not persist in soil " *Transgenic Research Electronic Preprint September 2007*(--).10.1007/s11248-007-9133-8 AND <http://www.botanischergarten.ch/Bt/Icoz-Bt-notpersist-2007.pdf>

The Cry3Bb1 protein, insecticidal to the corn rootworm complex (*Diabrotica* spp.), of *Bacillus thuringiensis* (Bt) subsp. *kumamotoensis* was released in root exudates of transgenic Bt corn (event MON863) in sterile hydroponic culture ( $7.5 \pm 1.12$  ng/ml after 28 days of growth) and in nonsterile soil throughout growth of the plants ( $2.2 \pm 0.62$  ng/g after 63 days of growth). Kitchawan soil, which contains predominantly kaolinite (K) but not montmorillonite (M), was amended to 3 or 6% (vol./vol.) with K (3K and 6K soils) or M (3M and 6M soils) and with 1, 3, 5, or 10% (wt./wt.) of ground biomass of Bt corn expressing the Cry3Bb1 protein and incubated at  $25 \pm 2$  °C at the -33-kPa water tension for 60 days. Soils were analyzed for the presence of the protein every 7 to 10 days with a western blot assay (ImmunoStrip) and verified by ELISA. Persistence of the protein varied with the type and amount of clay mineral and the pH of the soils and increased as the concentration of K was increased but decreased as the concentration of M was increased. Persistence decreased when the pH of the K-amended soils was increased from ca. 5 to ca. 7 with CaCO<sub>3</sub>: the protein was not detected after 14 and 21 days in the pH-adjusted 3K and 6K soils, respectively, whereas it was detected after 40 days in the 3K and 6K soils not adjusted to pH 7. The protein was detected for only 21 days in the 3M soil and for 14 days in the 6M soil, which were not adjusted in pH. These results indicate that the Cry3Bb1 protein does not persist or accumulate in soil and is degraded rapidly. (Icoz & Stotzky, 2007)

**Icoz, I., D. Saxena, D. A. Andow, C. Zwahlen and G. Stotzky (2008).** "Microbial Populations and Enzyme Activities in Soil In Situ under Transgenic Corn Expressing Cry Proteins from *Bacillus thuringiensis*." *J Environ Qual* %R 10.2134/jeq2007.0352 37(2): 647-662. <http://jeq.scijournals.org/cgi/content/abstract/joenq;37/2/647> AND <http://www.botanischergarten.ch/Bt/Icoz-Microbial-Populations-2007.pdf>

Transgenic Bt crops produce insecticidal Cry proteins that are released to soil in plant residues, root exudates, and pollen and that may affect soil microorganisms. As a continuation of studies in the laboratory and a plant-growth room, a field study was conducted at the Rosemount Experiment Station of the University of Minnesota. Three Bt corn varieties that express the Cry1Ab protein, which is toxic to the European corn borer (*Ostrinia nubilalis* Hubner), and one Bt corn variety that expresses the Cry3Bb1 protein, which is toxic to the corn rootworm complex (*Diabrotica* spp.), and their near-isogenic non-Bt varieties were evaluated for their effects on microbial diversity by classical dilution plating and molecular (polymerase chain reaction-denaturing gradient gel electrophoresis) techniques and for the activities of some enzymes (arylsulfatases, acid and alkaline phosphatases, dehydrogenases, and proteases) involved in the degradation of plant biomass. After 4 consecutive years of corn cultivation (2003-2006), there were, in general, no consistent statistically significant differences in the numbers of different groups of microorganisms, the activities of the enzymes, and the pH between soils planted with Bt and non-Bt corn. Numbers and types of microorganisms and enzyme activities differed with season and with the varieties of corn, but these differences were not related to the presence of the Cry proteins in soil. The Cry1Ab protein of Bt corn (events Bt11 and MON810) was detected in most soils during the 4 yr, whereas the Cry3Bb1 protein was not detected in soils of Bt corn (event MON863) expressing the cry3Bb1 gene. (Icoz et al., 2008)

**Kreutzweiser, D. P., J. L. Gringorten, D. R. Thomas and J. T. Butcher (1996).**

"Functional effects of the bacterial insecticide *Bacillus thuringiensis* var *kurstaki* on aquatic microbial communities." *Ecotoxicology and Environmental Safety* **33**(3): 271-280. <Go to ISI>://A1996UK98100010 AND <http://www.botanischergarten.ch/Bt/Kreutzweiser-Functional-Effects-1996.pdf>

Epilithic microbial communities were colonized on leaf disks and exposed to commercial preparations of *Bacillus thuringiensis* var. *kurstaki* (Btk) in aquatic microcosms. Responses in terms of microbial respiration, bacterial cell density, protozoan density, and microbial decomposition activity were measured. Test concentrations for treatments with Dipel 64AF and Dipel 8AF in microcosms were the expected environmental concentration (EEC) of 20 IU/ml, 100X the EEC, and 1000X the EEC. Bacterial cell density in the biofilm of leaf disks was significantly increased at concentrations as low as the EEC. There were no concomitant alterations in protozoan density. Microbial respiration was significantly increased, and decomposition activity was significantly decreased, but only at the artificially high concentration of 1000X the EEC. This effect was attributed to the spore-crystal component rather than formulation ingredients. Microbial decomposition of leaf material was also determined in outdoor stream channels treated at concentrations ranging from the EEC to 100X the EEC. Although there tended to be reduced decomposition activity in treated channels, there were no significant differences in mass loss of leaf material between treated and control channels. Various regression, classification, and ordination procedures were applied to the experimental data, and none indicated significant treatment effects. These results from laboratory and controlled field experiments indicate that contamination of watercourses with Btk is unlikely to result in significant adverse effects on microbial community function in terms of detrital decomposition. (C) 1996 Academic Press, Inc. (Kreutzweiser et al., 1996)

**Krogh, P. H. and B. Griffiths (2007).** "ECOGEN-Soil ecological and economic evaluation of genetically modified crops, SPECIAL ISSUE SOIL ECOLOGICAL AND ECONOMIC EVALUATION OF GENETICALLY MODIFIED CROPS - ECOGEN." *Pedobiologia* **51**(3): 171-173. <http://www.sciencedirect.com/science/article/B7CW5-4NTHKYM-2/2/e0f4e52a6128e3b2d70f15a8bf816276> AND <http://www.botanischergarten.ch/Bt/Krogh-ECOGEN-intro-2007.pdf>

The biodiversity of, and processes performed by soil organisms make up a crucial part of the natural basis for agricultural production and, therefore, have subsequent economic consequences. ECOGEN was a research initiative funded under the European Commission Framework 5 programme, designed to integrate the combined soil ecological and economic effects of introducing systems including genetically modified (GM) crops by performing data mining and building decision support systems. The project involved eight academic partners from five EU countries and an input from Monsanto. Maize expressing an insecticidal protein from *Bacillus thuringiensis* (Bt-maize) was chosen as the model GM crop due to its availability, while studies using GM herbicide tolerant (HT) maize were initiated in the latter stages of the project. Single species tests under laboratory conditions (not presented in this issue) showed conclusively that Bt toxin has no deleterious effects on protozoa, nematodes, earthworms and collembolans. The agrochemicals characteristics of the cropping systems studied were similarly non-toxic at field concentrations. More detailed measurements in mesocosms revealed that the slight effects of Bt-maize or a conventional insecticide on nematodes, protozoa and microorganisms were less pronounced than effects due to soil and plant growth stage (Griffiths et al., 2006), and less than the variation seen between eight maize cultivars (Griffiths et al., 2007a). No effects could be attributed to the Bt-maize on snails, microarthropods or mycorrhizal fungi in a separate mesocosm experiment, but the detection of Bt protein in snail faeces was identified as a novel route into the soil food web (de Vauffleury, 2007). Field experiments were established at four sites across three European climatic zones and showed the effectiveness of Bt-maize against the European Corn Borer, where this pest was present (Andersen et al., 2007). These field experiments point to the conclusion that Bt-maize (Mon 810 event) could have a significant, but small and transient, negative effect on soil protozoa, nematodes and microorganisms (Griffiths et al., 2005; 2007b) but no effects on organic matter (wheat straw) decomposition (Cortet et al., 2005). Generally Bt-maize did not affect earthworms (Krogh et al., 2007) and microarthropods (Cortet et

al., 2007), while a data mining approach revealed the interplay between factors affecting earthworms and microarthropods (Debeljak et al., 2007). The fact that we conducted experiments using the same organisms and soils across a range of scales (i.e. laboratory, glasshouse and field) allowed for a comparison of results from these scales and an assessment of their utility. While it was not possible to predict the outcome between scales there was useful information and insights to be had from each of the experimental approaches (Birch et al., 2007). The complexity of soil organisms and their functioning was collectively summarised in soil quality attributes and a multi-attribute model, and used in assessment of new agricultural technologies including GM crops. We developed a quantitative, multi-attribute, model to summarise the effects of the different cropping systems on soil quality (Bohanec et al., 2007), which has considerable potential for application for other aspects of soil management. Preliminary results of our economic research indicate that, while irreversible benefits from Bt-maize are very small (less than one Euro per hectare planted), the benefits foregone from non-adoption are several million Euros per year for the EU-15 (Wesseler et al., 2007). The concept and approach of ECOGEN was to combine and integrate the ecological and economic information to give an overall outcome, as summarised in Fig. 1.

In conclusion, Bt-maize did not have deleterious effects on the soil biota. When effects were observed these were likely to be caused by differences between the maize varieties. Bt-maize in the agricultural systems studied did not decrease soil quality due to the GM crop itself, but changes in the agricultural techniques used along with the GM crop could improve (reduced tillage) or reduce (increased use of pesticides) the soil quality. The EU-15 forgo several million Euros of net social benefits per year by postponing the introduction of Bt-maize, although this can be justified, if decision makers assume that the willingness-to-pay by household for not having those crops being introduced is about one Euro on average per year. (Krogh & Griffiths, 2007)

**Krogh, P. H., B. Griffiths, D. Demsar, M. Bohanec, M. Debeljak, M. N. Andersen, C. Sausse, A. N. E. Birch, S. Caul, M. Holmstrup and L. H. Heckmann (2007).** "Responses by earthworms to reduced tillage in herbicide tolerant maize and Bt maize cropping systems SPECIAL ISSUE SOIL ECOLOGICAL AND ECONOMIC EVALUATION OF GENETICALLY MODIFIED CROPS - ECOGEN." *Pedobiologia* 51(3): 219-227. <http://www.sciencedirect.com/science/article/B7CW5-4NNPB70-4/2/25971e5aa6055a8cc566f7621076b1eb> AND <http://www.botanischergarten.ch/Bt/Krogh-Earthworms-ECOGEN-2007.pdf>

The population dynamics of soil organisms under agricultural field conditions are influenced by many factors, such as pedology and climate, but also farming practices such as crop type, tillage and the use of pesticides. To assess the real effects of farming practices on soil organisms it is necessary to rank the influence of all of these parameters. Bt maize (*Zea mays* L.), as a crop recently introduced into farming practices, is a genetically modified maize with the Cry1Ab gene which produces a protein toxic to specific lepidopteran insect pests. To assess the effects of Bt maize on non-target soil organisms, we conducted research at a field site in Foulum (Denmark) with a loamy sand soil containing 6.4% organic matter. The study focused on populations of springtails (*Collembola*) and earthworms (*Oligochaeta*) from samples taken at the beginning and at the end of the maize crop-growing season during 2 consecutive years. Farming practices, soil parameters, the biological structure of soil communities, and the type and age of the crop at the time of sampling, were used as attributes to predict the total abundance of springtails and biomass of earthworms in general and the abundance or biomass for specific functional groups (epigeic, endogeic and anecic groups for earthworms, and eu-, eu to hemi-, hemi-, hemi to epi- and epiedaphic groups for *Collembola*). Predictive models were built with data mining tools, such as regression trees that predict the value of a dependent variable from a set of independent variables. Regression trees were constructed with the data mining system M50. The models were evaluated by qualitative and quantitative measures of performance and two models were selected for further interpretation: anecic worms and hemi-epiedaphic *Collembola*. The anecic worms ( $r^2 \frac{1}{4} 0.83$ ) showed preferences for less clay and more silt soil with medium pH but were not influenced directly by farming practices. The biomass of earthworms was greater in early autumn than in spring or late autumn. Biomass of hemi-epiedaphic *Collembola* ( $r^2 \frac{1}{4} 0.59$ ) increased at the end of the maize growing season, while higher organic matter content and pH tended to increase their biomass in spring. Greater abundance of *Collembola* was also noted in early autumn if the crop was non-Bt maize. The models assessed by this research did not find any effects of the Bt maize cropping system on functional groups of soil fauna. (Krogh et al., 2007)

**Meek, B. D., D. L. Carter, D. T. Westermann and R. E. Peckenpaugh (1994).** "Root-Zone Mineral Nitrogen Changes as Affected by Crop Sequence and Tillage." Soil Science Society of America Journal **58**(5): 1464-1469.<Go to ISI>://A1994PH24600027 AND NEBIS 20080302

Crop sequence and tillage affect soil mineral N (NH<sub>4</sub> plus NO<sub>3</sub>) and NO<sub>3</sub> leaching below the root zone following alfalfa (*Medicago sativa* L.). A 2-yr field experiment was conducted in south-central Idaho to determine the effect on soil NO<sub>3</sub> levels of a corn (*Zea mays* L.)wheat (*Triticum aestivum* L.) rotation compared with a bean (*Phaseolus vulgaris* L.)-bean rotation and to demonstrate improved N utilization with a corn-wheat rotation. Alfalfa, growing on an irrigated Portneuf silt loam (coarse-silty, mixed, mesic Durixerollic Calciorthid), was killed in October 1989 with herbicide. Treatments were: (i) BT-BT: conventional tilled bean grown in 1990 and 1991; (ii) CNT-WNT: no-till silage corn grown in 1990, and no-till winter wheat grown in 1990-1991; and (iii) CT-WT: same as CNT-WNT but under conventional tillage. Similar amounts of soil N were mineralized the first (275 kg N ha(-1)) and second (213 kg N ha(-1)) year after killing the alfalfa in all treatments. The BT-BT treatment had the highest growing-season soil mineral N (up to 251 kg ha(-1), 0-0.45-m depth) because the N uptake by bean was lower (187 kK N ha(-1)) than corn (252 kg N ha(-1), average of CT-WT and CNT-WNT treatments) in 1990 and later than winter wheat uptake in 1991. Most wheat N uptake had occurred by late June when bean uptake was just starting. A rotation that follows alfalfa with corn or a crop with a similar N uptake pattern, instead of bean, will save N fertilizer, lower soil NO<sub>3</sub> levels, and reduce NO<sub>3</sub> leaching potential. (Meek et al., 1994)

**Motavalli, P. P., R. J. Kremer, M. Fang and N. E. Means (2004).** "Impact of genetically modified crops and their management on soil microbially mediated plant nutrient transformations." Journal of Environmental Quality **33**(3): 816-824.<Go to ISI>://000221509200003 AND <http://www.botanischergarten.ch/Motavalli-Impact-GM-Soil-2004.pdf>

One of the potential environmental effects of the recent rapid increase in the global agricultural area cultivated with transgenic crops is a change in soil microbially mediated processes and functions. Among the many essential functions of soil biota are soil organic matter decomposition, nutrient mineralization and immobilization, oxidation-reduction reactions, biological N fixation, and solubilization. However, relatively little research has examined the direct and indirect effects of transgenic crops and their management on microbially mediated nutrient transformations in soils. The objectives of this paper are to review the available literature related to the environmental effects of transgenic crops and their management on soil microbially mediated nutrient transformations, and to consider soil properties and climatic factors that may affect the impact of transgenic crops on these processes. Targeted genetic traits for improved plant nutrition include greater plant tolerance to low Fe availability in alkaline soils, enhanced acquisition of soil inorganic and organic P, and increased assimilation of soil N. Among the potential direct effects of transgenic crops and their management are changes in soil microbial activity due to differences in the amount and composition of root exudates, changes in microbial functions resulting from gene transfer from the transgenic crop, and alteration in microbial populations because of the effects of management practices for transgenic crops, such as pesticide applications, tillage, and application of inorganic and organic fertilizer sources. Possible indirect effects of transgenic crops, including changes in the fate of transgenic crop residues and alterations in land use and rates of soil erosion, deserve further study. Despite widespread public concern, no conclusive evidence has yet been presented that currently released transgenic crops, including both herbicide and pest resistant crops, are causing significant direct effects on stimulating or suppressing soil nutrient transformations in field environments. Further consideration of the effects of a wide range of soil properties, including the amount of clay and its mineralogy, pH, soil structure, and soil organic matter, and variations in climatic conditions, under which transgenic crops may be grown, is needed in evaluating the impact of transgenic crops on soil nutrient transformations. Future environmental evaluation of the impact of the diverse transgenic crops under development could lead to an improved understanding of soil biological functions and processes. (Motavalli et al., 2004)

**Muchaonyerwa, P., S. Waladde, P. Nyamugafata, S. Mpeperekwi and G. G. Ristori (2004).** "Persistence and impact on microorganisms of *Bacillus thuringiensis* proteins in some Zimbabwean soils." Plant and

Soil **266**(1-2): 41-46.<Go to ISI>://000226385500005 AND <http://www.botanischergarten.ch/Bt/Muchaonyerwa-Persistence-Zimbabwe-2004.pdf>

The persistence of the *Bacillus thuringiensis* subsp. *kurstaki* (Btk) toxin (Cry1Ab protein) from Bt maize (MON810, Yieldgard(R)) residues incorporated in a vertisol (739 g clay kg<sup>-1</sup>) was investigated. The maize residues were incubated in the soil for 4 weeks, and activity of the toxin in the residues was bioassayed using larvae of the diamondback moth, *Plutella xylostella* (Lepidoptera: Yponomeutidae). Corrected mortality of *P. xylostella* in the bioassays decreased from 76% to 30% in less than a week of incubation in the soil. In addition to the above observations, the effects of Btk, Bt subsp. *israelensis* (Bti), and Bt subsp. *tenebrionis* (Btt) proteins on the soil microbiota were examined using a vertisol, an alfisol, and an oxisol. The pre-incubated soils (7 days after moisture adjustment) were treated with crystal proteins of Btk, Bti, and Btt and incubated for further a 7-day period. Microbial biomass carbon (MBC) and counts of culturable bacteria and fungi were determined. The proteins did not show effects on MBC or bacterial and fungal counts, possibly as a result of adsorption of the proteins on soil particles, which could have rendered the proteins inaccessible for microbial utilization. Microbial biomass carbon and counts arranged in decreasing order were vertisol>oxisol>alfisol, similar to the amounts of organic C and clay in the soils. However, bacteria and fungi counts were higher in the vertisol than in the alfisol and the oxisol soils. Our observations suggest that larvicidal proteins produced by different subspecies of Bt and Bt maize could persist in tropical soils as a result of adsorption on soil clays but that there were no observable effect on the soil microbiota. (Muchaonyerwa et al., 2004)

**Muchaonyerwa, P. and S. M. Waladde (2007).** "Persistence of the pesticidal *Bacillus thuringiensis* protein expressed in Bt maize plant materials in two soils of the Central Eastern Cape, South Africa." South African Journal of Plant and Soil **24**(1): 26-31.<Go to ISI>://BIOSIS:PREV200700499556 <http://www.botanischergarten.ch/Bt/Muchaonyerwa-Persistence-Bt-2007.pdf>

Environmental effects of genetically modified plants are not yet fully understood. Experiments were conducted to determine relative amounts of the bioactive Bt protein in roots, leaves and stems of Bt maize and persistence of the protein in two soil forms (Shortlands and Oakleaf). The Bt protein activity was bioassayed using 2<sup>nd</sup> to 3<sup>rd</sup> instar larvae of diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). Bioassay results showed that the extracts from different parts of Bt maize plants were equally toxic and caused overall larval motility of over 70%. It was also found that two years' storage of dried Bt maize material at room temperature, did not reduce the pesticidal activity of the protein toxin. When Bt maize plant materials were incubated in soils for two weeks under glasshouse conditions, the extracted toxin caused 20% larval mortality and within 7 weeks of incubation in both soils, the extracts obtained caused < 10% larval mortality. However, extracts obtained from Bt maize plant materials incubated in the field showed decreased larval mortality, from 60% to 30% in two weeks and remained > 25% after 12 weeks of incubation in the Shortlands soil and from 55% to 15% within four weeks and eventually down to 10% in 23 weeks of incubation in the Oakleaf soil. The findings suggest that Bt maize plant parts contribute comparable amounts of Bt protein toxin to the soil, and toxin persistence in the soil appears to depend on soil type, and temperature and moisture conditions. (Muchaonyerwa & Waladde, 2007)

**Mulder, C., D. De Zwart, H. J. Van Wijnen, A. J. Schouten and A. M. Breure (2003).** "Observational and simulated evidence of ecological shifts within the soil nematode community of agroecosystems under conventional and organic farming." Functional Ecology **17**(4): 516-525.<Go to ISI>://000184573900012 AND <http://www.botanischergarten.ch/Nematodes/Mulder-Nematodes-Comparison-2003.pdf>

1. Soil sustainability implies a sufficient diversity and abundance of organisms to perform soil functions and to resist environmental stress. Previous studies have shown the importance of functional biodiversity for soil organisms. 2. Soil samples have been collected within the framework of a long-term monitoring programme in the Netherlands. Nematological and microbiological techniques were combined to facilitate a more comprehensive understanding of possible below-ground effects of land management. 3. A possible bias due to stochastic circumstances was investigated. The Mantel test showed that the diversity at species level is largely related to air temperature, but at genus level the effect of temperature disappears. No direct influence of rainfall on the soil biodiversity was found in our model. 4. To extrapolate our data to a national level, habitat-response relationships for soil organisms have been derived. Generalized linear models (GLMs) and Monte Carlo simulation allowed the estimation of the probability of

occurrence at a given abundance for 95 nematode genera. 5. Our study describes the influence of abiotic conditions and land use intensity on the composition of nematode communities in grasslands on sand. The results obtained reveal a major influence of pH and livestock density on the diversity of the nematode community at both taxonomic levels as well as at different trophic levels (feeding habits). The presence and abundance of soil nematodes decrease with cattle pressure. 6. Functional diversity decreases with increasing management intensity. It is shown that the Shannon diversities of bacterial feeding nematodes and fungal feeding nematodes are strictly related to cattle pressure, whereas the bacterial biomass occurring under organic farming scores higher than in other farming systems. (Mulder et al., 2003)

**Mulder, C., M. Wouterse, M. Raubuch, W. Roelofs and M. Rutgers (2006).** "Can transgenic maize affect soil microbial communities?" *Plos Computational Biology* 2(9): 1165-1172. <Go to ISI>://000240867500017 AND <http://www.botanischergarten.ch/Bt/Mulder-Bt-Straw-Soil-2006.pdf>

The aim of the experiment was to determine if temporal variations of belowground activity reflect the influence of the Cry1Ab protein from transgenic maize on soil bacteria and, hence, on a regulatory change of the microbial community (ability to metabolize sources belonging to different chemical guilds) and/or a change in numerical abundance of their cells. Litter placement is known for its strong influence on the soil decomposer communities. The effects of the addition of crop residues on respiration and catabolic activities of the bacterial community were examined in microcosm experiments. Four cultivars of *Zea mays* L. of two different isolines (each one including the conventional crop and its *Bacillus thuringiensis* cultivar) and one control of bulk soil were included in the experimental design. The growth models suggest a dichotomy between soils amended with either conventional or transgenic maize residues. The Cry1Ab protein appeared to influence the composition of the microbial community. The highly enhanced soil respiration observed during the first 72 h after the addition of Bt-maize residues can be interpreted as being related to the presence of the transgenic crop residues. This result was confirmed by agar plate counting, as the averages of the colony-forming units of soils in conventional treatments were about one-third of those treated with transgenic straw. Furthermore, the addition of Bt-maize appeared to induce increased microbial consumption of carbohydrates in BIOLOG EcoPlates. Three weeks after the addition of maize residues to the soils, no differences between the consumption rate of specific chemical guilds by bacteria in soils amended with transgenic maize and bacteria in soils amended with conventional maize were detectable. Reaped crop residues, comparable to post-harvest maize straw (a common practice in current agriculture), rapidly influence the soil bacterial cells at a functional level. Overall, these data support the existence of short Bt-induced ecological shifts in the microbial communities of croplands' soils. (Mulder et al., 2006)

**Mulder, C., M. Wouterse, M. Rutgers and L. Posthuma (2007).** "Transgenic Maize Containing the Cry1Ab Protein Ephemeraly Enhances Soil Microbial Communities." *Ambio* 36(4): 363-365. <http://www.botanischergarten.ch/Bt/Mulder-Bt-Residue-2007.pdf>

*Bacillus thuringiensis* is a Gram<sup>+</sup> sporeforming bacterium that produces parasporal crystals during sporulation that are pathogenic to insect and some other organisms (1). Preparations of bacterial spores and crystalline proteins are widely used as Bt-insecticides for the control of insect pests of crops. Bt-toxins are classified based on their specific activity against invertebrates, with proteins Cry1 and Cry2 being lethal to Lepidoptera, Cry3 to Coleoptera, Cry 2 and Cry4 to Diptera, and Cry5 to Nematoda (2, 3). Cry1Ab is a toxin commonly used against the European and Asian corn borer complex, particularly the species *Ostrinia nubilalis* and *O. furnacalis* (4). Besides reports of facultative phytophagous insects that seem to have acquired resistance to larvicidal toxins belonging to the Cry1 protein family, nontarget effects of the toxin produced by the insecticidal cry1Ab gene released in the root exudates of *B. thuringiensis* maize (*Zea mays* L.) are unclear. Microbial communities occurring below ground under genetically engineered Bt-crops are less investigated, and possible effects on microbes remain a concern. Toxins may accumulate in soil after postharvest maize straw is plowed under (a common practice in agriculture), and high concentrations of the Cry1Ab toxin seem to persist for several months (5). In this paper, the following questions will be addressed:

– Is there microbial evidence of environmental disturbance in relation to plowed leaves and straw of Bt-maize in soils?

– Can a better ecological insight in the microbial community be obtained using metabolic fingerprints of soil bacteria? (Mulder et al., 2007)

**Naef, A. and G. Defago (2006).** "Population structure of plant-pathogenic *Fusarium* species in overwintered stalk residues from Bt-transformed and non-transformed maize crops." European Journal of Plant Pathology **116**(2): 129-143.<Go to ISI>://WOS:000240396600006 AND <http://www.botanischergarten.ch/Bt/Naef-Population-Structure-2006.pdf>

Bt-transformed maize contains genes from *Bacillus thuringiensis* encoding for insecticidal crystal proteins. Less insect damage on Bt maize stalks can cause a reduced infection by *Fusarium* species through plant injuries. This could affect the presence of plant-pathogenic *Fusarium* species on maize residues which serve as an inoculum source for subsequent crops. We collected overwintered maize stalks of four different Bt maize hybrids and their corresponding non-Bt lines in two consecutive years in a field trial in Germany. *Fusarium* spp. were isolated from 67% of 648 collected maize stalks. Identification with new multiplex PCR assays showed that *F. graminearum*, *F. avenaceum*, and *F. proliferatum* were the most abundant *Fusarium* species, isolated from 42%, 26%, and 15% of the stalks, respectively. Species abundances varied between varieties and collection years. No consistent difference was found between Bt and non-Bt stalks. *Fusarium graminearum* isolates were subject to a population genetic structure analysis with eight newly developed microsatellites. Significant association of loci and overrepresentation of repeated multilocus haplotypes indicated a substantial asexual component of reproduction, supporting selection of haplotypes. The data suggested selection of particular *F. graminearum* haplotypes by collection years but not by maize Bt transformation. Haplotypic changes between years caused no divergence in the distribution of alleles, suggesting that gene flow beyond the field scale prevented substructuring. We present evidence for gene flow between our saprophytic *F. graminearum* population on maize residues and a wheat-pathogenic population from a field 100 km distant. (Naef & Defago, 2006)

**Naef, A., T. Zesiger and G. Defago (2006).** "Impact of transgenic Bt maize residues on the mycotoxigenic plant pathogen *Fusarium graminearum* and the biocontrol agent *Trichoderma atroviride*." Journal of Environmental Quality **35**(4): 1001-1009.<Go to ISI>://000239189900005 AND <http://jeq.scijournals.org/cgi/content/abstract/joenq;35/4/1001> AND <http://www.botanischergarten.ch/Bt/Naef-Impact-Transgenic-Residues-2006.pdf>

Transformation of maize with genes encoding for insecticidal crystal (Cry) proteins from *Bacillus thuringiensis* (Bt) could have an impact on the saprophytic survival of plant pathogens and their antagonists on crop residues. We assessed potential effects on the mycotoxin deoxynivalenol (DON)-producing wheat and maize pathogen *Fusarium graminearum* and on the biocontrol agent *Trichoderma atroviride*. Purified Cry1Ab protein caused no growth inhibition of these fungi on agar plates. Cry1Ab concentrations above levels common in Bt maize tissue stimulated the growth of *F. graminearum*. The fungi were also grown on gamma-radiation-sterilized leaf tissue of four Bt maize hybrids and their non-transgenic isolines collected at maize maturity on a field trial in 2002 and 2003. Both fungi degraded the Cry1Ab protein in Bt maize tissue. Fungal biomass quantification with microsatellite-based polymerase chain reaction (PCR) assays revealed differential fungal growth on leaf tissue of different maize varieties but no consistent difference between corresponding Bt and non-Bt hybrids. Generally, year of maize tissue collection had a greater impact on biomass production than cultivar or Bt transformation. The mycotoxin DON levels observed in maize tissue experiments corresponded with patterns in *F. graminearum* biomass, indicating that Bt transformation has no impact on DON production. In addition to bioassays, maize leaf tissue was analyzed with a mass spectrometer-based electronic nose, generating fingerprints of volatile organic compounds. Chemical fingerprints of corresponding Bt and non-Bt leaf tissues differed only for those hybrid pairs that caused differential fungal biomass production in the bioassays. Our results suggest that Cry1Ab protein in maize residues has no direct effect on *F. graminearum* and *T. atroviride* but some corresponding Bt/non-Bt maize hybrids differ more in composition than Cry protein content alone, which can affect the saprophytic growth of fungi on crop residues. (Naef et al., 2006)

**Nielsen, K. M., A. M. Bones, K. Smalla and J. D. van Elsas (1998).** "Horizontal gene transfer from transgenic plants to terrestrial bacteria - a rare event?" Fems Microbiology Reviews **22**(2): 79-103

Today, 12 years after the first field release of a genetically modified plant (GMP), over 15 000 field trials at different locations have been performed. As new and unique characteristics are frequently

introduced into GMPs, risk assessment has to be performed to assess their ecological impact. The possibilities of horizontal gene transfer (HGT; no parent-to-offspring transfer of genes) from plants to microorganisms are frequently evaluated in such risk assessments of GMPs before release into the field. In this review we indicate why putative HGT from plants to terrestrial (soil and plant associated) bacteria has raised concern in biosafety evaluations. Further, we discuss possible pathways of I-IGT from plants to bacteria, outline the barriers to HGT in bacteria, describe the strategies used to investigate HGT from plants to bacteria and summarize the results obtained. Only a few cases of HGT from eukaryotes such as plants to bacteria have been reported to date. These cases have been ascertained after comparison of DNA sequences between plants and bacteria. Although experimental approaches in both field and laboratory studies have not been able to confirm the occurrence of such HGT to naturally occurring bacteria, recently two studies have shown transfer of marker genes from plants to bacteria based on homologous recombination. The few examples of HGT indicated by DNA sequence comparisons suggest that the frequencies of evolutionarily successful HGT from plants to bacteria may be extremely low. However, this inference is based on a small number of experimental studies and indications found in the literature. Transfer frequencies should not be confounded with the likelihood of environmental implications, since the frequency of HGT is probably only marginally important compared with the selective force acting on the outcome. Attention should therefore be focused on enhancing the understanding of selection processes in natural environments. Only an accurate understanding of these selective events will allow the prediction of possible consequences of novel genes following their introduction into open environments, (Nielsen et al., 1998)

**Oliveira, A. R., T. R. Castro, D. M. F. Capalbo and I. Delalibera (2007).** "Toxicological evaluation of genetically modified cotton (Bollgard((R))) and Dipel (R) WP on the non-target soil mite *Scheloribates praeincisus* (Acari : Oribatida)." *Experimental and Applied Acarology* **41**(3): 191-201.<Go to ISI>://000245132500004 AND <http://www.botanischergarten.ch/Bt/Oliveira-Bollgard-Dipel-2007.pdf>

Insecticides derived from the bacterium *Bacillus thuringiensis* (Bt) and plants genetically modified (GM) to express *B. thuringiensis* toxins are important alternatives for insect pest control worldwide. Risk assessment of *B. thuringiensis* toxins to non-target organisms has been extensively studied but few toxicological tests have considered soil invertebrates. Oribatid mites are one of the most diverse and abundant arthropod groups in the upper layers of soil and litter in natural and agricultural systems. These mites are exposed to the toxic compounds of GM crops or pesticides mainly when they feed on vegetal products incorporated in the soil. Although some effects of *B. thuringiensis* products on Acari have been reported, effects on oribatid mites are still unknown. This study investigated the effects of the ingestion of Bt cotton Bollgard (R) and of the *B. thuringiensis* commercial product Dipel (R) WP on the pantropical species *Scheloribates praeincisus* (Scheloribatidae). Ingestion of Bollgard and Dipel did not affect adult and immature survivorship and food consumption (estimated by number of fecal pellets produced daily) or developmental time of immature stages of *S. praeincisus*. These results indicate the safety of Bollgard and Dipel to *S. praeincisus* under field conditions where exposition is lower and other food sources besides leaves of Bt plants are available. The method for toxicological tests described here can be adapted to other species of Oribatida, consisting on a new option to risk assessment studies. (Oliveira et al., 2007)

**Pagel-Wieder, S., F. Gessler, J. Niemeyer and D. Schroder (2004).** "Adsorption of the *Bacillus thuringiensis* toxin (Cry1 Ab) on Na-montmorillonite and on the clay fractions of different soils." *Journal of Plant Nutrition and Soil Science-Zeitschrift Fur Pflanzenernahrung Und Bodenkunde* **167**(2): 184-188.<Go to ISI>://000221004400008 AND <http://www.botanischergarten.ch/Bt/Pagel-Wieder-Adsorption-2004.pdf>

The adsorption of the toxin from *Bacillus thuringiensis* (Bt-toxin), which is synthesized in genetically modified maize, on sterilized Na-montmorillonite and on H<sub>2</sub>O<sub>2</sub>-treated and untreated clay fractions of three soils from different sites were studied. All adsorption isotherms can be described by a linear isotherm. Although all clay fractions from the different soils show nearly the same mineralogical composition, we found different affinities ranging from  $k = 47.7$  to  $k = 366.7$  of the adsorbates for the Bt-toxin. The H<sub>2</sub>O<sub>2</sub>-treated clay fractions show no correlation between the adsorption affinity and the amount of soil organic matter. On the other hand, there is a correlation between the content of organic carbon and the adsorption affinity of the untreated clay fractions. This can be explained by the fact that due to the coatings of soil organic matter on aggregates, the Bt-toxin polymers are not able to adsorb within the clay aggregates.

**Pagel-Wieder, S., J. Niemeyer, W. R. Fischer and F. Gessler (2007).** "Effects of physical and chemical properties of soils on adsorption of the insecticidal protein (Cry1Ab) from *Bacillus thuringiensis* at Cry1Ab protein concentrations relevant for experimental field sites." *Soil Biology & Biochemistry* **39**: 3034-3042.<Go to ISI>://WOS:000250181500006 AND <http://www.botanischergarten.ch/Bt/Pagel-Wieder-physical-chemical-2007.pdf>

The adsorption of the insecticidal Cry1Ab protein of *Bacillus thuringiensis* (Bt) on Na-montmorillonite (M-Na) and soil clay fractions was studied. The aim of this study was not to find the adsorption capacity of the soils from the experimental field site, where Bt corn (MON810) was cultivated, but rather to characterize the adsorption behavior of the Cry1Ab protein at concentrations typically found at experimental field sites. In kinetic experiments, the Cry1Ab protein adsorbed rapidly (< 60 min) on M-Na. As the concentration of M-Na was varied and the added Cry1Ab protein concentration was kept constant (20 and 45 ng ml<sup>-1</sup>), the adsorption per unit weight of Cry1Ab protein decreased with increasing concentrations of M-Na. Adsorption of Cry1Ab protein on M-Na decreased as the pH value of the suspension increased. All adsorption isotherms could be described mathematically by a linear regression with the parameter *k*, the distribution coefficient, being the slope of the regression line. Although their mineralogical composition was nearly identical, the soil clay fractions showed different *k* values. The different *k* values were correlated with the physical and chemical properties of the soil clay fractions, such as the organic carbon content, the specific external surface area, and the electrokinetic charge of the external surfaces of the clays, as well as with the external surface charge density. An increase in the amount of soil organic matter, as well as an increase in the electrokinetic external surface charge of the soil clays, decreased the distribution coefficient *k*. An increase of the specific external surface areas of the soil clays resulted in a higher distribution coefficient *k*. Less than 10% of adsorbed Cry1Ab protein was reversibly adsorbed on the soil clays and, thus, desorbed. The desorption efficiency of distilled water was higher than that of a solution of CaCl<sub>2</sub> (2.25 mmol) and of dissolved organic carbon (50 mg Cl<sup>-1</sup>). (c) 2007 Elsevier Ltd. All rights reserved.

**Rauschen, S. and I. Schuphan (2006).** "Fate of the Cry1Ab protein from Bt-maize MON810 silage in biogas production facilities." *Journal of Agricultural and Food Chemistry* **54**(3): 879-883.<Go to ISI>://000235249800043 AND <http://www.botanischergarten.ch/Bt/Rauschen-Fate-Cry1Ab-Silage-2006.pdf>

Biogas plants fuelled with renewable sources of energy are a sustainable means for power generation. In areas with high infestation levels with the European corn borer, *Ostrinia nubilalis* (Hbn.), it is likely that transgenic Bt-maize will be fed into agricultural biogas plants. The fate of the entomotoxic protein Cry1Ab from MON810 maize was therefore investigated in silage and biogas production-related materials in the utilization chains of two farm-scale biogas plants. The Cry1Ab content in silage exhibited no clear-cut pattern of decrease over the experimental time of 4 months. Mean content for silage was 1878 +/- 713 ng Cry1Ab g<sup>-1</sup>. After fermentation in the biogas plants, the Cry1Ab content declined to trace amounts of around 3.5 ng g<sup>-1</sup> in the effluents. The limit of detection of the employed ELISA test corresponded to 0.75 ng Cry1Ab g<sup>-1</sup> sample material. Assays with larvae of *O. nubilalis* showed no bioactivity of the reactor effluents. The utilization of this residual material as fertilizer in agriculture is therefore deemed to be ecotoxicologically harmless. (Rauschen & Schuphan, 2006)

**Rossi, F., M. Moschini, L. Fiorentini, F. Masoero and G. Piva (2003).** "Analytical composition and rumen degradability of isogenic and transgenic corn varieties." *Journal of the Science of Food and Agriculture* **83**(13): 1337-1341.<Go to ISI>://000185655500010 AND <http://www.botanischergarten.ch/Bt/Rossi-Effect-Broilers-2005.pdf>

Two different corn cultivars were compared with their genetically modified counterparts containing the gene coding for the Cry1A(b) protein of *Bacillus thuringiensis* (Bt). There were no analytical differences between the conventional and transgenic genotype kernels, whereas stovers from Bt(+) plants had higher sugar (148.3 g kg<sup>-1</sup> versus 115.9 g kg<sup>-1</sup>); *P* < 0.01) and lower NDF (592.7 g kg<sup>-1</sup> versus 631.5 g kg<sup>-1</sup>); *P* < 0.05) contents than Bt(-) maize. A comparison of the amino acid profiles showed higher phenylalanine content in kernels from the Bt(+) plants (49.1 g kg<sup>-1</sup> vs 47.8 g kg<sup>-1</sup>); *P* < 0.05) which was, however, not reflected in the protein content. The initial dry matter rumen degradability of the isogenic

kernels was higher than that of Bt(+) varieties (569.5 g kg<sup>-1</sup>) vs 543.7 g kg<sup>-1</sup>); P &LT; 0.05), whereas the lower fibre content increased the dry matter (548.6 g kg<sup>-1</sup>) vs 526.6 g kg<sup>-1</sup>); P &LT; 0.01) and protein (695.6 g kg<sup>-1</sup>) vs 647.9 g kg<sup>-1</sup>); P &LT; 0.01) degradability after 24 h of incubation in stovers from Bt(+) plants. The NDF degradability was higher in Bt(-) corn varieties because of the higher proportion of hemicellulose in the total fibre. (Rossi et al., 2003)

**Saxena, D., S. Flores and G. Stotzky (1999).** "Transgenic plants: Insecticidal toxin in root exudates from Bt corn." *Nature* **402**(6761): 480-480.<http://dx.doi.org/10.1038/44997> AND <http://www.botanischergarten.ch/Bt/Stotzky-Insecticidal-Toxin-1999.pdf>

Bt corn is corn (*Zea mays*) that has been genetically modified to express insecticidal toxins derived from the bacterium *Bacillus thuringiensis* to kill lepidopteran pests feeding on these plants. Here we show that Bt toxin is released into the rhizosphere soil in root exudates from Bt corn. (Saxena et al., 1999)

**Saxena, D., S. Flores and G. Stotzky (2002).** "Bt toxin is released in root exudates from 12 transgenic corn hybrids representing three transformation events." *Soil Biology & Biochemistry* **34**(1): 133-137.<Go to ISI>://000173528800017 AND <http://www.botanischergarten.ch/Bt/Saxena-Bt-toxin-released-2002.pdf>

The anti-lepidopteran toxin (Cry1Ab protein) encoded by truncated genes from *Bacillus thuringiensis* was released in the root exudates from all hybrids of Bt corn studied and which represented three transformation events (Bt11, MON810, and 176). In vitro and in situ studies indicated that the toxin released in root exudates accumulates in soil, as it adsorbs and binds rapidly on surface-active particles (e.g. clays and humic substances), and retains insecticidal activity for at least 180 d, the longest time studied. The results indicated that the release of the Cry1Ab protein by roots is a common phenomenon with transgenic Bt corn and is not restricted to only the one Bt corn hybrid (NK4640Bt) and transformation event (Bt11) studied initially. (Saxena et al., 2002)

**Saxena, D., C. N. Stewart, I. Altosaar, Q. Y. Shu and G. Stotzky (2004).**

"Larvicidal Cry proteins from *Bacillus thuringiensis* are released in root exudates of transgenic *B. thuringiensis* corn, potato, and rice but not of *B. thuringiensis* canola, cotton, and tobacco." *Plant Physiology and Biochemistry* **42**(5): 383-387.<Go to ISI>://000222281800004 AND <http://www.botanischergarten.ch/Bt/Saxena-Larvicidal-Cry-2004.pdf>

Larvicidal proteins encoded by cry genes from *Bacillus thuringiensis* were released in root exudates from transgenic *B. thuringiensis* corn, rice, and potato but not from *B. thuringiensis* canola, cotton, and tobacco. Nonsterile soil and sterile hydroponic solution in which *B. thuringiensis* corn, rice, or potato had been grown were immunologically positive for the presence of the Cry proteins; from *B. thuringiensis* corn and rice, the soil and solution were toxic to the larva of the tobacco hornworm (*Manduca sexta*), and from potato, to the larva of the Colorado potato beetle (*Leptinotarsa decemlineata*), representative lepidoptera and coleoptera, respectively. No toxin was detected immunologically or by larvicidal assay in soil or hydroponic solution in which *B. thuringiensis* canola, cotton, or tobacco, as well as all near-isogenic non-*B. thuringiensis* plant counterparts or no plants, had been grown. All plant species had the cauliflower mosaic virus (CaMV) 35S promoter, except rice, which had the ubiquitin promoter from maize. The reasons for the differences between species in the exudation from roots of the toxins are not known. The released toxins persisted in soil as the result of their binding on surface-active particles (e.g. clay minerals, humic substances), which reduced their biodegradation. The release of the toxins in root exudates could enhance the control of target insect pests, constitute a hazard to nontarget organisms, and/or increase the selection of toxin-resistant target insects. (Saxena et al., 2004)

**Saxena, D. and G. Stotzky (2001).** "Bacillus thuringiensis (Bt) toxin released from root exudates and biomass of Bt corn has no apparent effect on earthworms, nematodes, protozoa, bacteria, and fungi in soil." *Soil Biology & Biochemistry* **33**(9): 1225-1230.[www.elsevier.com/locate/soilbio](http://www.elsevier.com/locate/soilbio) and <http://www.botanischergarten.ch/Bt/Saxena-Stotzky-2001.pdf>

There were no significant differences in the percent mortality and weight of earthworms (*Lumbricus terrestris*) after 40 days in soil planted with Bt (NK4640Bt) or non-Bt corn or after 45 days in soil amended with biomass of Bt or non-Bt corn. The toxin was present in the guts and casts of earthworms in soil planted with Bt corn or amended with biomass of Bt corn, but it was cleared within 2-3 days from the guts after placing in fresh soil. There were no significant differences in the colony-forming units of culturable

bacteria (including actinomycetes) and fungi and in the numbers of protozoa and nematodes between rhizosphere soil of Bt and non-Bt corn or between soil amended with biomass of Bt and non-Bt corn. The CryIAb protein in root exudates and biomass of Bt corn appears not to be toxic to earthworms, nematodes, protozoa, bacteria, and fungi. The presence of the toxin in the guts and casts of earthworms confirmed that the toxin released in root exudates and from transgenic biomass was bound on surface-active particles in soil, which protected the toxin from biodegradation, as has been observed in this laboratory with purified toxin. (Saxena & Stotzky, 2001)

**Sims, S. R. and L. R. Holden (1996).** "Insect bioassay for determining soil degradation of *Bacillus thuringiensis* subsp *kurstaki* CryIA(b) protein in corn tissue." *Environmental Entomology* **25**(3): 659-664.<Go to ISI>://WOS:A1996UR93900015 AND <http://www.botanischergarten.ch/Bt/Sims-Bioassay-Bt-Soil-1996.pdf>

A bioassay using larval growth inhibition of *Heliothis virescens* (F.) was used to study the environmental fate (=soil degradation) of *Bacillus thuringiensis* subsp, *kurstaki* CryIA(b) protein in transgenic corn plant tissue (transgenic corn). Transgenic corn was incubated for up to 43 d, with and without contact with soil, in a chamber maintaining warm (24-27 degrees C) and humid (>80% RH) conditions. Incubation was terminated by moving the transgenic corn to -80 degrees C. A series of dilutions was made from each incubated sample, mixed into artificial insect diet, and fed to neonate *H. virescens*. Weights of treated larvae were scored after 7 d growth at approximate to 28 degrees C, and the bioactivity of CryIA(b) protein in the samples was determined using regression analysis of the dose-weight response data. The insect bioassay approach allowed for nearly complete recovery of CryIA(b) protein bioactivity in a transgenic corn + soil matrix or transgenic corn incubated without soil contact. CryIA(b) protein added to soil as a component of transgenic corn tissue had an estimated DT50 (50% dissipation time = 'half-life') of 1.6 d and a DT90 (90% dissipation time) of 15 d. CryIA(b) protein in transgenic corn tissue incubated without soil contact, had an estimated DT50 of 25.6 d and a DT90 of 40.7 d. The results suggest that CryIA(b) protein, as a component of postharvest transgenic corn plants, will dissipate readily on the surface of, or cultivated into, soil. (Sims & Holden, 1996)

**Sims, S. R. and J. W. Martin (1997).** "Effect of the *Bacillus thuringiensis* insecticidal proteins CryIA(b), CryIA(c), CryIIA, and CryIIIA on *Folsomia candida* and *Xenylla grisea* (Insecta: Collembola)." *Pedobiologia* **41**(5): 412-416.<Go to ISI>://A1997YA11200004 AND <http://www.botanischergarten.ch/Bt/Sims-Effect-Folsomia-1997.pdf>

The dietary toxicity of four *Bacillus thuringiensis* insecticidal proteins (CryIA(b), CryIA(c), CryIIA, and CryIIIA) was evaluated against two Collembola species, *Folsomia candida* (Willem) (Isotomidae) and *Xenylla grisea* Axelson (Hypogastruridae). Test diets were prepared by adding the respective purified *B. thuringiensis* proteins to aqueous suspensions of Baker's yeast (*Saccharomyces cerevisiae*) and lyophilizing the mixtures to obtain final test concentrations of 200 µg B, *thuringiensis* protein per gram of diet. Collembola were tested inside petri dish microcosms containing a moistened 9:1 plaster of paris:charcoal mix. The numbers of surviving adults and progeny were recorded following 21 days exposure to the test diets. None of the *B. thuringiensis* proteins significantly reduced Collembola survival or reproduction compared to the negative yeast control. The insecticide chlorpyrifos was used as a positive control for assay validation. Chlorpyrifos significantly reduced *F. candida* adult survival and reproduction at diet concentrations greater than or equal to 20 µg g<sup>-1</sup> *X. grisea* was not significantly affected by chlorpyrifos concentrations up to 200 µg g<sup>-1</sup>. The test results demonstrate that the relatively low concentrations of *B. thuringiensis* insecticidal proteins present in the tissues of transgenic crop plants do not pose a toxicological risk to soil Collembola. (Sims & Martin, 1997)

**Stotzky, G. (2000).** Release, Persistence, and Biological Activity in Soil of Insecticidal Proteins from *Bacillus thuringiensis*. The Environmental Implications Of Genetically Modified Plants With Insect Resistance Genes, Bern, ESF/AIGM Workshop - BERNE, SWITZERLAND.pp <http://www.botanischergarten.ch/Bt/StotzkyAbstr-2000.pdf>

Insecticidal proteins produced by various subspecies of *Bacillus thuringiensis* bind rapidly and tightly on clays, both pure mined clay minerals and soil clays, and on humic acids extracted from soil. This

binding reduces the susceptibility of these proteins to microbial degradation, and the bound toxins retain their larvicidal activity. Both purified toxins and toxins released from the biomass of transgenic Bt corn and in root exudates of growing Bt corn exhibit binding, persistence, and larvicidal activity in soil. The biomass of transgenic Bt corn decomposes less in soil than does biomass of isogenic non-Bt corn. The toxins do not appear to have any consistent effects on organisms in soil (earthworms, nematodes, protozoa, bacteria, fungi) or on microorganisms in vitro. The toxins are not taken up from soil by non-Bt corn grown in soil in which Bt corn has been grown or into which biomass of Bt corn has been incorporated. The persistence of these bound insecticidal toxins may enhance the control of target pests, constitute a hazard to nontarget organisms, and result in the selection and enrichment of toxin-resistant target insects. Because of the large differences in the chemical composition and structure between clays and humic acids, these studies can serve as models for the potential fate and effects of other biomolecules, which are also chemically and structurally diverse, that will be introduced to soil from "factories" of transgenic plants and animals genetically engineered to produce vaccines, hormones, antibodies, toxins, pharmaceuticals, and other bioactive compounds. (Stotzky, 2000)

**Stotzky, G. (2004).** "Persistence and biological activity in soil of the insecticidal proteins from *Bacillus thuringiensis*, especially from transgenic plants." *Plant and Soil* **266**(1-2): 77-89.<Go to ISI>://000226385500009 AND <http://www.botanischergarten.ch/Bt/Stotzky-Persistence-2004.pdf>

Insecticidal proteins produced by various subspecies (*kurstaki*, *tenebrionis*, and *israelensis*) of *Bacillus thuringiensis* (Bt) bound rapidly and tightly on clays, both pure mined clay minerals and soil clays, on humic acids extracted from soil, and on complexes of clay and humic acids. Binding reduced susceptibility of the proteins to microbial degradation. However, bound proteins retained biological activity. Purified Cry1Ab protein and protein released from biomass of transgenic Bt corn and in root exudates of growing Bt corn (13 hybrids representing three transformation events) exhibited binding and persistence in soil. Insecticidal protein was also released in root exudates of Bt potato (Cry3A protein) and rice (Cry1Ab protein) but not in root exudates of Bt canola, cotton, and tobacco (Cry1Ac protein). Vertical movement of Cry1Ab protein, either purified or in root exudates or biomass of Bt corn, decreased as the concentration of the clay minerals, kaolinite or montmorillonite, in soil increased. Biomass of transgenic Bt corn decomposed less in soil than biomass of near-isogenic non-Bt corn, possibly because biomass of Bt corn had a significantly higher content of lignin than biomass of non-Bt corn. Biomass of Bt canola, cotton, potato, rice, and tobacco also decomposed less than biomass of the respective near-isogenic non-Bt plants. However, the lignin content of these Bt plants, which was significantly less than that of Bt corn, was not significantly different from that of their near-isogenic non-Bt counterparts, although it was consistently higher. The Cry1Ab protein had no consistent effects on organisms (earthworms, nematodes, protozoa, bacteria, fungi) in soil or in vitro. The Cry1Ab protein was not taken up from soil by non-Bt corn, carrot, radish, or turnip grown in soil in which Bt corn had been grown or into which biomass of Bt corn had been incorporated. (Stotzky, 2004)

**Visser, S., J. A. Addison and S. B. Holmes (1994).** "Effects of Dipel(R)-176, a *Bacillus-Thuringiensis* Subsp *Kurstaki* (Btk) Formulation, on the Soil Microflora and the Fate of Btk in an Acid Forest Soil - a Laboratory Study." *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere* **24**(3): 462-471.<Go to ISI>://A1994NL14500005 AND NEBIS 20080120

The effects of DiPel(R) 176, a commercially available *Bacillus thuringiensis* Berliner subsp. *kurstaki* (B.t.k.) formulation, on microbially mediated carbon and nitrogen mineralization processes, and the persistence of B.t.k. following application of DiPel(R) 176 to an acidic, coniferous forest soil were evaluated in the laboratory using simple microcosms. Litter (L) and fermentation-humus (FH) material were exposed to DiPel(R) at the recommended field application rate (FA), DiPel(R) at 1000 X the field application rate (1000 X FA), or left untreated. Respiration, substrate induced respiration (SIR), microbial biomass C, metabolic quotients (qCO<sub>2</sub>), NH<sub>4</sub>-N, NO<sub>3</sub>-N, cellulose decay, and B.t.k. viability were monitored regularly over 8 weeks. The FA treatment had no significant impact on soil processes in either the L or FH. The 1000 X FA treatment increased SIR and biomass C and decreased qCO<sub>2</sub> consistently in both the L and FH. No other effects of the 1000 X FA treatment were evident in the L, while in the FH this treatment stimulated respiration initially, then reduced it below control levels; it enhanced cellulose decay; and it inhibited ammonification and nitrification after 8 weeks incubation. In both the L and FH there was no significant loss in viability of B.t.k. in either of the DiPel(R) treatments over 8 weeks. The microcosms used in this study were simple, inexpensive, and effective, with respiration, SIR, biomass C, and qCO<sub>2</sub>

being the least variable measurements and the most sensitive to perturbation. This approach is recommended for ecotoxicological and fate testing as outlined in the Guidelines for Registration of Naturally Occurring Microbial Pest Control Agents. (Visser et al., 1994)

**Wandeler, H., Bahylova J, Nentwig W (2002).** "Consumption of two Bt and six non-Bt corn varieties by the woodlouse *Porcellio scaber*." *Basic Appl Ecol* **3**: 357-365.  
<http://www.botanischergarten.ch/Bt/Wandeler-Consumption-Porcellio-2002.pdf>

Studies of the degradation of transgenic *Bacillus thuringiensis* corn were limited to date, to a comparison between one Bt corn variety and its isogenic control line. Laboratory experiments using six non-transgenic and two transgenic Bt corn varieties were carried out to study the effect of Bt protein Cry1Ab and corn variety on the consumption of the decomposer *Porcellio scaber* (Latreille). The Cry1Ab toxin concentration in the Bt corn leaves was quantified at the beginning and at the end of the trial. Further, *P. scaber* and their faeces were analysed for presence of the Cry1Ab toxin after feeding on Bt corn using ELISA. During a feeding period of 20 days, *P. scaber* fed significantly less on the transgenic Bt corn (Bt+) than the control corn variety (Bt-). Comparing all eight corn varieties, the consumption depended significantly on the corn variety. The transgenic corn variety N4640Bt equalled the poorly consumed corn varieties; the second transgenic variety, Max88, which contained much less of the Cry1Ab protein, was one of the most consumed varieties. No differences in the nitrogen content but varying energy content were detected across the eight corn varieties. Neither the nitrogen, nor the energy content showed a significant correlation to the consumption rate. The Cry1Ab toxin concentration decreased in both Bt corn varieties during the time period of 20 days, but only significantly in one variety. The Cry1Ab protein could be detected in both the body of *P. scaber* and its faeces, showing that *P. scaber* ingested and excreted the Cry1Ab protein only to some extent. These results suggest that corn varieties, including conventional ones, differ with respect to degradation. Therefore, it is difficult to draw conclusions about the effective consequences from just one isogene test system. This study also supports earlier reports on the slow degradation of Bt corn. (Wandeler, 2002)

**Widmer, F., R. J. Seidler and L. S. Watrud (1996).** "Sensitive detection of transgenic plant marker gene persistence in soil microcosms." *Molecular Ecology* **5**(5): 603-613.<Go to ISI>://A1996VK76400001 NOT IN EZB, NEBIS 20080120

Genetic engineering offers the opportunity to generate plants with useful new traits conferred by genes originating from a variety of organisms. The objectives of this study were to establish methods for investigating persistence of recombinant plant marker DNA after introduction into soil and to collect data from controlled laboratory test systems. As a model system, we studied the stability of DNA encoding recombinant neomycin phosphotransferase II (rNPT-II), a neomycin/kanamycin resistance marker, used in plant genetic engineering. The recombinant nature of the target (i.e. fusion of nopaline synthase promoter and NPT-II coding region) allowed us to design a rNPT-II-specific PCR primer pair. DNA preparation and quantitative PCR protocols were established. Effects of temperature and moisture, on DNA persistence in soil were determined in two laboratory test systems. In the first system, purified plasmid DNA was added to soil and incubated under controlled conditions. Up to 0.08% of the rNPT-II target sequences were detectable after 40 days. In the second system, fresh leaf tissue of transgenic tobacco was ground, added to soil, and incubated under controlled conditions. After 120 days, up to 0.14% of leaf tissue-derived genomic rNPT-II sequences were detectable. Under most experimental conditions, leaf tissue-derived and plasmid DNA were initially degraded at a high rate. A small proportion of the added DNA resisted degradation and was detectable for several months. We hypothesize that this DNA may have been adsorbed to soil particles and was protected from complete degradation. (Widmer et al., 1996)

**Zwahlen, C., A. Hilbeck, P. Gugerli and W. Nentwig (2003).** "Degradation of the Cry1Ab protein within transgenic *Bacillus thuringiensis* corn tissue in the field." *Molecular Ecology* **12**(3): 765-775.  
<http://www.botanischergarten.ch/Maize/Zwahlen-2003a.pdf>

Large quantities of *Bacillus thuringiensis* (Bt) corn plant residue are left in the field after harvest, which may have implications for the soil ecosystem. Potential impacts on soil organisms will also depend on the persistence of the Bt toxin in plant residues. Therefore, it is important to know how long the toxin persists in plant residues. In two field studies in the temperate corn-growing region of Switzerland we investigated degradation of the Cry1Ab toxin in transgenic Bt corn leaves during autumn, winter and spring using an enzyme-linked immunosorbent assay (ELISA). In the first field trial, representing a tillage system, no

degradation of the Cry1Ab toxin was observed during the first month. During the second month, Cry1Ab toxin concentrations decreased to approximately 20% of their initial values. During winter, there was no further degradation. When temperatures again increased in spring, the toxin continued to degrade slowly, but could still be detected in June. In the second field trial, representing a no-tillage system, Cry1Ab toxin concentrations decreased without initial delay as for soil-incorporated Bt plants, to 38% of the initial concentration during the first 40 days. They then continued to decrease until the end of the trial after 200 days in June, when 0.3% of the initial amount of Cry1Ab toxin was detected. Our results suggest that extended pre- and post- commercial monitoring are necessary to assess the long-term impact of Bt toxin in transgenic plant residues on soil organisms. (Zwahlen et al., 2003a)

**Zwahlen, C., A. Hilbeck, R. Howald and W. Nentwig (2003).** "Effects of transgenic Bt corn litter on the earthworm *Lumbricus terrestris*." *Molecular Ecology* 12(4): 1077-1086. <http://www.botanischergarten.ch/Bt/Zwahlen-Lumbricus-litter-2003.pdf> AND <http://www.botanischergarten.ch/Bt/Zwahlen-Bt-Lumbricus-2003-corrigendum.pdf>

A 200-day study was carried out to investigate the impact of transgenic *Bacillus thuringiensis* (Bt) corn on immature and adult *Lumbricus terrestris* in the field and in the laboratory. Another objective of this study was to develop test methods that could be used for standard testing of the impact of transgenic plants on different earthworm species in the field and in the laboratory. For this purpose two different experiments were involved, a laboratory experiment with adult *L. terrestris* and a field experiment with immature *L. terrestris*. No lethal effects of transgenic Bt corn on immature and adult earthworms were observed. Immature *L. terrestris* in the field had a very similar growth pattern when fed either (Bt+) or (Bt-) corn litter. No significant differences in relative weights of (Bt+) and (Bt-) corn-fed adult *L. terrestris* were observed during the first 160 days of the laboratory trial, but after 200 days adult *L. terrestris* had a significant weight loss of 18% of their initial weight when fed (Bt+) corn litter compared to a weight gain of 4% of the initial weight of (Bt-) corn-fed earthworms. Further studies are necessary to see whether or not this difference in relative weight was due to the Bt toxin or other factors discussed in the study. Degradation of Cry1Ab toxin in corn residues was significantly slower in the field than at 10 degreesC in the laboratory. Enzyme-linked immunosorbent assay results indicated that earthworms in both experiments were exposed to the Bt toxin throughout the whole experimental time. (Zwahlen et al., 2003b)

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Soil microbial communities associated with Bt and non-Bt corn in three soils. *Journal of Environmental Quality*, 33, 3, pp 832-836  
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Subacute effects of Cry1Ab Bt corn litter on the earthworm *Eisenia fetida* and the springtail *Folsomia candida*. *Environmental Entomology*, 35, 4, pp 1121-1129  
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Subacute Effects of Transgenic Cry1Ab *Bacillus thuringiensis* Corn-Litter on the Isopods *Trachelipus rathkii* and *armadillidium nasatum*. *Environmental Toxicology and Chemistry*, 25, 10, pp 2653-2661  
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