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The Impact of Genetically Modified Crops on Soil Microbial Communities

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Key words. Transgenic plants, non-target organisms, beneficial microorganisms, gene transfer, *Bt* toxin.

Abstract. *Genetically modified (GM) plants represent a potential benefit for environmentally friendly agriculture and human health. Though, poor knowledge is available on potential hazards posed by unintended modifications occurring during genetic manipulation. The increasing amount of reports on ecological risks and benefits of GM plants stresses the need for experimental works aimed at evaluating the impact of GM crops on natural and agro-ecosystems. Major environmental risks associated with GM crops include their potential impact on non-target soil microorganisms playing a fundamental role in crop residues degradation and in biogeochemical cycles. Recent works assessed the effects of GM crops on soil microbial communities*

on the basis of case-by-case studies, using multimodal experimental approaches involving different target and non-target organisms. Experimental evidences discussed in this review confirm that a precautionary approach should be adopted, by taking into account the risks associated with the unpredictability of transformation events, of their pleiotropic effects and of the fate of transgenes in natural and agro-ecosystems, weighing benefits against costs.

1. INTRODUCTION

Genetically modified (GM) plants represent a potential benefit for environmentally friendly agriculture and human health. Though, poor knowledge is available on the interactions among the different components of agro- and natural ecosystems and on potential hazards posed by unintended modifications occurring during genetic manipulation. The increasing amount of reports on ecological risks and benefits of GM plants stresses the need for experimental works aimed at evaluating the environmental impact of GM crops, taking also into account the fact that little is known of the fate of transgenes after their field release, except that in nature “everything goes everywhere” and genes can flow from one organism to another (Heinemann and Sprague [1989]; Hooykaas [1989]; Doolittle *et al.* [1990]; Courvalin [1995]; Ellstrand *et al.* [1999]; Intrieri and Buiatti [2001]; Giovannetti [2003]).

A clear example of the occurrence of unintended effects of genetic manipulation is represented by gene insertion mediated by *Agrobacterium tumefaciens*, which provoked host DNA disruption in over one-third of events (Szabados *et al.* [2002]; Forsbach *et al.* [2003]), and induced loss of about 12 genes, duplication/translocation of DNA fragments (up to 40 kbp) and random insertion of plasmid DNA at insertion sites (Kaya *et al.* [2000]; Tax and Vernon [2001]; Kim *et al.* [2003]). This kind of mutations may pass unnoticed during the different characterisation stages of transformants and could give rise to unexpected phenotypes (Windels *et al.* [2001]). Such effects of genetic transformation were previously detected in *Bt* corn lines, which display an altered pattern in the shikimic acid pathway leading to a higher lignin

content, compared to non transformed plants (Saxena and Stotzky [2001]).

When assessing the ecological risks of GM crops, their impact on soil microbes fundamental for the completion of biogeochemical cycles and soil fertility, should be considered, since plants release into the rhizosphere as much as 20% of their assimilates as root exudates and are often incorporated as crop residues into the soil at the end of production cycles (Whipps [1990]).

In this review we discuss the available data concerning (a) how GM crops can affect soil microbes, (b) methods for evaluating GM crops impact on soil microbial communities, (c) the impact on soil microbial communities and beneficial fungal symbionts, (d) risks of horizontal gene transfer, (e) long-term effects of GM crops.

2. HOW GENETICALLY MODIFIED (GM) CROPS AFFECT SOIL MICROBES

Different hypotheses have been considered as to how GM crops may exert direct and indirect effects on soil non-target microorganisms. Direct effects are produced by the activity of transgenic proteins and by their increasing amounts due to accumulation, whereas indirect effects may be ascribed to modifications occurring in GM crop plant metabolic pathways leading to changes in root exudate composition and altered expression in plant tissues.

Direct effects. Direct effects are usually easier to detect than indirect effects. For example, transgenic proteins, which have a range of activities against insects or pathogenic fungi and bacteria, may affect also non-target microorganisms, such as beneficial symbionts and/or microbial soil communities involved in organic matter decomposition. Most GM crops have been engineered to constitutively express transgenic proteins in plant tissues by using the Cauliflower Mosaic Virus (CaMV) 35S promoter, which may increase GM crops environmental hazard (Ho *et al.* [1999]). In fact, such viral promoter induces the continuous production in all plant tissues of transgenic proteins, which may accumulate in high amounts in the soil and remain stable for long periods, depending on their

bioavailability and persistence. An example of accumulation and persistence of transgenic proteins in the soil is represented by *Bt* plants constitutively expressing crystal proteins, which confer resistance to insect pests. It has been demonstrated that both *Bt* plant root exudates and *Bt* plant residues may lead to an accumulation of the transgenic protein in the soil, where it maintains its activity for a long period (Saxena *et al.* [1999]; Zwahlen *et al.* [2003]).

Indirect effects. Indirect effects are difficult to evaluate, since many different factors may interfere with root exudate composition and plant metabolic activity. It is well known that *in vitro* plant tissue culture, a fundamental step during genetic manipulation, may cause unintended alterations in plant characteristics. Such effect has been described as somaclonal variation, which is the result of genetic changes induced by *in vitro* cultural practices. Besides, as genetic transformation techniques cannot control the insertion site of the transgene, its position in the genome of transformed plants may lead to altered expressions and/or disturb the former genetic expression. This phenomenon is called pleiotropy and means that the insertion of a foreign gene in a new genomic context may be responsible for the development of unexpected phenotypes. For example, it has been shown that pleiotropic effects may enhance the competitiveness of transgenic crops once they are released into the environment. In fact, *Arabidopsis thaliana* plants transformed to express the gene for the acetolactate synthase, *Csr1-1*, which confers resistance to the herbicide chlorsulfuron, showed 20 times more ability to donate pollen to wild type plants, compared to mutants expressing the same mutant allele as the transgenic plants (Bergelson *et al.* [1998]). Other studies described further unintended features occurring in plant tissues or in root exudates that, for their particular characteristics, might affect soil microbial communities. For example, it has been demonstrated that transgenic *Bt* corn plants showed significant differences in fructose and soluble carbohydrates contents compared to non-transgenic plants (Escher *et al.* [2000]). Other works reported the presence of a higher lignin content in the tissues of *Bt* corn varieties compared with their non *Bt* isolines (Masoero *et al.* [1999]; Saxena and Stotzky [2001]; Flores *et al.* [2005]; Poerschmann *et*

al. [2005]). Donegan *et al.* [1995] reported that some *Bt* cotton lines affected total microbial population, whereas no effects were produced by the purified *Bt* toxins, suggesting that the impact on soil microorganisms was due to transformation events or tissue culture practices rather than to the products of the inserted genes.

All these findings should be taken into account since unintended new plant traits impact beneficial microbial population in the soil.

3. EVALUATION OF THE EFFECTS OF GM CROPS ON SOIL MICROBIAL COMMUNITIES

Many efforts have been recently made to develop new methods for the evaluation of the effects of GM crops on soil microbial communities. Five types of experimental methods have been proposed, which are able to describe both functional and structural composition of soil microbial communities (Table 1).

- Plating methods are utilised for the identification and characterisation of variations in functional groups, such as symbiotic nitrogen fixers and nitrifiers or organic matter decomposing bacteria. Such methods were not able to highlight significant effects produced by transgenic plants on soil microbial communities (Donegan *et al.* [1995]; Saxena and Stotzky [2001]; Wu *et al.* [2004]).

- Metabolic community profiles using BIOLOG microtiter plates (community-level physiological/catabolic profiling, CLPP or CLCP) represent another cultural method used to detect variations in microbial communities, although it does not allow to identify the specific microorganism responsible for the metabolic changes detected. This method has been largely utilised to study the effects of GM crops on soil microbes (Donegan *et al.* [1995], [1999]; Di Giovanni *et al.* [1999]; Griffiths *et al.* [2000]; Heuer *et al.* [2002]; Dunfield and Germida [2003]; Tesfaye *et al.* [2003]).

- PCR-based methods, such as Amplified Ribosomal DNA Restriction Analysis (ARDRA), Denaturing Gradient Gel Electrophoresis (DGGE), Single Strand Conformational Polymorphisms (SSCP) and automated ribosomal intergenic spacer analysis

(ARISA), which are used to amplify the ribosomal RNA genes from soil samples, are independent of cultural practices and useful to detect variations in the structure of soil microbial communities in the presence of GM crops (Donegan *et al.* [1995], [1999]; Heuer *et al.* [2002]; Lottmann and Berg [2001]; Dunfield and Germida [2003]; Sessitsch *et al.* [2003]; Castaldini *et al.* [2005]).

Table 1 – Methods for the evaluation of the impact of GM crops on soil microbial communities

Methods	Transgenic plants	References
Cultural methods	<i>Zea mays</i>	Donegan <i>et al.</i> [1995], [1999];
-Plating method	<i>Medicago sativa</i>	Di Giovanni <i>et al.</i> [1999];
-CLPP/CLCP	<i>Brassica napus</i>	Dunfield, Germida [2003];
	<i>Solanum tuberosum</i>	Griffiths <i>et al.</i> [2000]; Lottmann <i>et al.</i> [1999]; Lottmann, Berg [2001]; Saxena, Stotzky [2001]; Tesfaye <i>et al.</i> [2003]; Wu <i>et al.</i> [2004].
Methods for DNA analyses	<i>Zea mays</i>	Bruseti <i>et al.</i> [2004]; Castaldini <i>et al.</i> [2005]; Donegan <i>et al.</i> [1995], [1999]; Di Giovanni <i>et al.</i> [1999]; Dunfield, Germida [2003]; Gyamfi <i>et al.</i> [2002]; Heuer <i>et al.</i> [2002]; Schmalenberger, Tebbe [2002]; Sessitsch <i>et al.</i> [2003], [2004]; Tasfaye <i>et al.</i> [2003].
-DGGE	<i>Brassica napus</i>	
-RFLP	<i>Medicago sativa</i>	
-ARDRA	<i>Solanum tuberosum</i>	
-ARISA		
-SSCP		
Methods for lipid analyses	<i>Medicago sativa</i>	Cowgill <i>et al.</i> [2002]; Dunfield, Germida [2003].
-FAME	<i>Solanum tuberosum</i>	
-PFLA	<i>Brassica napus</i>	
Methods based on metabolic activities	<i>Zea mays</i>	Donegan [1999]; Griffiths <i>et al.</i> [2000]; Sessitsch <i>et al.</i> [2004]; Wu <i>et al.</i> [2004]; Castaldini <i>et al.</i> [2005].
-Enzymatic activities	<i>Oryza japonica</i>	
-Soil respiration	<i>Solanum tuberosum</i>	

- Microbial lipid analysis-based methods, such as Fatty Acid Methyl Ester profiles (FAME) and Phospholipid Fatty Acid analysis (PFLA) are independent of cultural practices and provide a quantitative assessment of viable microbial biomass. They were successful in the evaluation of the effects of transgenic crops on soil microorganisms (Cowgill *et al.* [2002]; Dunfield and Germida [2003]).

- Metabolic activities-based methods, including soil respiration and enzyme activities, give general information on functional soil microbial communities, and were able to reveal the effects of GM crops on soil microbes (Di Giovanni *et al.* [1999]; Sessitsch *et al.* [2004]; Wu *et al.* [2004]; Castaldini *et al.* [2005]).

Table 2 – Diverse experimental levels for the study of the effects of GM crops on soil microbial communities

Experimental conditions	Time scales of experimental sample analyses	References
Laboratory	weeks – months	Donegan <i>et al.</i> [1995]; Griffiths <i>et al.</i> [2000]; Medina <i>et al.</i> [2003]; Turrini <i>et al.</i> [2004]; Wu <i>et al.</i> [2004a, b].
Greenhouse	weeks – months	Castaldini <i>et al.</i> [2005]; Di Giovanni <i>et al.</i> [1999]; Griffiths <i>et al.</i> [2000]; Gyamfi <i>et al.</i> [2002]; Saxena, Stotzky [2001]; Sessitsch <i>et al.</i> [2003], [2004]; Turrini <i>et al.</i> [2004a, b]; Vierheilig <i>et al.</i> [1995].
Open field	Months – one year – several years	Cowgill <i>et al.</i> [2002]; Donegan <i>et al.</i> [1999]; Dunfield, Germida [2003]; Griffiths <i>et al.</i> [2000]; Heuer <i>et al.</i> [2002]; Lottmann <i>et al.</i> [1999]; Lottmann, Berg [2001]; Schmalemberger, Tebbe [2002]; Tesfaye <i>et al.</i> [2003].

Since each method offers differential advantages and disadvantages, only a combination of techniques may provide scientifically sound data, allowing accurate and extensive risk assessments. Moreover, since the studies performed to evaluate the impact of GM crops on soil microbes were carried out in different environmental conditions, i.e. laboratory, greenhouse and open field (Table 2), and produced different results, it would be appropriate to compare data originating from the three experimental levels (Castaldini *et al.* [2005]).

4. THE IMPACT OF GM PLANTS ON SOIL BACTERIA AND FUNGI

Field release of GM plants is highly controversial, since many concerns over their potential effects on human health and environment have been posed. Major environmental risks associated with GM crops include their potential impact on non-target soil microbial communities playing a fundamental role in crop residues degradation and in biogeochemical cycles.

Experiments aimed at evaluating the impact of GM plants on bulk soil and rhizospheric bacterial communities composition yielded diverse results, depending on the analytical method used: culture-dependent methods did not detect many changes, due to their reduced sensitivity with respect to culture-independent analyses.

Many studies have been performed on GM plants modified to constitutively express *Cry* genes, encoding *Bacillus thuringiensis* (*Bt*) insecticidal endotoxins, able to control stem borers and other insect pests. The impact of many *Bt* corn lines on soil has been largely studied, since these plants are able to release *Cry* proteins with root exudates, a widespread character of all the transformed plants analysed so far (Saxena *et al.* [1999]; Saxena and Stotzky [2000]; Saxena *et al.* [2002]). These works also demonstrated that the released toxin is able to bind to clays and humic acids and to maintain its insecticidal activity for at least 180 days, thus representing a potential risk for both target and non-target soil organisms (Saxena and Stotzky [2001]).

Some studies showed no apparent deleterious effect of the

CryIAb toxin released by *Bt* corn plants through root exudates or directly incorporated into soil on earthworms, nematodes, protozoa, culturable bacteria and saprophytic fungi, both *in vitro* and *in vivo* (Saxena and Stotzky [2001]; Koshella and Stotzky [2002]). Accordingly, no significant changes in culturable microflora were detected in the rhizosphere of *Cry*-expressing cotton, rice and potato (Donegan *et al.* [1995, 1996]; Wu *et al.* [2004]). Interestingly, also the release of the bacterium *Bacillus thuringiensis* in soybean plants did not affect rhizospheric culturable bacterial populations and saprophytic fungi (Ferreira *et al.* [2003]).

Some papers reporting data obtained by using culture-independent methods, such as analyses of PLFA, CLPP or CLCP, terminal restriction fragment length polymorphism (T-RFLP), DGGE and SSCP, did not show significant effects of *Bt* plants on soil microbial communities, claiming that plant age, soil type and texture represented the overriding factors affecting bacterial diversity (Blackwood and Buyer [2004]; Baumgarte and Tebbe [2005]; Fang *et al.* [2005]). Automated ribosomal intergenic spacer analysis has been used to compare rhizospheric bacterial communities of *Bt* and non *Bt* corn plants and to obtain fingerprinting from soil bacteria exposed to their root exudates: results have shown differences between profiles belonging to plants of different age and to transformed/non transformed plants. Interestingly, fingerprints of communities exposed to *Bt* and non *Bt* plant exudates clustered separately after principal component analysis, indicating an active selection exerted by the different root exudates (Bruseti *et al.* [2004]).

In a recent work, differences in total (16S rDNA) and metabolically active fractions (16S rRNA) of the rhizospheric eubacterial communities and in culturable rhizospheric heterotrophic bacteria have been found between *Bt* and non-*Bt* corn plants, by using DGGE analysis (Castaldini *et al.* [2005]). Microbial activity, assessed by measuring soil respiration (carbon released as CO₂) has been also reported to be affected by the presence of *Bt* crops and/or of their residues incorporated into the soil (Dinel *et al.* [2003]; Castaldini *et al.* [2005]).

Indirect effects of the *Cry* protein on microbiota have been also reported, such as the growth reduction of bacteria occurring on

faeces of the crustacean *Porcellio scaber* fed with GM corn (Escher *et al.* [2000]).

Crop plants harbouring genetic modifications other than *Bt* toxin expression have been developed to control invertebrate pests (lectin or proteinase inhibitors expression) and phytopathogenic fungi (plant-derived defensins, lysozymes, cecropins, pathogenesis-related proteins expression and expression of systemic acquired resistance). Studies on potato lines producing concanavalin A and *Galanthus nivalis* agglutinin showed 10% reduction in microbial activity and changes in physiological profiles of rhizospheric microbial communities at harvest, respectively (Griffiths *et al.* [2000]). Potato plants expressing the cysteine proteinase inhibitors, developed to control potato-cyst nematode, tested in the field for two growing seasons, clearly evidenced a 23% reduction in bacterial and fungal abundance during the second year of growth (Cowgill *et al.* [2002]). Phytopathogenic bacteria may be controlled by developing lytic peptides-expressing crops, like T4 lysozyme-producing potato and cecropin B-expressing plants, which did not induce significant alterations in bacterial communities (Lottmann *et al.* [1999]; Heuer *et al.* [2002]; Sessitsch *et al.* [2003]).

Genes encoding pathogenesis-related proteins like chitinases and glucanases have been introduced into plants to obtain enhanced resistance to pathogenic fungi. The analysis of chitinase-expressing rice showed a dramatic change in intraradical and root surface microflora: a reduction in number of intraradical fungi was paralleled by a ten-fold increase of bacteria, and the community composition was also affected by the genetic modification (Liu *et al.* [2004]).

The impact of modifications aimed at improving chemical characteristics of crops designed for industrial production has been poorly studied. An investigation on potato with altered starch composition reported that GM plants affected rhizospheric *Pseudomonas* populations (Milling *et al.* [2004]), whereas *Medicago sativa* modified with a gene from *Phanerochaete chrysosporium* to produce lignin peroxidase showed significant differences, with respect to non transformed plants, in rhizospheric bacterial communities composition and activity (Di Giovanni *et al.* [1999]; Donegan *et al.* [1999]). Qualitative changes in rhizospheric bacterial compo-

sition and differential substrate utilisation abilities were detected in soil where GM alfalfa plants, over-expressing malate dehydrogenase, were grown (Tesfaye *et al.* [2003]).

Many transformation events have been carried out with the purpose of developing plants resistant to non-selective herbicidal compounds, in order to improve weed control in the field. Glyphosate- and glufosinate-resistant *Brassica napus* and corn did not induce permanent alterations in the composition and diversity of soil and rhizospheric microbial communities (Siciliano and Germida [1999]; Gyamfi *et al.* [2002]; Schmalenberger and Tebbe [2002]; Dunfield and Germida [2003]), although cultivar-dependent differences were found in intraradical and rhizospheric microbial communities (Siciliano and Germida [1999]; Dunfield and Germida [2003]).

A pleiotropic effect of the *CryIAb* gene has been evidenced in *Bt* corn, since an alteration in shikimic acid pathway leading to a higher lignin content in the stem, compared with that of non-transgenic isolines, has been found in many transformation events (Saxena and Stotzky [2001]; Poerschmann *et al.* [2005]). However, beneficial or negative effects of the slower degradation rate – due to the higher lignin content – of such *Bt* plant residues and the resulting shifts in microbial community composition have to be assessed for these corn lines (Flores *et al.* [2005]).

All the results discussed above suggest that the evaluation of the impact of GM plants on natural and agricultural ecosystems should be carried out by testing specific effects of single transformation events on the basis of case-by-case studies, using different target and non-target organisms, and multimodal experimental approaches involving the assessment of biochemical, physiological and molecular parameters.

5. THE IMPACT OF GM PLANTS ON NON-TARGET BENEFICIAL FUNGAL SYMBIONTS

The effects of transgenic crops on non-target organisms establishing close relationships with plant roots, such as mycorrhizal symbionts, have been recently studied. Arbuscular mycorrhizal

(AM) fungi are an important group of beneficial microorganisms which establish mutualistic symbioses with the roots of most plant species, playing a fundamental role as biofertilizers (Smith and Read [1997]). Since AM fungi are strongly affected by agricultural practices and by changes in plant and soil characteristics, they represent key non-target soil organisms to be monitored in studies of GM crops impact (Fontanet *et al.* [1998]; Giovannetti and Avio [2002]).

An experimental model system developed using this group of plant symbionts was validated in two case-studies with transgenic corn and aubergine plants (Turrini *et al.* [2004a, b]). The experimental system allowed the detection of multiple effects on different stages of AM fungi life cycle: spore germination and pre-symbiotic growth, host recognition responses, infection structures differentiation and host root colonisation were assessed in concurrent analyses. Different assays were carried out on *Bt* transformation events 11 and 176, compared to the non transformed line NK4640 (Turrini *et al.* [2004a]), and on aubergine plants transformed to constitutively express the defensin Dm-AMP1 isolated from *Dahlia merckii* (Turrini *et al.* [2004b]). Defensins are able to inhibit the growth of phytopathogenic fungi by reducing hyphal elongation through binding to hyphal membranes at specific sphingolipid sites, and indirect evidences indicate that this binding is differential for each defensin type and is required for antifungal activity (Broekaert *et al.* [1995, 1997]; Terras *et al.* [1995]; Thevissen *et al.* [2000a, b, 2003, 2004]). GM aubergine plants expressing defensins were able to release in root exudates high levels of the antimicrobial protein, which maintained the capacity to reduce the growth of phytopathogenic fungi (Turrini *et al.* [2004b]).

Results obtained with this model system showed differential behaviour of the AM fungus *Glomus mosseae* in the presence of different GM plants. No differences in pre-symbiotic growth have been observed in the presence of exudates from Dm-AMP1, compared with control plants, whereas root exudates of *Bt* 176 line significantly reduced pre-symbiotic hyphal growth with respect to *Bt* 11 line and control. Interestingly, neither root exudates containing *Bt* toxin nor those carrying Dm-AMP1 interfered with the occurrence of fungal recognition responses in the presence of the

host plant (Turrini *et al.* [2004a, b]). Infection structures (appressoria and entry points) were developed on both plant lines, although their development was differentially affected by GM plants. In fact, *Bt* 11 and 176 lines reduced the amount of viable infection hyphae, and consequently the production of functional intraradical mycelium, by 72% and 67%, respectively, compared with non-*Bt* plants. Root colonisation was consistently affected, since the percentage of mycorrhizal root length of *Bt* plants was about 50% of that recorded in the non-transgenic line. On the contrary, although Dm-AMP1 was highly expressed in all GM plant tissues, roots included, and it was released in exudates, the development of infection structures and of mycorrhizal colonisation by *G. mosseae* were not affected (Turrini *et al.* [2004b]). Other studies had previously reported that *G. mosseae* was able to establish mycorrhizal infection in roots of *Nicotiana* spp. expressing chitinases and pathogenesis-related proteins: a delay in mycorrhizal colonisation was observed in the presence of plants expressing PR-2 protein, though its antifungal activity was low, whereas no differences were observed in the presence of plants expressing chitinases (Tahiri-Alaoui *et al.* [1993]; Vierheilig *et al.* [1993, 1995]). Moreover, tobacco plants modified for the expression of enhanced systemic acquired resistance showed a delayed root colonisation by AM fungi with respect to non transformed plants (Medina *et al.* [2003]).

The differences found in the effects of GM plants on symbiotic fungi confirm the need of case-by-case studies, involving different test organisms and experimental approaches. Moreover, the implementation of reliable model systems may lead to the development of bio-tests for the assessment of the impact of GM crops and their root exudates on important non-target soil microorganisms.

6. THE RISK OF HORIZONTAL GENE TRANSFER IN SOIL

The stability of inserted genes in GM plants has been claimed to be the same as that of plant's own DNA, though recent evidences show that in GM *Arabidopsis* transgenes are more likely to spread with pollen than genes carrying the same modification ob-

tained by mutagenesis (Bergelson *et al.* [1998]). It is well known that the expression of foreign genes in GM organisms is commonly achieved by transferring cassettes of genes encoding for the desired protein, a constitutive expression promoter and a drug resistance gene as selectable marker: the possibility of spreading of such genes in natural habitats represent a potential risk to take into account when assessing the environmental impact of GM plants. The difficulty of assessing gene transfer in natural settings, involving complex interactions among many different communities, is high, though recent advances in molecular techniques have allowed detailed analysis of genetic flow both between GM plants and microorganisms and among microorganisms.

Rhizospheric environments are “hot spots” for bacterial gene transfer, since enhanced nutrient availability and water/exudate fluxes stimulate bacterial activity and consequently horizontal gene transfer (HGT) processes, occurring by conjugation, transformation and transduction. Plasmid transfer between plant associated bacteria like pseudomonads (Lilley *et al.* [1994]; Kroer *et al.* [1998]; van Elsas *et al.* [1998]), phage-mediated gene transfer (Stephens *et al.* [1987]) and acquisition of plant-derived genes by rhizospheric bacteria (Kay *et al.* [2002]) have been observed in this ecological niche.

HGT is a fundamental strategy of bacterial genetic recombination and evolution occurring in all aquatic/terrestrial habitats, inside gastrointestinal tracts of different organisms and among many bacterial species (de Vries and Wackernagel [2004]). Transformation is the relevant mechanisms in DNA transfer from plants to bacteria, and its occurrence largely differs among strains and species, ranging over many orders of magnitude. Though, it is interesting to note that in bacteria an “improbable event” occurring at very low frequency may become a real risk, since more than one billion bacteria are found in one gram of fertile soil.

DNA release during plant litter decomposition is well documented in different systems (Widmer *et al.* [1997]; Paget *et al.* [1998]; Gebhard and Smalla [1999]; Ceccherini *et al.* [2003]). The release of DNA from transgenic potato plants into the soil has been monitored, showing that it spread into the rhizosphere at any plant growing stage and at distances of up to 2 m during flowering

stage (de Vries *et al.* [2003]). Although extracellular DNA persistence in soil was expected to be limited due to the lytic activity of microbes, released DNA has been detected in agricultural soil over a two-year period and it has been observed to persist in conserved soil for several years (Blum *et al.* [1997]; Widmer *et al.* [1997]; Paget *et al.* [1998]; Gebhard and Smalla [1999]; de Vries *et al.* [2003]; Meier and Wackernagel [2003]). Its long persistence may be explained by its ability to be absorbed and bound on clay minerals and other soil particles, which protect DNA against degradation (Aardema *et al.* [1983]; Khanna and Stotzky [1992]; Gallori *et al.* [1994]). Intact plasmidic DNA has also been detected in compost and manure to be incorporated into soil as fertilisers (Smalla *et al.* [2000]).

In soil, DNA retains its transforming activity towards naturally competent bacteria like *Bacillus*, *Pseudomonas* and *Acinetobacter* (Khanna and Stotzky [1992]; Lorenz and Wackernagel [1994]; Nielsen *et al.* [1997, 2000]). Rhizospheric bacteria belonging to the genus *Acinetobacter* have been reported to be able to capture and integrate a bacterial marker (kanamycin resistance) inserted in DNA of different plants (Gebhard and Smalla [1998]; de Vries and Wackernagel [2001]), though transformation frequencies were very low (below 10^{-9}) and the probability of integration of transgenes in bacterial genome were limited to the presence of homologue sequences, at least in transgene-flanking regions (Nielsen *et al.* [1997]).

Gene transfer between GM plants and fungi has been detected both in co-culture systems and *in planta* (Bryngelsson *et al.* [1988]; Hoffmann *et al.* [1994]), and could represent a potential risk in the case of fungal pathogens and symbionts living inside host plant cells.

These results suggest that gene transfer from GM crops to competent soil bacteria carrying homologous sequences is possible, and that the low frequencies of transformation events could be balanced by high numbers of active bacterial cells in “hot spots” and by the large quantities of DNA released in the environment during and after the cultivation of transgenic crops.

7. LONG-TERM EFFECTS OF GM CROPS: A REAL HAZARD?

Long-term effects of GM crops may be expected on soil macro- and microflora composition and functioning, since a large amount of compounds may accumulate by means of root exudates and residues incorporation into soil.

Studies on *Bt* toxin persistence have shown that this protein maintains its activity after absorption to clays or binding to humic acids (Saxena and Stotzky [2001]). In fact, *Bt* toxin released by corn and cotton retains its activity for 180-234 days both in laboratory and soil experiments, and its accumulation and persistence suggests possible long-term effects: further information is needed to evaluate how it affects non-target organisms, in particular those involved in biogeochemical cycles, and the selection of toxin-resistant insects (Tapp and Stotzky [1998]; Stotzky [2004]).

Studies on biodegradation of GM crop residues incorporated into soil have demonstrated slower litter decomposition for *Bt* corn, canola, cotton, potato, rice and tobacco, compared with non-*Bt* lines (Flores *et al.* [2005]). It is interesting to note that a significantly higher lignin content has been detected in *Bt* corn lines, than in non-*Bt* near-isolines. A lower degradation rate of litter might be beneficial for the maintenance of soil structure, although the longer persistence of *Bt* residues might enhance the risks related to toxin accumulation.

Differential enrichment in some bacterial groups like *Bacillus*, able to synthesize enzymes for the degradation of complex carbohydrates, has been found in the presence of *Bt* residues ploughed into soil, whereas different rhizobacteria have been detected in soil amended with non-*Bt* plants (Castaldini *et al.* [2005]). Furthermore, lower microbial activity, measured by CO₂ evolution, has been reported by many authors for *Bt*-amended soils (Stotzky [2000]; Flores *et al.* [2005]; Castaldini *et al.* [2005]). Since seasonal shifts evidenced in rhizospheric bacterial communities associated to corn, strawberry, potato and oilseed rape (Di Cello *et al.* [1997]; Smalla *et al.* [2001]) may complicate the analysis of results based on short-term studies or single-point harvests, prolonged time-course analyses should be performed to assess the occurrence of long-term modifications in microbial populations.

Germination ability and pre-symbiotic hyphal growth of the non-target beneficial AM fungus *G. mosseae* were not affected by *Bt* corn residues incorporated into soil, while a lower percentage of mycorrhizal colonisation by indigenous AM fungi was shown by plants grown in soil where *Bt* 11 corn residues were ploughed under, compared with wild type corn residues (Castaldini *et al.* [2005]). Two main questions remain as to whether mycorrhizal colonisation is reduced by direct toxicity of *Bt* corn litter towards AM fungal propagules, by indirect effects of microbial population alterations or by other factors, and as to whether prolonged permanence of litter in the soil could significantly affect inoculum potential of mycorrhizal fungi.

Further long-term studies in the field are necessary to evaluate the impact of GM plants on microbial communities fundamental for soil fertility and quality: in fact soil is a complex system where trophic and non trophic interactions among organisms are difficult to monitor by short-term researches. In particular, the hazard posed by GM plant residues to non-target beneficial soil microbes should be thoroughly investigated, since any reduction in their biodiversity might produce long-term effects, in space and time, on crops sequentially cultivated in the same soil in the years to come.

In conclusion, the experimental evidences discussed in this review confirm that a precautionary approach towards GM crops should be adopted, by taking into account the risks associated with the unpredictability of transformation events, of their pleiotropic effects and of the fate of transgenes in natural and agroecosystems, and weighing benefits against costs.

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IMPATTO DELLE PIANTE GENETICAMENTE MODIFICATE SULLE
COMUNITÀ MICROBICHE DEL SUOLO

Riassunto

Le piante geneticamente modificate (GM) rappresentano un mezzo

potenzialmente utile per la realizzazione di un'agricoltura a basso impatto per la salute umana e per l'ambiente. Tuttavia, poco si conosce dei possibili rischi costituiti dalle modificazioni genetiche inattese che avvengono durante le manipolazioni di ingegneria genetica. Il numero crescente di dati sui rischi e i benefici ecologici delle piante GM rende necessari lavori sperimentali mirati alla valutazione dell'impatto delle piante GM coltivate sugli ecosistemi naturali ed agrari. I maggiori rischi ambientali associati alla coltivazione di piante GM includono il loro possibile impatto sui microrganismi non-target del suolo, che svolgono un ruolo fondamentale nella degradazione dei residui vegetali e nel completamento dei cicli biogeochimici. Recenti ricerche, basate su studi caso-per-caso, hanno dimostrato effetti delle piante GM sulle comunità microbiche del suolo, usando approcci sperimentali multimodali e diversi organismi, sia target che non-target. I risultati sperimentali discussi nel presente articolo confermano l'utilità di un approccio precauzionale che tenga conto dei rischi associati alla imprevedibilità degli eventi di trasformazione genetica, dei loro effetti pleiotropici e del destino dei transgeni negli ecosistemi naturali e coltivati, e che valuti i possibili costi e benefici legati al rilascio ambientale delle piante GM.