Impact of Genetically Modified Crops on Soil- and Plant-Associated Microbial Communities

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ABSTRACT

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.

The debate surrounding the use and commercialization of genetically modified crops is ongoing. Scientific as well as ethical concerns about the implementation of transgenic plants have been raised in public forums such as the Royal Society Expert Panel on the Future of Food Biotechnology (2001), the Assessing the Impact of GM Plants (AIGM) program for the European Science Foundation and The European Environmental Agency (Eastham and Sweet, 2002), and the American Academy of Microbiology (Nester et al., 2002). The majority of the scientific concerns discussed involve the risks incurred when genetically modified plants are grown in uncontrolled environments, such as agroecosystems. The risks include plant invasiveness or dispersal of the plant itself into the native ecosystem causing indirect impacts on the diversity of crops, gene flow through pollen transfer or through horizontal gene transfer with associated microorganisms, development of resistance in target organisms, and nontarget effects on native flora and fauna including effects on the biodiversity of beneficial and antagonistic microorganisms (Eastham and Sweet, 2002; Nielsen et al., 2001; Wolfenbarger and Phifer, 2000; Riba et al., 2000). The National Research Council (2002) discusses some of the risks of growing transgenic plants in agroecosystems. In addition, gene flow through pollen transfer was reviewed by Eastham and Sweet (2002) and horizontal gene transfer in the rhizosphere of transgenic plants was reviewed by Nielsen et al. (2001). Despite the controversial environmental impact, the global transgenic crop hectareage and number of countries utilizing recombinant DNA technology is growing yearly. According to the International Service for the Acquisition of Agri-Biotech Applications (ISAAA), in 2002, the estimated global area of genetically modified crops was 58.7 million ha, and global hectareage of transgenic crops has increased 35-fold since 1996 (Fig. 1) (James, 2003). However, only 16 countries commercially grew genetically modified crops, and four countries (USA, Argentina, Canada, and China) were responsible for 99% of the area of global transgenic crops (James, 2003). Although the global hectareage of transgenic crops is increasing yearly, many countries are still in the process of drafting legislation to regulate the use of commercial genetically modified crops. This process has been slowed by both philosophical and scientific debate surrounding the introduction of transgenic plants. An argument against approving the growth of transgenic plants involves the dependence upon seeds protected by intellectual property rights and owned by major agrochemical companies, therefore enriching large corporations and stripping farmers of their rights to reuse their seed. Other concerns include the elimination of crop and herbicide rotations, the potential for seed dispersal through contamination, cross-pollination with wild plants creating "superweeds," and the ability of the public to be adequately informed about the presence of genetic manipulations in their food through methods such as mandatory labeling (Greenpeace, 2003). In many countries, scientific assessments of the environmental risks of genetically modified plants are currently underway. 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. Rhizosphere microorganisms play a major role in nutrient transformations and element cycling. Any impact that genetically modified plants have on the dynamics of the rhizosphere and root-interior microbial community could have either positive or negative effects on plant growth and health, and in turn ecosystem sustainability. This review assesses relevant studies that have examined

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Published in J. Environ. Qual. 33:806–815 (2004).

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Abbreviations: PCR, polymerase chain reaction.

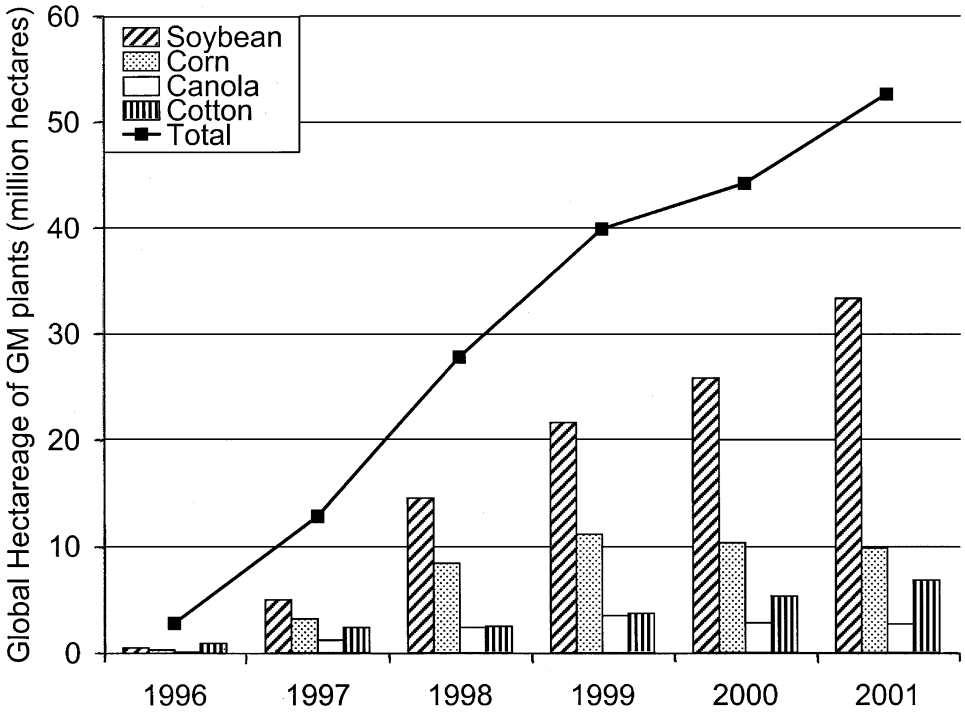

Fig. 1. Yearly global hectareage of genetically modified crops (James, 2003).

the impact of genetically modified plants on soil- and plant-associated microbial communities.

INTERACTION BETWEEN GENETICALLY MODIFIED PLANTS AND SOIL MICROBIAL COMMUNITIES

The first plants transformed by recombinant DNA technology were developed in the 1980s (Lal and Lal, 1993). Since then, DNA has been inserted into a number of crop species to achieve novel and desired traits. These genes are translated to novel proteins within the plant that will be released eventually into the soil ecosystem. Soil microbial communities have several opportunities to interact with novel plant gene products (Fig. 2). After harvest, decomposition of plant litter can release novel proteins into the soil environment (Donegan et al., 1997). The tillage system will influence the amount of interaction that occurs between the novel proteins and the microbial community (Angle, 1994). Under zero-tillage, crop residues are left concentrated on the soil surface, limiting the soil microorganisms that come in contact with the proteins to those at the soil surface. Under conventional tillage the plant litter will be incorporated into the soil, diluting the concentration of the gene products but increasing the number of organisms exposed (Angle, 1994). Transgene products have also been shown to be released directly from the plant roots from sloughed and damaged root cells as well as through root exudation. Transgenic Bt corn (*Zea mays* L.) was found to release a *Bacillus thuringiensis* insecticidal endotoxin from its roots (Saxena and Stotzky, 2000). The novel proteins then have the opportunity to interact with the soil microbial community throughout the growing season. For these reasons, it is inevitable that the novel gene products will eventually come into contact with the soil microbial community. However, the question remains whether those products will have any effect on soil microorganism function.

The Royal Society Expert Panel on the Future of Food Biotechnology (2001) suggested in a report that a consideration in the evaluation of genetically modified plants on ecosystem health is whether release of a single novel protein into the soil microbial community is significant in terms of the effect on soil function. In some cases changes to the microbial communities are inevitable. An example presented in the report was the transgenic corn cultivar NK4640Bt expressing the Bt toxin gene *cryIAb* that exudes some of the toxin protein from the root into the surrounding rhizosphere and soil, along with other proteins normally present in root exudates (Saxena et al., 1999). The report suggests that these routes of transgene product exposure are novel and will probably elicit a response from the rhizosphere and soil microbial community. Proteolytic microbes in the rhizosphere will respond to novel proteins or peptides present in the rhizosphere by degrading the novel proteins and assimilating the components.

Incorporation of transgenic plant products into the soil could alter soil microbial biodiversity due to variable responses by microorganisms to the novel proteins. Decreasing biodiversity is a concern because Tilman and Downing (1994) suggested that the preservation of biodiversity is essential for the maintenance of stable productivity in ecosystems. In general, most discussion of the impact of genetically modified crops on biodiversity has been focused on possible gene escape and gene flow

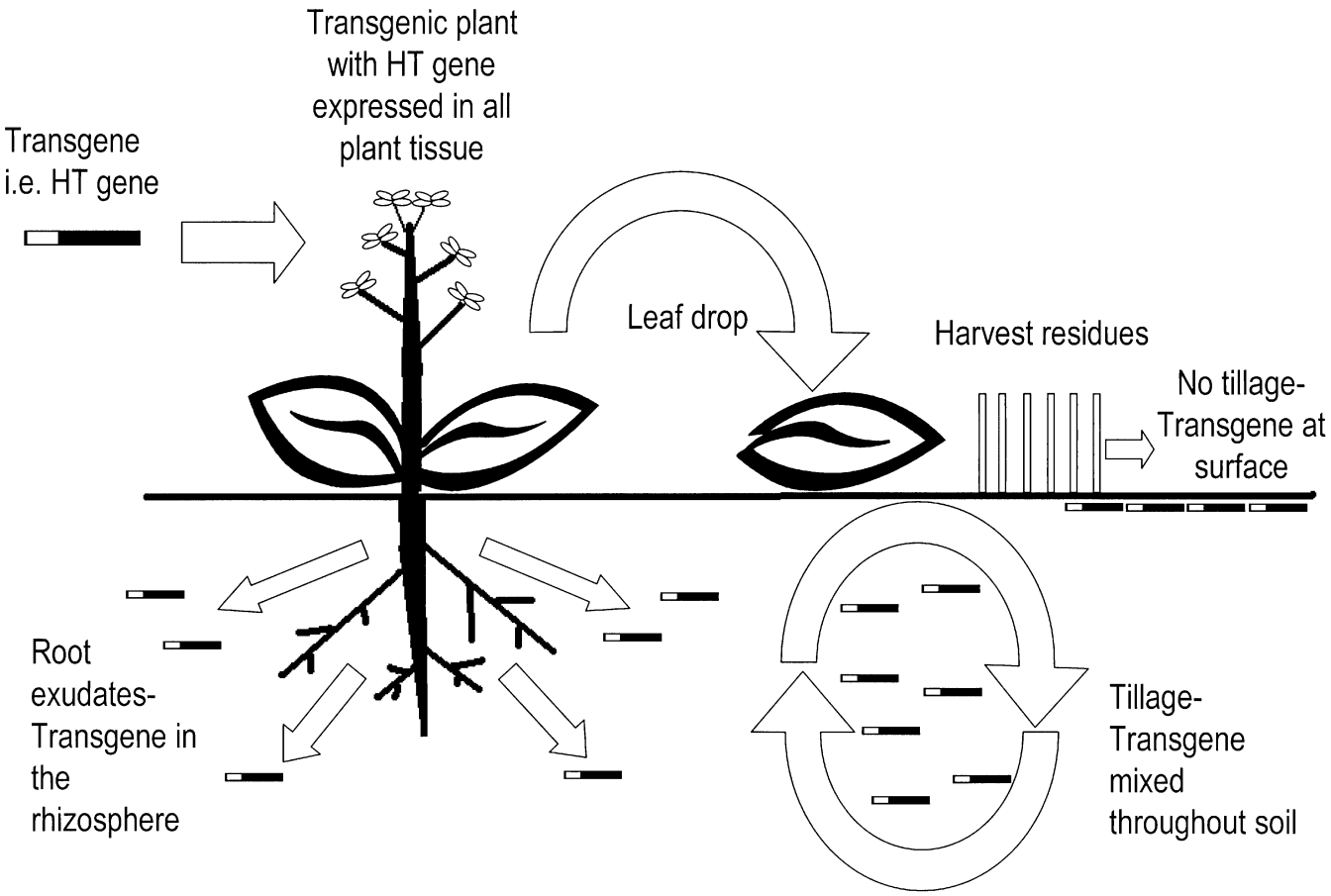

Fig. 2. Potential sites of interaction between transgenes and soil microbial community. HT, herbicide tolerance gene.

to wild relatives (Barton and Dracup, 2000; Eastham and Sweet, 2002), the replacement of traditional crop varieties with genetically modified crops (National Research Council, 2002), and possible effects on nontarget eukaryotic organisms, such as the effects of Bt corn pollen on the monarch butterfly (Losey et al., 1999). Because of the importance of soil microorganisms to soil ecosystem processes, it is important to examine the impact of genetically modified crops on the biodiversity of microorganisms. There are two main areas of study concerning the effects of transgenic plants on rhizosphere microorganisms: the possibility of horizontal gene transfer from transgenic plant to the microbial community, and the direct effect on the biodiversity of the microbial community through contact with novel proteins.

RISKS OF HORIZONTAL GENE TRANSFER TO BACTERIA IN THE SOIL ECOSYSTEM

A primary prerequisite for plant transformation research is the use of a selectable marker gene, so named because it confers the ability to survive in the presence of a normally toxic compound. Only transgenic cells and regenerating plants are thus selected for and maintained. The first selectable marker widely used in plant transformation work was a bacterial Tn*5* gene encoding neomycin phosphotransferase (*NPTII*) that confers resistance to the aminoglycoside antibiotics, kanamycin, neomycin, and G-418 (Dyer, 1996). One of the primary concerns about genetically modified crops is the presence of clinically important antibiotic resistance gene products in transgenic plants that could inactivate oral doses of the antibiotic. Another concern is that the antibiotic resistance genes could be transferred to pathogenic microbes in the gastrointestinal tract or soil, rendering them resistant to treatment with such antibiotics (Daniell et al., 2001).

Natural transformation is the uptake of naked DNA by competent cells (Reanney et al., 1982). This is an important method of genetic exchange in soil microorganisms because free DNA can be released into soil and bound to clays making it available for uptake by bacteria (Lorenz and Wackernagel, 1994). Through this mechanism soil organisms may be transformed by free DNA released from decomposing plant tissue and stabilized on soil particles. Natural transformation has been studied because it is one of the methods that may allow the dispersal of foreign transgenes, such as antibiotic resistance markers, to native soil bacteria (Paget et al., 1998; Widmer et al., 1996; Gebhard and Smalla, 1999; Nielsen et al., 2000a, 2000b; Nielsen and van Elsas, 2001).

A recent review by Nielsen et al. (2001) examined

horizontal gene transfer in the rhizosphere of transgenic plants. In order for natural transformation to occur in a soil environment, free DNA needs to be available and competent bacteria in the soil need to be in close vicinity to the DNA (Smalla et al., 2000). Transgenic plant DNA, such as the antibiotic resistance marker *NPTII*, has been shown to persist in field soil. Widmer et al. (1997) quantified marker gene persistence in the field, and found that marker genes from tobacco (*Nicotiana tabacum* L.) and potato (*Solanum tuberosum* L.) were detectable for 77 and 137 d, respectively. Similarly, Gebhard and Smalla (1999) showed that the DNA of transgenic sugar beet (*Beta vulgaris* L.) plants was detectable for several months in the soil under field conditions. The persistence of plant DNA in the soil is related to a number of abiotic and biotic factors. The content and type of clay minerals can affect DNA degradation by protecting free DNA from nucleases (Greaves and Wilson, 1970; Lorenz and Wackernagel, 1987; Khanna and Stotzky, 1992; Ogram et al., 1994; Widmer et al., 1997; Gebhard and Smalla, 1999). In addition, the presence of DNase in the soil can also affect the persistence of DNA in soil (Blum et al., 1997; Gebhard and Smalla, 1999).

While evidence for the persistence of transgenic plant DNA exists, the transformation of plant DNA to native soil microorganisms has not been found. Several studies attempted to assess natural transformation from plant DNA to soil microorganisms under field conditions and determined that while free DNA persisted in the soil, no proof of a plant gene being transferred to a soil bacteria was found (Widmer et al., 1997; Paget et al., 1998; Gebhard and Smalla, 1999). However, studies examining a naturally transformable bacterium (*Acinetobacter* sp. strain BD413) in sterile soil have shown that recombination can occur with transgenic plant DNA fragments (Gebhard and Smalla, 1999; Nielsen et al., 2000b). In combination, these results seem to suggest that a limiting factor in horizontal gene transfer of plant DNA is the availability of competent bacteria in the vicinity of the transformable DNA. Nielsen and van Elsas (2001) have shown that noncompetent *Acinetobacter* sp. strain BD413 cells in sterile soil could be stimulated to become competent in response to the presence of a variety of inorganic salts and simple carbon sources commonly found in root exudates. Due to the rapid initial degradation of plant DNA in different soil and field systems along with the persistence of marker DNA in the field for several months, possible transfer frequency of genes from plants to soil microorganisms may be very low and restricted to microhabitats that contain residual plant tissues and DNA complexed with soil particles (Widmer et al., 1997).

RISK OF TRANSGENIC PLANTS TO THE BIODIVERSITY OF THE SOIL MICROBIAL COMMUNITY

The effects of plant roots on soil microorganisms in the rhizosphere are well known [see Rovira (1965) for a review]. Furthermore, early studies by Neal et al. (1970, 1973) examined the genetic basis of rhizosphere effects (Table 1). Their studies found that the numbers and types of microorganisms inhabiting the rhizosphere of spring wheat (*Triticum aestivum* L.) were markedly affected by the substitution of a chromosome pair from a variety of spring wheat relatively resistant to common root rot, for the corresponding chromosome of S-615, a highly susceptible spring wheat variety. This simple genetic substitution produced a line unlike either parent with respect to most of the rhizosphere characteristics studied. While the lines used in this study were developed before modern genetic engineering techniques were available, traditional plant breeding techniques were used to achieve disomic substitution and develop lines that differed by one chromosome pair. This study showed that rhizosphere microflora characteristics can be qualitatively and quantitatively changed by substituting 1/21 of the genetic information from one variety for that of another through traditional plant breeding (Neal et al., 1970). Genetic engineering allows the formation of plants that differ by only one or two genes, resulting in the question of whether this small genetic difference is enough to influence the biodiversity of microorganisms in the rhizosphere of these genetically modified plants.

A more recent experiment by Oger et al. (1997) was designed to determine whether genetically engineered plants could influence rhizosphere microbial populations. The experimental model used the legume species bird's-foot trefoil (*Lotus corniculatus* L.) and bacteria indigenous to the soil. Plants were genetically engineered to produce low molecular weight compounds, opines, that may be used as growth substrates by a few of the root-associated bacteria. Oger et al. (1997) and Savka and Farrand (1997) demonstrated that opine-producing plants altered their microbial environment. In the rhizospheres of plants that were transformed to produce the opine, mannopine, the concentration of mannopine utilizers, was 80 times higher than in non-transformed plants, while the number of cultivable bacteria was not significantly different. They speculated that many metabolites overproduced by engineered plants would specifically stimulate the growth of bacteria degrading these metabolites. Their results demonstrate that the interaction between transgenic plants and their root-associated bacteria is highly specific. They speculate that any assessment study relative to the introduction of a given transgene into a genetically modified plant will be valid only for this transgene. Furthermore, they indicate that any transgene-associated biological effect will entirely depend on the identification of pertinent target populations.

In their 1997 study, Oger et al. demonstrated that genetically engineered plants might alter their biological environment, more precisely the root-associated bacterial populations. A response in the composition of the microbial population was observed after the introduction of a single genetic trait into the plant genome. A recent study has shown similar findings with different opines, with a second plant and in a second soil, showing

Table 1. Studies monitoring effects of transgenic plants on rhizosphere microorganisms.

Novel trait	Plant	Phenotype (gene)	Differences observed	Observed changes in rhizosphere of transgenic plants	Reference
Herbicide tolerant	rape (*Brassica napus* L.)	glyphosate tolerant (*EPSPS*†, *GOX*‡)	yes	community fatty acid and community-level physiological profiles (CLPP) altered	Siciliano et al. (1998)
			yes	taxonomic diversity of root-associated community altered	Siciliano and Germida (1999)
		glyphosate and glufosinate tolerant (*EPSPS*, *GOX*, *Pat*§)	some¶	community fatty acid and CLPP profiles differed for one line (Quest) out of four transgenic lines tested	Dunfield and Germida (2001)
	rape (*Brassica* spp.)	glufosinate tolerant (*Pat*)	yes	altered diversity of *Rhizobium leguminosarum* community	Becker et al. (2001)
			yes	minor differences in denaturing gel gradient electrophoresis (DGGE) pattern of eubacteria, different *Pseudomonas* population	Gyamfi et al. (2002)
	soybean [*Glycine max* (L.) Merr.]	glyphosate tolerant (*EPSPS*)	yes	increased colonization by *Fusarium* spp.	Kremer et al. (2000)
	corn (*Zea mays* L.)	glufosinate tolerant (*Pat*)	no	no differences with single strand conformation polymorphism (SSCP)–polymerase chain reaction (PCR)	Schmalenberger and Tebbe (2002)
Insect resistance	cotton (*Gossypium* spp.)	lepidopteran and coleopteran control (*Bacillus thuringiensis* var. *tenebrionsis* endotoxin)	yes	significant but transient stimulation of culturable bacteria and fungi and change in substrate utilization	Donegan et al. (1995)
	tobacco (*Nicotiana tabacum* L.)	insect resistance (Proteinase Inhibitor I)	yes	numbers of *Collembola* and nematodes	Donegan et al. (1997)
Insect resistance	potato (*Solanum tuberosum* L.)	invertebrate pest control (Con A and GNA lectins)	some¶	altered CLPP patterns	Griffiths et al. (2000)
Pathogen resistance	wheat (*Triticum aestivum* L.)	root-rot resistance (Chromosome S-615)	yes	composition of cultivable rhizosphere community	Neal et al. (1970, 1973)
	potato	resistance to phytopathogenic bacteria (T4-lysozyme)	some¶	different isolates identified in phyllosphere, no effect on DGGE fingerprints or catabolic profiles	Heuer and Smalla (1999)
			some¶	fewer species of antagonistic bacteria associated with plant, no differences in numbers and function of plant beneficial bacteria	Lottmann et al. (1999)
			yes	establishment of a T4-tolerant bacteria in rhizosphere	Lottmann et al. (2000)
			yes	higher bactericidal effect on *Bacillus subtilus* on root hairs	Ahrenholtz et al. (2000)
			no	no effect on antagonistic bacteria	Lottmann and Berg (2001)
			some¶	rhizosphere community structure associated with one line (DL4) differed from a second line tested and the control plants, no differences for other transgenic lines tested	Heuer et al. (2002)
Other	alfalfa (*Medicago sativa* L.)	α-amylase and lignin-peroxidase production	some¶	different populations of culturable microorganisms, altered enzyme activity and substrate utilization patterns; no differences in protozoa, nematodes, microarthropods, and DNA fingerprinting	Donegan et al. (1999)
			yes	enterobacterial repetitive intergenic consensus sequence (ERIC)–PCR patterns of communities in wells of GN BIOLOG plates	DiGiovanni et al. (1999)
	bird's-foot trefoil (*Lotus corniculatus* L.)	opine production	yes	increased numbers of opine utilizers	Oger et al. (1997)
			yes	increased numbers of opine utilizers	Mansouri et al. (2002)

† 5-Enolpyruvylshikamate-3-phosphate synthase.
‡ Glyphosate oxidoreductase.
§ Phosphinothricin acetyltransferase.
¶ Differences were observed in only some of the transgenic plant lines tested or with only some of the methodologies used to assess the microbial community.

that the effect was independent of the opine, plant, and soil (Mansouri et al., 2002). However, these studies were conducted using plants genetically engineered specifically to produce compounds known to be growth substrates for a few root-associated bacteria (Savka and Farrand, 1997). The question remained whether plants genetically modified for specific traits such as herbicide or insect resistance would have similar effects on the root-associated microbial community. There have been two major approaches to answer this question. The first is to examine the effects of genetically modified plants on specific groups of ecologically important soil microorganisms, and the second is to examine the effects of the genetically modified plants on the whole soil microbial community.

Effect of Genetically Modified Plants on Specific Groups of Soil Microorganisms

The implementation of genetically modified crops into crop rotations has provided a few indications that these new varieties may be affecting some important soil microorganisms. For example, glyphosate applied to Roundup Ready soybean [*Glycine max* (L.) Merr.] cultivars (Monsanto, St. Louis, MO) enhanced colonization by *Fusarium*, a known root pathogen (Kremer et al., 2000). This type of interaction could severely reduce the benefits of using these cultivars in a crop rotation.

A number of studies have been conducted in Germany to determine potential adverse effects of growing transgenic T4 lysozyme expressing potato plants, developed to enhance resistance against the bacterial pathogen *Erwinia carotovora*, on rhizobacterial populations (Lottmann and Berg, 2001). Initially it was found that many other bacteria and fungi are sensitive to T4 lysozyme in vitro (De Vries et al., 1999), also T4 lysozyme has been found to be active in the phyllosphere (Heuer and Smalla, 1999), the rhizosphere (Lottmann et al., 1999), and on the root hairs of transgenic potatoes (Ahrenholtz et al., 2000). Lottmann et al. (2000) questioned whether the T4 lysozyme from the transgenic potatoes would have an adverse impact on two potential biocontrol bacterial strains. Their eventual goal was to combine the two biocontrol methods (i.e., transgenic plants and biocontrol bacteria). They found that significantly more colony counts of T4-lysozyme tolerant *Pseudomonas putida* were recovered from the transgenic plants than from control plants. However, no negative effect of T4 lysozyme on the establishment of the biocontrol strains in the rhizo- and geocaulosphere was observed under field conditions.

Lottmann and Berg (2001) characterized antagonistic bacteria associated with transgenic potato. Their focus group of organisms was the fluorescent pseudomonads and enterobacterial rhizobacteria. Functional diversity of antagonistic bacteria was determined through fatty acid methyl esters (FAMEs), in vitro assays vs. phytopathogens, production of indole acetic acid, sensitivity to T4 lysozyme, and repetitive sequence polymerase chain reaction (BOX–PCR). The phenotypic analysis showed no correlation between bacterial genotypes and plant genotype, supporting the results of their previous investigations where the functions of rhizobacterial bacteria obtained from transgenic and non-transgenic potatoes were not influenced by the expression of T4 lysozyme (Lottmann et al., 1999, 2000; Lottmann and Berg, 2001).

Effect of Genetically Modified Plants on Microbial Communities

The second approach toward studying the impact of genetically modified plants on soil microorganisms is to study the structure or functioning of the whole community, rather than to focus on a specific group of microorganisms.

Initial work examined the effects of decomposing transgenic plant litter on soil ecosystems. Leaves of transgenic cotton (*Gossypium* spp.) containing the *Bacillus thuringiensis* toxin were placed into soil in a laboratory study (Donegan et al., 1995). A transient increase in culturable populations of bacteria and fungi was caused by two out of three transgenic cotton lines tested. Donegan et al. (1997) investigated the potential ecological impact of genetically engineered plants on soil ecosystems by burying litterbags containing leaves of transgenic tobacco that expressed Proteinase Inhibitor I into field plots. They found differences in carbon content between the decomposing parental and transgenic plant litter supporting the concern that genetic manipulation of plants may produce changes in plants that are unintended. They also found differences in nematode and *Collembola* numbers in the soil surrounding the transgenic plant litterbags.

Further work in this area has primarily focused on the potential that root exudates from genetically modified plants can influence rhizosphere microbial communities. One of the first studies by Siciliano et al. (1998) assessed the root-interior and rhizosphere bacterial communities associated with a field-grown genetically modified canola (oilseed rape, *Brassica* spp.) variety, Quest, and two conventional canola varieties. The carbon utilization patterns and fatty acid methyl ester profiles of the microbial community associated with the roots of the genetically modified canola variety differed from the profiles of two conventional canola varieties. Furthermore, isolation and characterization of representative bacteria showed that the composition of the cultivable microbial community associated with a genetically modified canola variety was significantly different than the conventional canola varieties (Siciliano and Germida, 1999). Follow-up work has confirmed that the root-interior and rhizosphere bacterial community associated with the genetically modified canola variety, Quest, was different from two conventional canola varieties tested; however, the finding was not generalized for other genetically modified canola varieties tested (Dunfield and Germida, 2001).

More work examining herbicide-resistant genetically engineered canola or oilseed rape also shows differences in the microbial communities associated with transgenic canola plants. Gyamfi et al. (2002) found minor differences in the denaturing gel gradient electrophoresis

(DGGE) patterns of the eubacterial population associated with transgenic canola; however, this was subject to seasonal variation. Furthermore, the transgenic plants hosted different *Pseudomonas* populations than wild-type plants throughout the growing season. Similarly, different populations of *Rhizobium leguminosarum* bv. *viceae* were associated with transgenic Basta-tolerant (glufosinate-tolerant) oilseed rape compared with their non-transgenic counterparts (Becker et al., 2001). In contrast, the microbial communities associated with glufosinate-tolerant transgenic maize were not different in their single strand conformation polymorphism (SSCP)–PCR patterns compared with those communities associated with wild-type maize plants (Schmalenberger and Tebbe, 2002).

Other transgenic plants have also been shown to have an impact on soil microorganisms. DiGiovanni et al. (1999) used enterobacterial repetitive intergenic consensus sequence (ERIC)–PCR to characterize bacterial communities associated with transgenic alfalfa (*Medicago sativa* L.) and present in Biolog (Hayward, CA) Gram-negative microplates. Using both cultural and molecular approaches, differences between the rhizosphere bacterial communities of the parental genotype and the alpha amylase and lignin peroxidase transgenic alfalfa were detected. Donegan et al. (1999) used these transgenic alfalfa lines in a field study that looked at the combination of transgenic alfalfa and recombinant microorganisms. They found that the metabolic fingerprints of the microbial community were different in the rhizosphere of transgenic compared with non-transgenic alfalfa.

Griffiths et al. (2000) determined whether transgenic potatoes producing the lectins Con A and GNA affected nontarget soil organisms and processes. The effects were assessed with respect to a range of organisms (bacteria, protozoa, nematodes, and plants) and processes (substrate utilization, microbial activity, decomposition, and plant growth). The microbial community in the soil under transgenic GNA lines consistently had a different community-level physiological profile from that of the control line at harvest. The fact that the range of Biolog substrates responsible for the differences was not consistent between lines or years suggests that the effect was due to a genotype–environment interaction leading to different chemical inputs into the soil (Griffiths et al., 2000). Dunfield and Germida (2001) also demonstrated that field site influenced microbial community composition and interacted with plant varieties in their influence on the microbial community. The effect of plant variety on the microbial community at one field site was sometimes entirely different at another field site, suggesting that the environment will play a major role in determining the potential ecological significance of growing genetically modified plants (Fig. 3). A timecourse study examining genetically modified plants over an entire field season suggests that changes to the microbial community structure associated with genetically modified plants are not permanent. Community-level physiological profiles and fatty acid methyl ester and 16S rDNA analysis all showed that a variety of transgenic canola

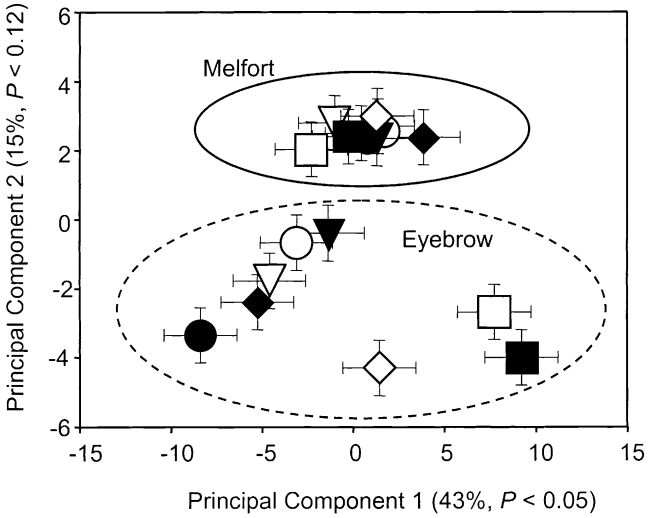

Fig. 3. Principal component analysis (PCA) of fatty acid methyl esters (FAMEs) obtained from the rhizosphere soil of canola cultivars grown at Eyebrow and Melfort, Saskatchewan, in 1998. Each symbol is the average of four replicates at one field site (n = 4). Closed symbols represent conventional varieties: triangles, Excel replicates; squares, Fairview replicates; diamonds, Hyola replicates; circles, 45A71 replicates. Open symbols represent genetically modified varieties: triangles, Exceed replicates; squares, Innovator replicates; diamonds, Invigor replicates; circles, Quest replicates. Error bars represent the standard error of the mean. Percent variation and P values are marked in parentheses. Reproduced with permission from Dunfield and Germida (2001).

significantly influenced microbial community structure over multiple field sites and years; however, there were no differences between the microbial communities associated with canola plants from the April sampling time (after plants were harvested in the preceding September (Fig. 4) (Dunfield and Germida, 2003). A study examining terminal restriction fragment length polymorphisms (T-RFLP) patterns associated with the rhizosphere of field-grown transgenic Barnase/Barstar and GUS potato plants also showed spatial effects, temporal effects, and spatial by temporal interactions (Lukow et al., 2000). Similarly, a three-year field study showed that the rhizosphere community structure associated with one transgenic line of T4-lysozyme potato (DL4) tested was different than the community structure associated with a second transgenic line (DL5) and the control line (DES); however, environmental factors had a more important influence on the microbial community structure associated with T4 lysozyme expressing transgenic plants than the transgenic nature of the plants (Fig. 5) (Heuer et al., 2002).

Collectively, these results seem to indicate that microbial diversity can sometimes be altered when associated with transgenic plants; however, these effects are minor in comparison with environmental factors such as sampling date and field site.

CONCLUSIONS

The environmental impact of transgenic crops on the environment has been a source of debate since their commercial introduction in 1996. Governments have chosen to address these concerns in different manners.

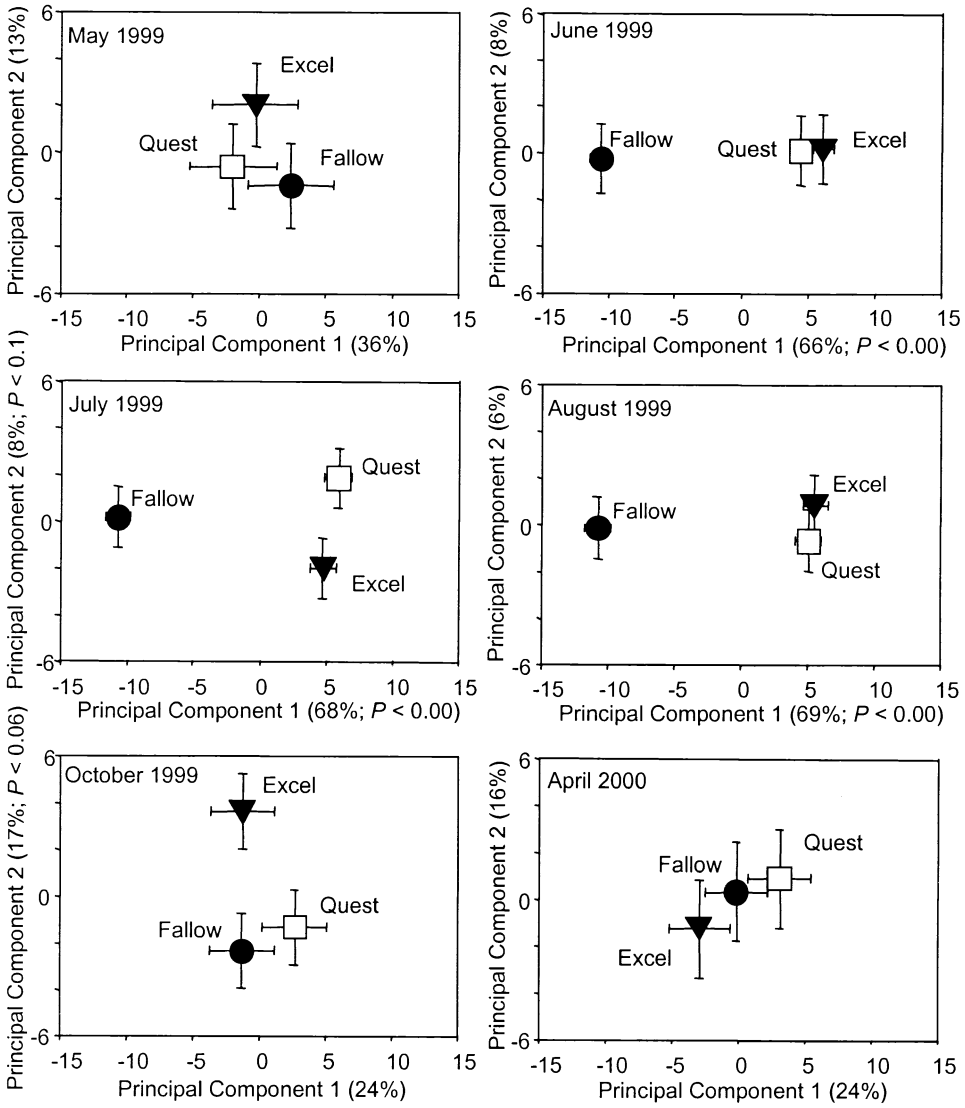

Fig. 4. Principal component analysis (PCA) of community-level physiological profiles (CLPP) obtained for microbial communities from fallow soil and rhizosphere microbial communities of canola varieties grown at Watson, Saskatchewan, sampled in May, June, July, August, and October 1999 and April 2000. Closed circles represent fallow soil (n = 4). Closed triangles represent conventional variety, Excel (n = 4). Open squares represent genetically modified variety, Quest (n = 4). Error bars represent the standard error of the mean. Percent variation explained by each PC is marked in parentheses. P values have been included when a significant variety effect is present, as determined by analysis of variance (ANOVA). Reproduced with permission from Dunfield and Germida (2003).

Countries such as Argentina, Canada, China, and the United States have rapidly adopted transgenic crops into their commercial agricultural operations, growing 99% of the transgenic crops worldwide, while the European Union and Japan have chosen to restrict their use until full environmental assessment can be made. More than 20 scientific assessments of transgenic plants have been published in peer-reviewed scientific journals. We now understand that transgenic plants and plant litter can influence the composition of the plant-associated microbial communities. Moreover, these effects have been shown in a variety of plants with different transgenes. However, it has also been shown that these effects are dependent on field site, seasonal variation, and method of analysis used to assess the community. The changes in microbial communities associated with growing transgenic crops are relatively variable and transient in comparison with some other well-accepted agricultural practices such as crop rotation, tillage, herbicide usage, and irrigation. Since minor alterations in the diversity of the microbial community, such as the removal or appearance of specific functional groups of bacteria such as plant-growth-promoting rhizobacteria, phytopathogenic organisms, or key organisms responsible for nutrient cycling processes, could affect soil health and ecosystem functioning, the impact that plant variety may have on the dynamics of rhizosphere microbial populations and in turn plant growth and health, and ecosystem sustainability, requires further study. Future work needs to address long-term effects of transgenic crops in rotation, while keeping in mind that these effects should not only be compared with a non-transgenic counterpart, but also to other acceptable changes in the agroecosystem, such as growing a novel non-transgenic plant or utilizing a new agronomic practice.

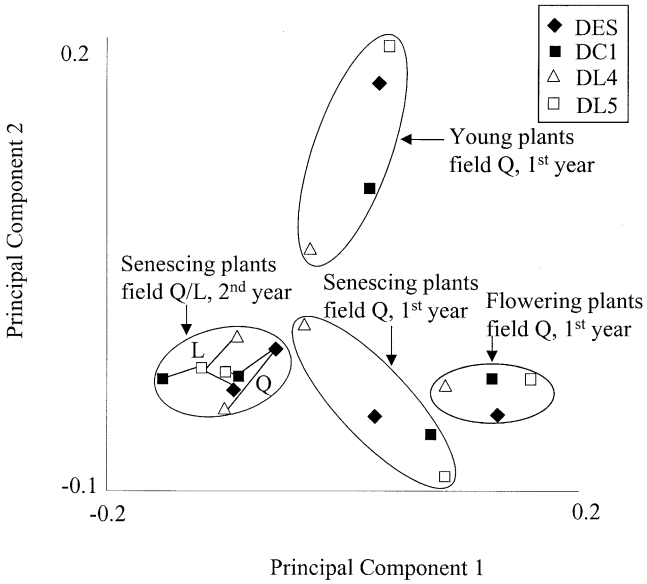

Fig. 5. Principal component analysis (PCA) of the relative species compositions (based on identification by fatty acids) of cultured rhizobacteria from the non-transgenic control (DES), transgenic control plant line (DC1), and transgenic lines (DL4 and DL5) in five samplings. The clustering of the species patterns was based on PCs 1 and 2, explaining 28 and 16% of the variance, respectively. Reproduced with permission from Heuer et al. (2002).

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