Genetically Engineered Crops - A Threat to Soil Fertility?

in

- **GE Failures & Contaminations** [1]

Editor: Jaan Suurkula M.D.
Published on March 21, 2001, Last revised on September 5, 104

The decisive role of Soil Ecology for fertility

Research has demonstrated the great importance of soil organisms for the fertility of the soil. In one gram of productive soil there is a complex web that can exceed over 100 million microorganisms that may represent over 1000 species. The main components are bacteria, fungi, algae, protozoa, nematodes, earthworms, and insects. Out of these, bacteria and fungi constitute about 80%, the proportions of these two depending on soil type. There is a complex ecological interdependence between all soil organisms. Together they are responsible for the cycle of decomposing and restructuring organic material so that it will be accessible to growing plants. It is also responsible for the nitrogen and water-retaining properties as well as for other factors of great importance for soil fertility. "Without the soil foodweb, plants would not obtain the nutrients necessary for growth, and the above ground foodweb would not long continue" according to Nannipieri, P. et al. (1990).

The knowledge about soil microecology is still incomplete but enough is known to conclude that it is of decisive importance for soil fertility. Ingham has established measurable criteria for predicting soil fertility on the basis of the microbial conditions in the soil, see Ingham, E. (1998).

Naseby D.C and Lynch J.M. (1998) wrote "Because of the importance of soil biota in mineralization and immobilization of nutrients, physical and biochemical degradation of organic matter, biological control of plant pests, and as food sources for other organisms, it is crucial to evaluate the potential impacts of transgenic plants on soil ecosystems".

Potential effects of Genetically Engineered (GE) crops on soil microbes

In genetic engineering, a package of novel genes are inserted into the recipient organism. In addition to the desired property gene, a number of other genes have to be added to ensure successful insertion.

Among the potentially problematic genes inserted into plants, those that help overcome the barriers against the introduction of foreign genes are of particular interest in the context of soil ecology. They function as vectors for successful insertion and prevent rejection of inserted foreign genes. These vector packages are chimaeric combinations of genetic elements commonly from pathogenic bacteria and viruses and from transposons.

Ho, M.W. & Tappeser, B. (1997) have proposed that the vector DNA in GE crops may promote horizontal transfer of genetic material between unrelated bacterial species. They warned that the result may be new human pathogenic bacteria.

This idea was further developed by Ho, M.W. et al. (1998). They refer to experimental observations indicating the possibility of gene transfer not only between related bacteria but also between bacteria of different species, as well as between bacteria and fungi and between bacteria and higher organisms, including mammals. They warn that the vector DNA may be transferred from GE plants to soil bacteria and soil fungi and contribute to increased horizontal transfer. They suggest that this may have contributed to the emergence of new human pathogenic bacteria during the last 10-15 years, some of which have been very harmful.
We want to extend this idea of Ho et al to Soil Ecology:

Hypothesis

Horizontal transfer of genes between soil micro-organisms may be facilitated by vector DNA from genetically engineered plants, resulting in such changes or disturbances in the functioning of the micro-organisms that soil ecology and fertility may be affected.

Evidence in support of the hypothesis

As important parts of the reasoning below is based on the hypothesis of Ho et al (1998), relevant parts of the text have been taken from there with permission.

Inductive

1. Direct experimental evidence of horizontal gene transfer, some between phylogenetically distant species, has been obtained in all natural environments, as well as in the gastrointestinal tract. These were all accomplished using artificially constructed vectors.
2. DNA released from dead cells (as well as live cells) is not readily broken down in the environment, nor in the gastrointestinal tract, where they may retain the ability to transform other bacteria.

Deductive

1. Genetic engineering is based on facilitating horizontal gene transfer between distant species by constructing vectors that break down barriers between species.
2. The artificial vectors constructed for genetic engineering are chimaeric combinations of viral pathogens and other invasive genetic elements that can generate new cross-species pathogens.
3. The artificial vectors constructed for genetic engineering are inherently unstable and prone to recombination, thereby enhancing horizontal gene transfer and recombination.
4. Shuttle vectors made by genetic engineering are essentially unstoppable, as they contain signals for transfer and replication in different species. Helper functions for mobilization and transfer can be supplied by viruses, plasmids and transposons, which occur naturally in bacteria in all environments.

Inductive evidence

1. Transfers have occurred from bacteria to higher plants and vice versa.

The best known example is the direct demonstration of transfer between the soil bacterium, Agrobacterium and plants. In a process bearing a strong resemblance to conjugation between bacteria, the tumour (T) segment of the tumour-inducing (Ti) bacterial plasmid is transferred and incorporated into the plant genome (Kado, C.I., 1993; Stachel, S.C., Timmerman, B. & Zambryski, P., 1986). However, it must be noted that nearly all cases of directly demonstrated horizontal gene transfer, especially those involving phylogenetically distant species, made use of already modified hybrid shuttle vectors that can transfer between species and replicate in both. These shuttle vectors possess signals for replication (origins of replication) in more than one species as well as the signal for DNA transfer (origin of transfer), and are hence much more likely to be successful in horizontal transfer than unmodified plasmids found naturally. The Ti plasmid is, indeed, the basis of a gene transfer vector system widely used for genetically engineering crop-plants.

Cross-Kingdom horizontal gene transfer by conjugation has also been demonstrated between bacteria and yeast using shuttle vectors derived from broad host-range promiscuous plasmid that are already transferable between many bacterial species (Heinemane, J.A. & Sprague, G.R., Jr., 1989; Sikorski R.S. & Hieter, P., 1989). Such vectors can even substitute for the Ti plasmid in transferring genes from Agrobacterium to plants (Buchanan-Wollaston, V. Passiatore, J.E. & Cannon, F., 1987).

The direct transfer of transgenes and marker genes from transgenic plants to soil fungi has been demonstrated (Hoffman, T., Golz, C. & Schieder, O., 1994).

Also transfer of transgenes from plant DNA to soil bacteria has been reported. The kanamycin resistance marker gene was transferred to the soil bacterium Acinetobacter in an experiment using DNA that was extracted from homogenized plant leaf from a range of transgenic plants, including...
Genetically Engineered Crops - A Threat to Soil Fertility?
Published on NW Resistance Against Genetic Engineering (http://nwrage.org)

potato, tobacco, sugar beet, oil-seed rape and tomato (De Vries, J. & Wackernagel, W., 1998). Furthermore In 1999, researchers in Germany reported from the first field-monitoring experiment in the world, that transgenic DNA had transferred from the GM sugar beet plant debris to bacteria in the soil (Gebhard F and Smalla K.1999).

Transfer to soil bacteria was also observed by Schluter et al 1995. Despite the title of their publication, Schluter, K. et al. (1995) actually observed a high "optimal" gene transfer frequency of 6.2 x 10-2 in the laboratory, from which they "calculated" a frequency of 2.0 x 10-17 under extrapolated "natural conditions". However such extrapolations are inevitably uncertain and the authors themselves admit that "synergistic effects cannot be ruled out" that would significantly increase the frequency.

Nielsen et al (1998) however could not confirm horizontal transfer to soil bacteria. However, less than one percent of all bacteria in the environment can be isolated and monitored for horizontal gene transfer (Pace, N. 1997). So negative results in the field cannot be taken as evidence of non-transfer. It cannot be excluded that among the 99% of bacteria that cannot be isolated, there are such that are very prone to horizontal transfer.

Transformation (pieces of genetic material taken up into the cell from the environment) may be a major route of horizontal gene transfer: frequencies obtained under different environmental conditions, using artificial vectors and markers are found to be generally quite high, many ranging between 10-2 to 10-5 transformant per viable cell. In fact, transformation frequencies are often higher under natural conditions than in the laboratory.

In a recent study in soil microcosms, the presence of earthworms significantly enhanced gene transfer between spatially separated bacterial species inoculated into the soil (Daane, L.L., Molina, J.A.E. & Sadowsky, M.J., 1997). A special form of bi-directional transformation by cell contact and fusion is now known to be widespread.

The roots of plants exudate DNA. So transgenic DNA is available here for horizontal transfer to soil bacteria. Timms-Wilson et al (1999) call the rhizosphere an environmental hotspot' for gene transfer.

The requirement for transformation, the presence of naked DNA in the soil (bound on surface active particles) is fulfilled, as explained in the following section.

2. DNA released from plant cells are not readily broken down in the general environment

Genes carried by vectors as naked DNA as well as chromosomal DNA, can survive for long times, especially when adsorbed to solid particles in the environment, e.g. on surfaces of sediment and in the soil, where they are efficiently taken up by other microbes (Crecchio, C. & Stotzky, G., 1996; Stotzky G, 2000; Jager, M.J. & Tappeser, B., 1995). Although DNA is rapidly broken down in waste water, adsorption to solid particles in the sludge, which happens very quickly, will stabilize it and prolong its transforming capacity. In addition, DNA has been found to persist for long periods in the laboratory by binding on many different surfaces.

Transformation by the uptake of DNA is a major route of horizontal gene transfer in the environment. DNA is not only released when cells die, but it is actively secreted by living cells, and even fragments of genes can have significant effects when transferred. Some species export DNA wrapped in membrane-bound vesicles. The DNA in a culture slime can be more than 40% of the dry weight. Thus, the environment is extremely rich in DNA. Fresh water contains between 0.5 to 7.8 mg per liter, while freshwater sediment has an upper concentration of 1 mg per gram. Although enzymes breaking down DNA (deoxyribonucleases, DNases) are found in the environment, DNA is protected from degradation by adsorbing to detritus, humic acid and, in particular, clay and sand particles. Adsorbed DNA is equally efficient in transforming cells. Thus, the half-lives of DNA (time for half of the DNA to be broken down) in soil is 9.1 hours for a loamy sand soil, 15.1 h for a silty clay soil and 28.2 h for a clay soil. While half-lives in waste water are typically fractions of an hour, those in freshwater and marine water are 3 to 5 hours, with high values of 45 to 83 h on the ocean surface, and extremely high values of 140 and 235 hours for the marine sediment (Lorenz, M.G. & Wackernagel, W., 1994).
Adsorption of DNA to solid particles is a very rapid process, which means that DNA released into the environment can survive and maintain its potential to transform other organisms.

**Deductive Evidence**

1. Genetic engineering is based on facilitating horizontal gene transfer between distant species by constructing vectors that break down species barriers.
2. The artificial vectors constructed for genetic engineering are chimaeric combinations of viral pathogens and other invasive genetic elements that can generate new cross-species viral pathogens.
3. The artificial vectors constructed for genetic engineering are inherently unstable and prone to recombination, thereby enhancing horizontal gene transfer and recombination.
4. Shuttle vectors made by genetic engineering are essentially unstoppable, as they contain signals for transfer and replication in different species; and helper functions for mobilization and transfer can be supplied by viruses, plasmids and transposons, which occur naturally in bacteria in all environments.

One main contributing factor that may increase the scope and frequency of horizontal gene transfer may be the deliberate attempts of genetic engineers to break down species barriers. These attempts include constructing a range of chimaeric vectors for cloning and transferring genes. These artificial vectors have the following important characteristics that enhance horizontal gene transfer.

- They are derived from elements that mediate horizontal gene transfer most effectively.
- Their chimaeric nature means that they possess sequence homologies to DNA from widely different species and their viral pathogens, plasmids and transposons, thus facilitating successful horizontal transfer and recombination.
- They often have origins of replication and transfer sequences, all of which facilitate horizontal gene transfer and recombination. In this context, the fact that they are "crippled", so that genes for mobility and/or virulence are removed, is irrelevant, as helper functions can be supplied by other viruses, plasmids and mobile genetic elements present in the donor, recipient or a third strain of bacteria. And virulence genes can be regained by recombination.
- It is well-known that chimaeric plasmids and viral vectors are subject to structural instabilities that make them more prone to recombine (Old, R.W. & Primrose, S.B., 1994). Vector instability is a continuing problem for genetic engineers and the biotech industry as far as the stability of the transferred genes is concerned. It also increases the probability and scope for unintended, secondary horizontal gene transfer, which has already been directly demonstrated.
- The now routine incorporation of strong promoters and enhancers in vectors to boost expression of transgenes is one main cause of structural instability, which is in addition to the instability arising from the attendant metabolic stress to the organism that, again, may increase unintended horizontal gene transfer (Ho, M.W. & Steinbrecher, R., 1997).
- Finally, vectors are designed to escape restriction (Hafle, M.G., 1994) thereby also enhancing the probability of successful horizontal gene transfer.

Although different classes of vectors are distinguishable on the basis of their main framework sequence, practically each of them is chimaeric. Important chimaeric vectors are the shuttle vectors that enable genes to be cloned (multiplied) in E. coli and transferred (transfected) into unrelated species in every Kingdom. Similarly, vectors used in manipulating plants and animals typically contain sequences from a range of plant and animal viral pathogens, as well as antibiotic resistance genes, often originating from promiscuous resistance plasmids and transposons. Phage vectors and plasmid vectors (hybrid of phage and plasmid) are also extensively used and may have special relevance for the evolution of pathogenicity islands in bacterial pathogens.

Thus, genetic engineering biotechnology has opened up highways for horizontal gene transfer and recombination, where previously, there was only restricted access through narrow, tortuous footpaths. Nielsen et al (1998) wrote:

" Transfer frequencies should not be confounded with the likelihood of environmental implications, since the frequency of [horizontal gene transfer] is probably only marginally important compared with the selective force acting on the outcome. Attention should therefore be focussed 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”.

We review further circumstantial evidence that artificial gene transfer vectors increase the scope and frequency of horizontal gene transfer.

Circumstantial evidence that artificial gene transfer vectors increase the scope and frequency of horizontal gene transfer.

It is not easy to transfer genes successfully between species, as we have already emphasized, because there are barriers to horizontal gene transfers (see Ingham, E.). That is why, apart from transposons which are promiscuous, such events were relatively rare in our evolutionary past.

Horizontal gene transfers have been directly demonstrated between bacteria in marine environments (Frischer, M.E., Stewart, G.J. & Paul, J.H., 1994; Lebaron, Ph., Batailler, N. & Baleux, B., 1994; Sandaa, R.A. & Enger, Å., 1994), in freshwater environments (Ripp, S., Ogunseitan, O.A. & Miller, R.V., 1994) and in soil (Stotzky, G. & Babich H., 1986; Stotzky G., 1989; Neilson, J.W., Josephson, K.L., Pepper, I.L. et al., 1994). Again, in some of the experiments, horizontal gene transfers were mediated by specially constructed hybrid plasmid vectors, of the sort used in genetic engineering. Also conjugation and transduction occurred.

Horizontal gene transfer occurs preferentially in interfaces between air and water and in the sediment and, especially under nutrient depletion conditions (Goodman, A.E., Marshall, K.C. & Hermansson, M., 1994), thus refuting the claim that nutrient-rich media are necessary to support horizontal gene transfer. Horizontal gene transfer of antibiotic resistances has even been demonstrated in wastewater treatment ponds, the effluent from which is increasingly being used for irrigation in developing countries (Mezrioui, N. & Echab, K., 1995). As pointed out in Ho, M.W. et al. (1998, section 7.1), frequencies of horizontal gene transfer may be greater under natural conditions than in the laboratory (Daane, L.L., Molina, J.A.E. & Sadowsky, M.J., 1997).

Horizontal gene transfer occurs preferentially in interfaces between air and water and in the sediment and, especially under nutrient depletion conditions (Goodman, A.E., Marshall, K.C. & Hermansson, M., 1994), thus refuting the claim that nutrient-rich media are necessary to support horizontal gene transfer. Horizontal gene transfer of antibiotic resistances has even been demonstrated in wastewater treatment ponds, the effluent from which is increasingly being used for irrigation in developing countries (Mezrioui, N. & Echab, K., 1995). As pointed out in Ho, M.W. et al. (1998, section 7.1), frequencies of horizontal gene transfer may be greater under natural conditions than in the laboratory (Daane, L.L., Molina, J.A.E. & Sadowsky, M.J., 1997).

Stephenson & Warnes (1996, p.5) wrote, "The threat of horizontal gene transfer from recombinant organisms to indigenous ones is very real and mechanisms exist whereby, at least theoretically, any genetically engineered trait can be transferred to any prokaryotic organism and many eukaryotic ones.”.

A year later, another molecular geneticist, who works on transgenic plants, admitted that, "..the potential for horizontal [gene] transfer may be greater than thought previously." (Harding, K., 1996). Potential agricultural consequences

The great problem in predicting potential outcomes here is that the suggested horizontal transfer mechanism would have very diverse results, impossible to predict. Only by multiple experiments will it be possible to get some idea whether this can effect soil fertility.

We can only speculate on some potential scenarios that might be the result if our hypothesis is correct:

1. The scenario that would seem most likely is that there occurs an accumulation of vector DNA in the soil microbiota with repeated GMO cultivation. This would enable horizontal transfer between unrelated species, leading to a cumulative loss of soil biodiversity over repeated harvests. Diversity has been found to be important for soil fertility, see e.g. Hawksworth, DL, 1991.

This might be difficult, if not impossible, to repair if there is a large reservoir of horizontal transfer-promoting vectors in the soil microbiota. If so, permanent decrease of fertility might be the end result.

2. A second possible complication might come from horizontal transfer of the toxin gene from Bacillus thuringiensis (BT) to soil bacteria from GE crops with this gene (this is a common GE application).
Stotzky et al have recently shown that BT toxins from GE crops will, unlike natural BT toxins not disappear when added to soil, but become rapidly bound to soil particles and are not broken down by soil microbes. They warn that engineered BT toxins could build up in the soil. See Koskella J. and Stotzky G. (1997) and Tapp H. and Stotzky G. (1998). Stotzky warns that the BT toxins bound in soil "could pose a potential hazard to non-target organisms" (Stotzky G. 2000). Also it has been warned that transgenic cyanobacteria carrying the BT gene might transfer this toxin gene to other microbes (Entwistle P.F. et al 1993).

If the BT gene is taken up by soil bacteria, some might perhaps produce BT toxins. In the worst case, after repeated cultivation of BT-GMOs, a cumulatively increasing production of BT-toxins might ensue in the soil, which could damage soil organisms important for fertility.

3. A third scenario might be that a new variety or species arises that is able to overgrow or damage some essential species of soil microorganism so that the ecological balance would be disrupted. If it has a good survival and multiplication capacity, including sporulation ability, it might spread widely through wind erosion and through the ground water. New virulent and harmful species might cause reduction of fertility in infected soils.

Summary

Some studies suggest the possibility of horizontal transfer of DNA from transgenic crops to soil microorganisms. The persistence of naked DNA in the soil is well documented. It has been shown that such DNA can be taken up by soil microorganisms. Transgenic DNA contains vectors designed to promote uptake of genes by DNA from unrelated species. Some experimental evidence indicate that such vectors can promote interchange of genetic material between micro-organisms.

If these mechanisms work, our hypothesis is that there might occur an increased transfer of genetic material between unrelated microorganisms that might cause disturbances in their functioning so that they cannot make their normal contribution to the complex interplay between soil microorganisms that is important for soil fertility. The diversity of the soil ecology, which is associated with good fertility, may be lost to an increasing extent. It seems likely that such a change might be irreversible.

Another potential complication might be the uptake of BT toxin genes by soil microbes. If this occurs, it needs to be elucidated if this might lead to BT toxin production in the soil to such an extent that it is harmful to soil insects that contribute to soil fertility.

If transgenic DNA is transferred to soil microorganisms and can persist, it might accumulate as long as transgenic crops are cultivated, which would increase the risk for the suggested kinds of damage.

Only if the transgenic DNA confers a competitive disadvantage to the microorganisms, will it gradually disappear. Otherwise it is likely to remain for a long time, if not permanently after cultivation of transgenic crops has ceased.

We want to emphasize that the presented evidence is indirect and does not confirm the hypothesis. It is impossible to judge, without direct experimental investigation, how probable it is for the proposed mechanism to work in practice to a significant extent and to result in altered soil microorganisms and in disturbed soil ecology to such a degree that it will result in a decrease of soil fertility.

As:

1. The suggested mechanism might in the worst case cause irreparable, widespread, cumulative and persistent damage to soil fertility
2. The genes causing the complication might in the worst case spread uncontrollably over vast areas
3. The evidence supporting this possibility is not insignificant
4. None of presently grown GE crops are of any significant value to mankind,
we find that it is unjustifiable to continue the culture of any transgenic crops until it has been established experimentally beyond reasonable doubt that the proposed mechanism may not result in disturbances of soil ecology.

About the contributors

For reasons explained elsewhere, the list of authors can not presently be disclosed. So it is presently published in the name of PSRAST.

Among the contributors to this paper have been

Three experts on Soil Biology and Soil Ecology.
An expert on Microbial Ecology who has done research on horizontal transfer problems.
A an expert on Virology and horizontal transfer specially in connection with transgenes
A an expert on Microbiology and Molecular genetics
An expert on Gene flow.
An expert on Genetics with extensive experience of transgenic organisms.
An Agricultural expert

Related articles:

# Soil Effects of Transgenic Agriculture: Biological Processes and Ecological Consequences. By soil scientists Neil Macgregor and Max Turner
"We are concerned about the unevaluated effects of these technologies and the possible long-term residual effects on essential soil biological processes."

# "Is there sufficient knowledge about environmental effects to justify release of GE organisms?"
[ML]
# Does science have enough knowledge about DNA to be able to predict and master the effects of genetic engineering? [ML]

References


Genetically Engineered Crops - A Threat to Soil Fertility?
Published on NW Resistance Against Genetic Engineering (http://nwrage.org)


Smalla, K. 1997, personal communication.


Copyright Â© Physicians and Scientists for Responsible Application of Science and Technology (PSRAST) 2001.

For copying the whole article, mail requests to the editor, Dr. Jaan Suurkula at xpsrchair@psrast.org [3], but erase the x first (this is to confuse mail spam adress collectors).

Part of the text may be quoted, provided the source is given as follows: "Excerpt from "Genetically

Source URL: http://nwrage.org/content/genetically-engineered-crops-threat-soil-fertility

Links:
[3] mailto:xpsrchair@psrast.org