GM CONTAMINATION:

Can biological containment work for crops and society?

GeneWatch UK
Briefing Number 33
December 2005

One of the potential risks of GM crops is that the introduced genes will be passed to other non-GM crops or related wild plants. This could result in the contamination of foods or the evolution of new, more competitive weeds, causing problems for farmers or ecosystems. If the gene transferred was coding for a drug, as part of an attempt to make medicines in plants, the consequences of contamination of food could be particularly serious.

One response to this threat has been to develop further genetic modifications of the plant that attempt to reduce or eliminate gene flow by altering the plant's reproductive processes. The most notorious of these is 'Terminator technology', where a crop produces sterile seeds, but a range of other approaches is being developed. This briefing reviews the different approaches and considers their effectiveness and practicality. This is important because 'biological containment' is being promoted as a biosafety issue.[1] While biological containment systems to prevent gene flow may be presented as safety mechanisms for PR purposes, their main purpose is an economic one - to prevent farmers keeping seed for future use or to reduce possible liability claims for contamination, for example.[2]

How do GM crops cause contamination?

There are a number of ways in which a GM crop may cause contamination of other non-GM crops of the same species or of wild related species, including the following:

- **Cross-pollination of neighbouring crops or related wild species.** The extent of this will depend on an array of factors including distance between plants, whether they are flowering at the same time, how compatible they are, landscape and the relative contribution of wind or insects to pollen movement.
- **Seed spilt at harvest that germinates and contaminates later crops grown in the field.** This will depend upon the extent of seed spillage and seed pod shattering, and whether the seed can survive in the soil to germinate in the future.
- **Seed split around fields and on verges during transport after harvest.** Again, this will be influenced by the characteristics of the crop, how the seed is handled and where it is transported.
- **Mixing of GM and non-GM crops in storage or during distribution.** Grain stores or equipment may not be cleaned out properly, or mistakes may be made by operators leading to mixing or errors in labelling.

The GeneWatch UK and Greenpeace worldwide register of GM contamination incidents (www.gmcontaminationregister.org) lists over 100 incidents which illustrate the different ways in which GM contamination has arisen in practice.

What is biological gene containment?

Biological containment uses GM techniques aimed at preventing cross-pollination or preventing seed from a GM crop, or a cross with a GM crop, being viable or persistent. Biological containment systems cannot help reduce or eliminate contamination of food, feed or seed caused as a result of accidental mixing after harvest or during transport and processing. Although a range of different techniques for biological containment has been proposed, none of them is in commercial use for containment purposes and most are far from being ready to apply. For a variety of technical reasons, none of them will ever be 100% effective or applicable in all situations. The approaches under investigation are described below.[1,3]

Limiting gene flow via pollen

Pollen is the plant equivalent of sperm and fertilises the egg or ovum to produce seed. Crops, such as wheat, can be largely self-pollinating, whereas others, such as oilseed rape, outcross to varying degrees with pollen moving on wind or insects to fertilise neighbouring plants. Two approaches to limiting gene flow by pollen are described below.

Chloroplast/plastid transformation

Plastids are organelles found in the cells of plants. These include chloroplasts where photosynthesis takes place and mitochondria where energy generation occurs. Other plastids may be involved in the production and storage of oils or carbohydrates. These organelles are thought to have evolved millions of years ago when bacteria were taken up into cells and eventually became a part of the cell. Therefore, plastids have their own genes derived from the original micro-organisms. During evolution, many of the original plastid genes have migrated to the nucleus of the cell and become part of the main plant nuclear genome.[4]

Chloroplast transformation has some advantages which make it attractive to genetic engineers, but there are some technical problems and limitations

In many (but not all) plants, during formation of pollen, plastids are excluded or degraded so pollen does not contain plastid DNA which is inherited maternally through the ovum. Therefore, if plastid DNA is genetically modified in plants where its inheritance is maternal, the introduced genes (known as transgenes) will not be found in the pollen of the plant and they will not be found in crosses with other crops or wild relatives where the GM crop is the 'father'.

Most of the research on plastid genetic modification has involved chloroplasts; it is one of the most advanced attempts to develop a biological method of gene containment (although this is not necessarily the primary driver of this type of research). Chloroplast transformation has some other advantages which make it attractive to genetic engineers, but there are some technical problems and limitations on how useful it could ever be.

The advantages of plastid genetic modification include the following.[5,6]

- The techniques used can control where in the plastid DNA the new genes are inserted. In contrast to genetic modification of the nuclear genome, disruption of other genes does not take place.
- There are many copies of the plastid genome in each plastid and many plastids in each plant cell. This means that many copies of a gene can be incorporated and higher levels of a product, such as a drug, obtained.Transgene silencing, when an introduced gene does not function because several copies of the gene have been inserted, does not appear to occur with plastid transformation in contrast to genetic modification of the nuclear genome.
- It has been possible to accumulate quite high levels of new proteins (between 1 and 44%) inside modified plastids without causing toxic effects on the plants. This is because the wall surrounding the plastid protects the rest of the cell. Following nuclear transformation to produce drugs for example, plants often have poor growth because the product harms the cells.

The technical problems and limitations include the following.[7]

The technique is much more time consuming than genetic modification of the nuclear genome

- The technique is much more time consuming than genetic modification of the nuclear genome. To modify the plastid DNA, DNA is coated on microscopic gold particles and fired into the cells, by a process known as 'biolistics'. The DNA has the gene coding for the desired trait between two segments of DNA which match DNA sequences found in the plastid genome that are located between gene sequences. In a small proportion of cases, the matching sequences will recognise each other and the new gene will be inserted through a process known as 'homologous recombination'. Marker genes are used to identify cells where plastids have incorporated the genes. Because there are many plastids in every cell, and only one or two may have been modified, many cycles of selection are needed to obtain a plant where all the plastids contain the introduced gene in all their genomes. This takes four to five weeks for an easily transformed plant, such as tobacco, but months for others.[8]

Plastid transformation is efficient only in tobacco, although cotton,[9] carrot, soybean, tomatoes[10] and oilseed rape[11] have been successfully transformed but at very low rates. It has not been possible to modify the plastids of monocot plants (the grasses including rice, maize and wheat) at all.

Plastid transformation is efficient only in tobacco

- Even in plants where the procedure is successful, situations can arise where the technique does not prevent gene transfer in pollen. This 'leakage' can be for one of two reasons.

  ⇒ Not all plastids may be excluded from pollen because the mechanism excluding or degrading plastids during pollen formation is not always 100% effective. For some species, like alfalfa, paternal inheritance of chloroplasts is the norm.

  ⇒ There may be transfer of the plastid gene into the nucleus. This has happened over evolutionary timescales in the past. However, recent studies indicate that this may happen at much higher rates and over shorter timescales than was once supposed.[4,12,13] This research suggests a transfer rate of 1 in16,000 for tobacco pollen and 1 in 5 million for cells in culture. However, proponents of plastid transformation have argued that the research may have been flawed and there was accidental transformation of the nuclear genome.[14] They say that even if transfer did take place, it would not be important because the plastid genetic modifications introduced will not operate in the nucleus because they are based on the bacterial systems that plastids originated from.[15]

- Antibiotic resistance marker genes are widely used in plastid modification although they can be removed or other markers employed.[16,17]

- Although a crop with plastid transformation will usually not contain the modified plastids in its pollen, if it is pollinated by a neighbouring wild plant or non-GM crop (rather than the other way around), the resulting hybrid would carry the transgenes in its plastids. Alternatively, some of the GM crop could survive and form feral populations which could pass on the GM plastids if they crossed with wild relatives.[18] How successful the wild or crop hybrid would be depends on whether it is fertile and could persist. In 1999, researchers from Reading University calculated that there would inevitably be some movement of GM plastids from feral oilseed rape (*Brassica napus*) populations to the wild relative (*Brassica rapa*), but this would be at very low levels because mixed populations are scattered and uncommon.[19] This finding was based on a low estimate of common occurrence (sympatry) of the two species derived from a study of only one area. The same researchers revised their estimate of sympatry in 2003 following a UK-wide survey of *Brassica rapa*, and concluded that 'any procedure seeking to preclude hybrids over the 10-year life-span of a GM cultivar claiming 10% of rapeseed acreage needs to repress hybrid formation by a factor of at least 10^{-5} [1 in 100,000]'.[20]

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Therefore, plastid transformation is a technique which is at the developmental stage and remains routine only in tobacco. It has some clear advantages for use in GM plants to produce drugs and other proteins because of the large number of gene copies that can be introduced into plastids and the high levels of product that can be achieved without harming the plant. However, doubt remains about whether gene containment will be complete and, although the likelihood of hybridisation with related neighbouring plants will be reduced, the wild plant could act as the pollinator of the GM crop or feral population and, over time, gene flow is likely to take place.

Male sterility

Pollen has also been modified by making it sterile. Male sterility has long been an aspiration of plant breeders for reasons other than gene containment. By preventing

cross-pollination, breeders can encourage outcrossing and higher yields through 'hybrid vigour', where the product of two genetically different plants performs better than the parents. A male sterile line is grown among a male fertile line producing pollen to form the F1 hybrids for sale to farmers. However, this is not entirely straightforward. For farmers to use them, these hybrids usually have to be fertile to produce the required grain or seed so the usefulness of male sterility in gene containment is very restricted. The male sterile line also has to be reproduced and maintained so there needs to be some means of reversibility or suppression of the sterility and the difficulties of propagating male sterile lines seriously limits the practical application of the approach.[21] The GM approaches to producing male sterility include:

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- **Disrupting the process of pollen formation.** The best known example of this is Bayer CropScience's commercial GM hybrid oilseed rape that is grown commercially in Canada. A gene coding for an enzyme, barnase, that damages the cells involved in pollen formation, is transferred to make the male sterile line. To produce a fertile hybrid for sale to farmers, the male sterile line is grown alongside a second GM line (known as fertility restorer) containing the barstar gene which codes for a protein that blocks the action of barnase.[22] If GM male sterile lines that do not produce pollen were used commercially, a source of pollen would have to be provided if the final product required seed formation.
- **Linking the male sterility gene to a chemical inducer.** A chemical switch system is included in the GM crop that can control male fertility (or possibly another plant function – see Box) allowing fertility to be switched on or off by the application of an external chemical. This would facilitate maintenance of the male sterile line.[23]
- **Altering the levels of metabolites needed for pollen formation.** This may involve carbohydrates[24] or other essential nutrients. By supplying the nutrient, sterility can be reversed.
- **Having a two-component system** where crossing between male sterile and male fertile plants is needed to produce the desired product.[25] In this case, the male sterile line contains the gene coding for the desired product but these are not operational until pollinated by a male fertile plant containing the activation genes. The seed produced will contain a product such as a pharmaceutical protein. Further modifications could be introduced to make the seed unlikely to germinate.
- **Chloroplast male sterility**. Tobacco chloroplasts have been genetically modified to give male sterility.[26] A beta-ketothiolase gene was introduced and hyper-expressed in chloroplasts leading to male sterility by depleting a substrate needed for fatty acid synthesis essential for pollen production. This was reversible by prolonged light exposure.
- **Interference with flowering.** Some plants undergo self-fertilisation without their flowers opening, so pollen does not become dispersed. There have been suggestions that genetic modification of the flowering process could be achieved to restrict pollen movement. While this may restrict pollen flow, it could have a damaging impact on insects which depend on open flowers for feeding.

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- **Apomixis.** Here fertilisation is not needed for a seed to be produced, so it is another approach to preventing pollen movement.[27] Apomixis is complex and does not occur widely and, even in those species where it does occur, is not the exclusive means of reproduction. Therefore, the adoption of this approach would require further understanding of the process and its introduction into other plants. If pollen was produced, it could lead to the dissemination of the trait into wild populations.

Except for the barnase/barstar system, all GM approaches to male sterility are experimental and untested, and issues of practicability remain. In terms of gene containment, there are several limitations to the use of male sterility. Because the major crops grown by farmers have to produce seed (except fruits and vegetables), complete gene containment will not be achieved. As with chloroplast transformation, the male sterile plant could be pollinated by neighbouring wild relatives or crops, severely limiting its usefulness in some cases.

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What are Genetic Use Restriction Technolgies (GURTs)?
There are two types of GURTs:

- **v**-GURTs: where the use of the GM crop **variety** is controlled through seed sterility
- **t**-GURTs: where the use of a GM **trait** (such as disease resistance) is controlled.

GURTs were designed because conventional ways of preventing copying, such as patent protection, are difficult to enforce for plants which are self-reproducing. GURTs use a chemical sentisitve genetics switch system which is turned on or off by the external application of a chemical. This switch is linked to either a sterility trait in v-GURTs or the GM trait in t-GURTs. The company controls the seed or trait via access to the chemical to be applied. Both types of GURT are still in the development and testing stage.

GURTs as a whole are also known as '**Traitor technolog**y' and v-GURTs as '**Terminator technology**.

Limiting gene flow via seeds

A second mechanism to limit gene flow from GM crops is to prevent any seed that is produced being able to germinate or persist in the environment. All the approaches described below are experimental, and a considerable way from being proven.

Seed sterility (Terminator technology)

Terminator crops will still produce pollen and could cross with neighbouring non-GM or organic crops

This is one of the most contested applications of GM to crops. It involves genetic modification of a plant so that the seed from the crop will not germinate if farmers keep it for resowing.[28] Like Traitor technology (see Box), Terminator technology uses a chemical sensitive genetic switch (responsive, for example, to alcohol or the antibiotic tetracycline) linked to a gene for an enzyme which activates a toxin gene.[29,30] When the toxin gene is switched on, it becomes active in the late stage of seed formation; it does not prevent the seed forming but will prevent it germinating. The geneticl switch may act either to suppress or activate the enzyme and toxin so switches germination either on or off. It is anticipated that the switch would generally be used to suppress germination – the chemical would be applied to the seed before it is sold to farmers to prevent seed saving and resowing. In terms of gene containment, because the seed is sterile, any hybrids formed will be sterile and seed shed at harvest will not survive and germinate in later years: one dimension of gene flow is limited. However, the system is complex and largely experimental and has several shortcomings in terms of gene containment.[3]

- Terminator crops will still produce pollen and could cross with neighbouring non-GM or organic crops. The GM traits could therefore contaminate non-GM food or feed and compromise fertility if farmers had been intending to save seed from their crops.
- Treatment of seeds before sale may not be completely effective. In fact, for any use of genetic switches, it is difficult to imagine that sufficient chemical

could be applied to millions of seeds in sufficient concentrations to reliably trigger the switch in every case. The effect may be sufficient to make saving seed an unreliable exercise for farmers, but not enough for complete gene containment.

- There may be gene silencing or instability of one component leading to failure of the system. Depending on which gene was affected, there would be seed sterility at the wrong time (during seed production), or it would not occur when required (after sale to farmers). Gene silencing is one phenomenon seen in GM crops that arises from the introduction of foreign genes.
- The chemical sensitive genetic switch may be activated by some of the plant's own chemicals or may not be completely switched off all the time. This 'leakage' could lead to fertile seeds being produced. Some of the chemicals used in studies using such switch technology can be harmful to the plant.[31]
- The genes forming the Terminator system have to be linked together to work properly. If they split during reproduction, the system would fail.

Terminator technology has social and economic implications for the millions of poor farmers in developing countries who rely on farm-saved seed for survival

Terminator technology brings potential social and economic implications for the millions of poor farmers in developing countries who rely on farm-saved seed for survival. Terminator should never be allowed on these grounds alone. However, it is also not a reliable gene containment system for both technical and practical reasons.

Recoverable Block of Function (RBF)

This approach has been developed by researchers in Finland and is, in fact, a version of Terminator technology although presented as a mechanism of mitigating against gene flow.[32, 33] In this approach a 'blocking construct' is linked to the gene of interest. A 'recovering construct' is also introduced at a separate location; this is externally controllable. The blocking construct codes for a lethal or harmful effect which can be reversed by switching on the recovering construct. However, RBF suffers from the same limitations as Terminator technology in relation to gene containment.

Limiting introgression into wild plants

A third mechanism to limit the movement of transgenes is to reduce the likelihood of the gene becoming established in the wild population including by:

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Chemical control of transgene expression

This is another form of Traitor technology as described for male and seed sterility. In this case the Genetic Use Restriction technology (t-GURT s- see Box)) is linked to the trait. In this case, the chemical sensitive genetic switch is linked to the introduced characteristic of the crop, such as disease resistance or herbicide tolerance. The trait can be switched on when needed by the application of a chemical. If a hybrid is formed with a wild related plant, the trait will not be expressed and so any competitive advantage will not be gained, making the foreign genes less likely to persist.

Tandem constructs/transgenic mitigation

In this approach a gene is introduced into the GM crop which would be a disadvantage to any wild plants but have no effect on performance of the crop.[34] This is intended to be used as a mitigation measure in addition to other gene containment strategies which allow some gene leakage.

Conclusions

The main approaches which are being proposed for gene containment by biological methods are at the experimental or conceptual stage, with only a male sterility system in use commercially to facilitate hybrid production, not for gene

containment. No single approach gives complete containment because:

- Male sterility and plastid transformation do not prevent gene escape via seed or into wild populations if the GM crop is the maternal parent.
- Male sterility has very restricted usefulness depending on the product of the crop.
- Seed sterility does not limit first-generation contamination of neighbouring crops.
- Chemical treatments may not be completely effective in giving seed sterility in GURT systems.
- Efforts to limit spread of transgenes if they do enter wild populations, may have ecological impacts which have not yet been fully considered. Researchers have modelled gene escape over time and their findings suggest that, depending on the scale of growing and sympatry with wild relatives, containment approaches with leakage rates greater than 1 in 1000 may fail relatively quickly.[36]
- Bringing several systems together may limit gene 'leakage', but such approaches are yet to be developed and evaluated so are many years away even if they do work.

Male sterility and plastid transformation do not prevent gene escape via seed or into wild populations if the GM crop is the maternal parent

However, the decision about gene containment is not purely technical. There has to be a debate about what level of contamination is acceptable, if any. The consequences of failures will have to be considered: in the case of crops being used to produce drugs, for example, they could be serious. But one of the most important questions concerns the intentional or secondary social and economic consequences of the various approaches. The potential for seed sterility and systems which are controlled by the external application of chemicals to increase dependency on seed corporations is extremely worrying. Ten multinational corporations are estimated to control around half of all the world's seed supply with Monsanto now the largest seed corporation globally.[37] This consolidation has been facilitated by biotechnology and the advent of patents on genes and seeds allowing corporate control. Genetic Use Restriction technology would further add to this control.

Delta and Pine Land, the company behind Terminator, presents its technology as 'enhancing biosafety and biodiversity'.[36] This company and the many others, including Monsanto and Syngenta, developing systems to control the reproduction and use of GM crops for economic reasons, were damaged by the international protest against their plans. Forced to make statements that they would not develop Terminator crops, they now seem to be seeking a more acceptable image for their intentions. To allay fears about contamination they present the technology as for 'biological containment'.

Because of the serious social and economic consequences of Terminator technology, especially on poor farmers, there is an *ad hoc* international moratorium on its development under the Convention on Biological Diversity (CBD). However, in February 2005, during a meeting of the CBD, a leaked memo revealed that the Canadian government was seeking to reverse this position. Several governments began to attack a report of an advisory group which had concluded that the disadvantages of Terminator far outweighed any potential advantages in terms of gene containment.[38] The International Seed Federation, representing the world's largest seed producers who stand to benefit from an end to farm-saved seed, are also still interested in Terminator.[39]

Genetic Use Restrictions technologies should be ruled out of discussions about biosafety

GeneWatch UK believes that governments must not allow corporations to drive forward technologies with damaging social consequences under a smokescreen of preventing genetic contamination. Genetic Use Restriction technologies should be ruled out of discussions about biosafety, and only those approaches which do not threaten food security should be options for the future. Even for these, there needs to be wider debate about what levels of contamination and failure are acceptable.

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