Genetically engineered trees: why biocontainment won't work

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Genetically engineered (GE, also called genetically modified, GM) trees pose specific environmental risks that are even higher than those of annual crops such as maize or soy. Trees are long lived, wild and undomesticated species that are part of natural food webs and ecosystems, and hence pose long-term environmental threats to biodiversity-rich ecosystems that are difficult, if not impossible, to foresee and assess.

These facts are also acknowledged by proponents of GE trees. As a risk mitigation strategy, they are now proposing genetic technologies to prevent gene flow from GE trees. Most so-called biocontainment methods introduce new genes that, in theory, prevent the GE trees from flowering or producing fertile seed and hence contain the new genes within the target population. However, here we present scientific evidence that biocontainment will not work and that genes from GE trees will eventually find their way into wild populations and pristine ecosystems.

Biocontainment strategies include Terminator technologies or GURTs (Genetic Use Restriction Technologies), for which a *de facto* moratorium exists under the CBD[1]. It would be misguided to open the debate on Terminator again under the disguise of an attempted containment strategy for GE trees – biocontainment does not work, and the Terminator debate should be terminated once and forever, as these technologies are a threat to farmer's rights and food security.

Greenpeace urges parties attending the 9th Conference of the Parties (COP9) of the UN Convention on Biological Diversity (CBD) to

- **recognise that the use of any biocontainment methods, including GURTS will not remove the threat of GE trees to forest biodiversity,**
- **call on parties to apply the precautionary approach to the use of GE trees, and**
- **to adopt a moratorium on environmental releases, including field trials, of GE trees.**

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GE trees pose even higher environmental risks than GE crops

Trees are very different to the annual crops that have been subject to commercial genetic engineering, such as soy, rapeseed, maize, or cotton. Even GE papaya, commercialised in Hawaii, is botanically a herb[2] and has lifespan of just a few years. Long-lived trees have survival, reproduction and adaptation strategies that differ significantly from short-lived food crops; they display greater functional attributes, e.g. seasonal adaptations, asexual reproduction through twigs or root suckers, and the ability to repair damage within the life-span of the individual.

Different from food crops, trees are not domesticated and hence mate more readily with wild relatives, significantly increasing the risk that new genes are transferred to wild populations. In addition, feral populations of GE trees will likely to be much more persistent than most GE annual crops, as they have not yet lost their wild survival capacities.

Feral populations of escaped GE trees could adversely affect ecological systems (e.g. if the trait was insect resistance), or have, as yet unknown, adverse effects. Ultimately, escaped GE trees could affect the genetic make-up of wild species of trees. This might be in some way important for the survival of that species or for other organisms that depend on that tree for their survival[3].

If GE trees are equipped with sterility genes to prevent flowering or seed production, this will pose additional environmental risks. Tree flowers (pollen) and seeds are important food sources for many wild animals and an indispensable link in forest food webs. Hence, in addition to other novel traits, GE trees with genes inserted to prevent flowering or production of seeds, will have direct and potentially severe impacts on the forest ecosystem.

Biocontainment will not work

There have been suggestions to prevent the outcrossing of GE trees by using or biocontainment technologies, including Terminator/GURTs and genetic sterilization. In theory, these suppress flowering and/or seed production, preventing the spread of GE genes. They are based on the introduction of one or more additional genes into the tree genome that interfere with the tree's reproduction (see box).

However, in practice, no sterilization technique is 100 % effective. This is especially true for GE trees because of the instability of gene expression over time and the long life-time of trees. Even a small amount of gene flow from one GE tree can have enormous consequences for the genetic make up of wild trees. Consider poplar trees that produce up to 25 million seeds annually[4]. Even if a biocontainment strategy would work in 99.9 % of all cases this would result in the case of poplars in the production of 25,000 fertile seeds for every single tree in every single year, enough for a GE trait to escape from the target population into the wild, forever.

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> **Biocontainment, Terminator and GURTs**
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> Genetic use restriction technologies (GURTs), are varied but generally are an additional part of the GE process designed to prevent gene flow from the GE plant. Initially, they were developed to prevent farmers from re-seeding their harvest and better enforce patents on seeds.
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> Two different types of GURTs can be distinguished: The trait GURTs (t-GURTs) suppress the GE trait, e.g. insect resistance, in future generations without interfering with the reproduction of the plant. With t-GURTs, plants will continue to produce pollen and seeds, i.e. reproduce. Hence they are not relevant for biocontainment strategies and not considered any further here.
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> The other type is so-called varietal GURTs, or v-GURTs, which interfere with the reproduction of a plant (or variety). Types of v-GURTs for biocontainment are normally those that allow the GE plant to produce seed, but the seed is infertile[6]. There are several types of v-GURTS, including "conditional" or "reversible" GURTs, where the tree is sterile unless a certain chemical is present. These are all Terminator technologies.
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> Additional biocontainment methods are being considered for GE trees. These consist of GE inserts that prevent the plant flowering, producing pollen or seed. These additional GE-sterility methods are being considered for GE trees because unlike GE crops, where seed is harvested, seeds are not necessarily harvested from GE trees (e.g. if they are for timber or paper).

Terminator or sterility genes interfere with the primary directive of all living beings: to propagate and multiply. In short, they challenge the very concept of evolution. Therefore, it is predictable that GE plants containing Terminator or sterility genes will develop strategies to negate their effects, i.e. reverse the sterilisation/infertility. In addition, many trees, especially poplars, have the ability to reproduce vegetatively, to form sprouts from root suckers often distant from the parent. This would allow the GE tree to circumvent any biocontainment strategy put in place.

Genes can be silenced

An essential part of most, if not all, Terminator and sterility technologies is that additional genes are inserted into the DNA of the tree during the genetic engineering process, along with the genes for the novel trait. As with any other inserted GE gene, Terminator or sterility genes inserted into the tree genome can be switched off, or silenced, at any time during the lifetime of the tree.

Experiments on GE trees have shown that expression of the inserted genes is variable[7]. Gene expression has also been shown to vary between greenhouse and field conditions[8]. This means that experiments performed in the lab may not be good predictors of what might happen in the field, should the GE tree ever be grown outdoors. Expression of the

GE genes in poplar trees (*Populus*) can vary between different constructs; between different GE plants carrying the same GE construct and between the different organs in the same plant[9]. This variability, in combination with the environmental (e.g. drought) and biological (e.g. virus infection) stresses that trees are exposed to throughout their lifetime, means that expression of GE genes in trees is much more complex than in food crops. In both cases, "predictions" cannot be made.

Generally, experiments with GE trees are only conducted over a few years, whereas the life time of trees is of the order of decades or even longer[10]. Hence, although gene expression instabilities may be rare over the period of an experiment, these could be important in the long term. Science simply does not have the capacity to make any long-term assurances regarding biocontainment methods of trees:

> ***"Complete prevention of sexual reproduction with 100% certainty is a daunting technical and social challenge. The long time frames and large numbers of potential reproductive meristems in transgenic [GE] tree plantations provide many opportunities for reversion to fertility, such that rare events become probable."***[11]

Many mechanisms of gene silencing

There are a variety of mechanisms that could silence Terminator or sterility genes. More and more of these gene silencing mechanisms have been found to involve RNA interference (RNAi). Various types of RNA, discovered in the past few years, are now thought to cause RNAi (e.g. miRNA, siRNA). They are transient and intermediary gene products but are core element of genome regulation. They play a crucial role in gene silencing but are poorly understood[12]. In addition, the functions of many types of RNA that are not involved in protein production are still unknown[13], and these could also contribute to gene silencing.

The scientific knowledge of gene silencing is far from complete and is constantly evolving. Known mechanisms that could silence these Terminator or sterility genes include:

1. **Genetic interactions between the plant genome and GE insert;**
2. **stress induced;**
3. **virus induced;**
4. **life plan induced.**

1) Genetic interactions: Interactions between the plant's own genes and the GE insert can cause either to be silenced. Ever since the first genetic engineering experiments were performed on plants, silencing of the GE insert and plant genes has been observed. For example, in the late 1990s, genetic engineering to deepen the colour of petunia caused both variegated and white flowers to appear[14]. This surprising result was caused by silencing (or switching off) of both the plant's own pigment genes and the GE insert.

Multiple GE inserts increase the chances silencing of the inserted genes. The probability of gene silencing increases with multiple or repeated copies of the GE insert[15]. It is now well known that the genetic engineering process is not precise – it is crude. The number of copies of a GE insert that integrate into the plant's genome and the position of the integration site cannot be predicted, regardless of the method used[16]. Hence, as a consequence of the crude methods used to create GE trees, the Terminator or sterility genes could be switched off.

2) Response to stress. Stresses can cause RNAi. Recently, RNAi in poplar trees was found to be induced by mechanical stress[17]. Similarly, environmental stress, such as drought can also induce RNAi. Such RNAi could silence the Terminator or sterility genes at some point (e.g. in response to stress) during the long-life span of a tree.

3) Viruses can both initiate and be a target of gene silencing defence mechanisms. In both GE and non GE plants, virus-plant interactions are varied and include gene silencing. These modes of interaction are constantly evolving and could interfere with the operation of the Terminator or sterility genes, if not immediately, then possibly at a later date in the GE tree's life[18].

4) As a plant matures, flowers and ages, genes are activated and deactivated (silenced) in order to regulate growth form, sexual maturity, seasonal adaptations and aging[19]. These patterns are an on-going process of selection and adaptation to the species' living and non-living environment. Silencing of biocontainment genes may occur during these processes. Recent studies have shown that RNAi can involve complex mechanisms[20] and it's conceivable that this silencing may occur inadvertently.

Inserting DNA into different parts of the cell is not an effective sterilisation technique.

It has been suggested that inserting DNA into the chloroplast will prevent gene flow in pollen as this DNA is only inherited maternally[21]. Hence, the GE genes will not spread via the (male) pollen of the plant. However, because there is "leakage" of DNA from the chloroplast to the nuclear genome[22], so this approach is unlikely to be 100 % effective. The inserted genes could migrate into the nuclear genome, where genes are inserted during normal genetic engineering processes. From this nuclear genome, they could be readily spread by pollen and seed. In addition, this technique does not prevent pollen inflow from wild plants that could fertilize the GE trees and thus produce viable seeds containing the GE trait[23].

Trees can reproduce vegetatively

Trees, especially poplars, which are the favoured tree species for genetic engineering, can reproduce vegetatively, e.g. through suckers. This invalidates any claim of biocontainment as vegetative propagation can take place quite a long way from the GE tree (e.g. if a branch breaks off and travels downstream)[24]. In addition, several studies on gene expression have been found to be even more unstable in vegetatively propagated trees, meaning that any sterility could be less effective[25].

Conclusions

- **Biocontainment strategies for GE trees will never be 100 % effective because they are either conceptually flawed (e.g. chloroplast engineering), or cannot be relied on to be active throughout the lifetime of the tree.**
- **Gene flow from even just one GE tree is enough to change the genetic make up of wild trees, forever.**
- **The ecological consequences of gene escape from GE trees are potentially very severe. They include impacts on species that depend on specific trees for their survival and changes at the forest ecosystem level.**

References

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