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Genetically Engineered Trees & Risk Assessment

An overview of risk assessment and risk management issues.

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> *"Forests are not only trees, and forest ecosystems are more fragile, longer-lived and less closely controlled than crop fields."*
> *(El-Lakany 2004)*

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Abstract

Trees differ in a number of important characteristics from field crops, and these characteristics are also relevant for any risk assessment of genetically engineered (GE) trees. A review of the scientific literature shows that due to the complexity of trees as organisms with large habitats and numerous interactions, currently no meaningful and sufficient risk assessment of GE trees is possible, and that especially a trait-specific risk assessment is not appropriate. Both scientific literature and in-field experience show that contamination by and dispersal of GE trees will take place. Transgenic sterility is not an option to avoid the potential impacts posed by GE trees and their spread. Regulation of trees on a national level will not be sufficient because due to the large-scale dispersion of reproductive plant material, GE trees are likely to cross national borders. All this makes GE trees a compelling case for the application of the precautionary principle.

1. Trees differ from field crops

Comparisons are frequently made between genetically engineered (GE; or genetically modified - GM) agricultural crops and trees in order to understand or anticipate the risks and adverse effects arising from the release and use of GE trees.

However, comparisons reveal significant differences between agricultural crops and trees, such as grade of domestication, time scale, complexity of ecosystems, symbiotic partners and/or spatial distribution. More detailed comparison highlights that the knowledge gained from GE crops is clearly insufficient to understand or assess the risks posed by GE trees.

> *“While there are many different types of forest ecosystems, most of them are markedly different from those typically used for agricultural production in developed countries. Agronomic inputs to forested areas are relatively low in all but the most intensively managed plantations, and species-level biodiversity in these ecosystems is very high. […] The interactions among forest species are, in a relative sense, much more complex than those in an agricultural system where species abundance and distribution are normally highly regulated.* ***While the complexity of the forest ecosystem is well recognized, it is not well understood****.”* (Mullin and Bertrand, 1998)

1.1 Trees have a low level of domestication

Agricultural crops like maize (corn), wheat and soy are highly domesticated and have been under cultivation and selective breeding for many centuries. As a result of domestication most field crops need agricultural practices to enable them to compete with wild plants and/or to survive winter periods. In many cases, domestication and breeding of wild plants into field crops led to a development away from their wild relatives. Combined with agricultural practises, this means that field crops can be cultivated in regions with few or no wild hybridization partners (e.g. maize cultivation in Europe).

Trees, especially forest trees, have a low level of domestication and a rich genetic diversity. (FAO 2004). Domestication of forest trees began just 6 decades ago. Therefore very few trees are removed more than one to three generations from their wild relatives (El-Kassaby 2003 in Sedjo 2006, Libby 1973 in FAO 2004). Unlike (domesticated) field crops, trees can persist and establish in the wild, in unmanaged ecosystems.

> *“However, forest trees may have more complex and less understood interactions with their environments than their agricultural counterparts. Many species are capable of dispersing large quantities of viable pollen to great distances and may cross with many sexually compatible individuals and species of naturally occurring plants.* ***And unlike many agricultural crops, forest trees are genetically very diverse within a species, are long-lived, and are capable of persisting in unmanaged ecosystems****.”* (Finstad *et al.*, 2007)

1.2 Trees have life cycles of decades or centuries

Nearly all agricultural crops are annual plants and complete their life cycle within (less then) one year, i.e. they go from seed to seed in one growing season. Only very few crops are biennial plants, such as sugar beet.

Trees in contrast are perennial plants, with life spans ranging from 150-300 years (Balsam Poplar, Silver Birch, Loblolly Pine, American Elm) to 3000-3500 years (Giant Sequoia, Alaska Yellow Cedar). Even managed trees in plantations have a life span of up to several decades. Depending on tree species, seed production may start as early as at age 4 or as late as 30. Pollen and seed production increase greatly with age and height.

Any effect caused by trees can take place and last over a very long time. Effects can also accumulate.

Due to their long life span trees are repeatedly exposed to and have to respond to a variety of abiotic stresses (e.g. cold, heat, drought, flood, fire, storms) and biotic stresses (e.g. attacks by herbivores, pests, diseases). The ability to react to such often extreme stresses are often linked to secondary metabolic pathways.

1.3 Pollen, seed and other reproductive plant material are dispersed over long distances

Forest trees have evolved to produce high quantities of pollen and seed, both of which are largely adapted for dispersal by wind and can travel great distances.

> Long-distance dispersal of seed from conifers has been reported over distances as far as 600 to 1200 km (Katul *et al.* 2006). Pollen of *Pinus* and *Picea* for example may travel as far as 600 to 1000 km. Under rare conditions transport distances for pine and spruce pollen of up to 3000 km have been recorded (Gregory 1973[1], Campbell *et al.* 1999).
>
> The ability of pollen to survive long-distance transport and to remain viable was shown for example for *Pinus sylvestris* pollen after long-distance transport in northern Sweden (Lindgren *et al*. 1975).

Birds and squirrels, but also other animals like bats and deer help trees to conquer distant habitats. (Nathan 2006, Richardson *et al.* 2000)

Trees also reproduce vegetatively through roots, suckers, shoots or from broken branches, that can be transported by wind, water, animals and humans, and that can set root again.

This is especially relevant for risk assessment of GE trees. (See 3.0 for propagule dispersal).

1.4 Trees have a large spatial distribution

Many trees are present over a large geographical area and hybridisation is common. This is especially true for the genus *Populus*.

> *"The genus Populus is widely distributed throughout the Northern hemisphere, in both temperate and subtropical zones. Representative species are found from Alaska and Labrador south to northern Mexico, as well as Europe, North Africa, the Himalayas, mainland China and Japan (Schreiner 1974). Some species are very widely distributed. P. tremuloides, for example is the most broadly distributed tree species in North America [...] and the most second widely distributed in the world (Jones 1985, Barnes and Hahn 1993)."* OECD 2006
>
> *"Disagreement over the species classification of poplars shows no sign of abatement. [...] Thus species counts for the genus range from the low 20's to over 80, depending on the authority. The classification suggested by Eckenwalder (1996), which enjoys the transitory advantage of being the most recently published, recognizes 29."* (OECD 2006)

1.5 Trees are integral part of complex ecosystems – forests

Field crops are part to mostly tightly controlled cropping systems, with reduced or minimized interaction with other organism (plant, animal, fungi or bacteria). Trees, however, are a major part of complex ecosystems (forests), also providing ecosystems, habitats and food to symbiotic partners, such as mycorrhiza, and for animals and other plants. Unlike most agricultural plants,

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[1] in OECD consensus document vol 2, p.208

forest trees can persist and thrive in unmanaged ecosystems.

1.6 Trees affect water and climate systems

Trees are integral part of complex systems (forests), which play essential roles in managing water supply and rainfall, carbon sequestration and also climate regulation.

2. It is currently not possible to conduct a meaningful and sufficiently comprehensive risk assessment for GE trees

The complexity of forest ecosystems combined with the complexity of trees themselves is well recognised as a major challenge for the ability to perform reliable and scientifically robust risk assessments of GE trees.

> *"The complexity of woody biomass crop systems and associated wild populations precludes simple answers about environmental risks of transgenic [tree] varieties."* (James *et al.* 1998)
>
> *"There are no regulations, however, specific to the use of genetic modification in forestry. Although policies and regulations adopted for agricultural crops are also likely to be used for forest trees, forest trees present special challenges (long time frames, and life spans, wild resource, major constituents of an ecosystem)."* (El-Lakany 2004)

The following gives an overview of issues that need to be taken into consideration for a meaningful and sufficiently comprehensive risk assessment, and outlines why lack of crucial knowledge makes such a risk assessment impossible at present.

2.1 General consideration for risk assessment

It has to be stressed that risk assessment of GE trees must be multi-layered and therefore more complex than those for field crops. The list of points presented below is preliminary and will need to be extended as knowledge and understanding emerges.

A sufficiently comprehensive and robust risk assessment should, for example, include interactions with other organisms (e.g. fungi, plants (e.g. undergrowth) and animals). Since GE trees provide feed to a large number of species, risk assessment has to take effects on whole food webs into account.

Since GE trees or their prodigy will travel or spread, effects on different habitats need to be assessed both in the location where the GE tree is originally grown, but also in the much wider vicinity, including across national borders. Risk assessment needs to address the effects of the growing of GE trees inside forest ecosystems and other natural habitats as well as in plantations and managed forests. A risk assessment also needs to cover potential invasiveness.

Impacts need to be understood for both above and below ground, with special regard to soil organisms such as mycorrhiza as symbiotic partner of trees. (Snow *et al.* 2005)

Effects need to be considered on running water and on ground water, especially for fast growing trees.

Because forests play an important role in weather and climate, effects on micro and macro climate have to be assessed.

Risk assessment cannot yet be undertaken at the level of specific scenarios, instead it has to be taken at the larger categories of spatial levels where risk multiplies or changes are not defined or yet understood.

2.2 Short-comings of trait-confined risk assessment

A survey of scientific papers on potential harm arising from GE trees has revealed a tendency toward limiting the identification of the potential adverse effect solely to the GE trait. According to these papers, GE trees can be classified as either "no-risk", "low-risk" or "high-risk" according to the trait and its intended effect.

One of the earlier proponents of this approach are James *et al.* (1998), who stated for example:

> *"... transgenes and the phenotype they impart are typically known in great detail. Therefore a tiered approach is suggested where only those plants [trees] whose transgenes pose significant risks based on knowledge of the phenotypes they impart be required to undergo special evaluation before commercial use."*

Such risk perception assumes knowledge of behaviour, processes and interactions that in fact have remained largely unknown.

Furthermore, such a simplification fails to see or consider two crucial aspects of genetic engineering namely (1) the pleiotropic or other unintended effects of the transgene and its protein and (2) the mutational effect of the genetic engineering processes themselves, which have been shown to lead to hundreds of mutations throughout the genome of the plant. The existence and effects of both processes are well documented in the scientific literature but are not yet fully understood.

Additionally, the same gene may behave differently in different organisms or give rise to different proteins or effects.

> Such changes were observed by an Australian group who transferred a gene (alpha-AI or alpha-amylase inhibitor gene) from the common bean to peas. Unexpectedly, the protein product from the bean gene changed its characteristics and became immunogenic, i.e., causing immune reactions, when expressed in the pea (Prescott *et al.* 2005).
>
> Although the original gene and the modified transgene both coded for exactly the same protein, the pea produced a structurally different protein from the same genetic information. Furthermore, the transgenic protein also gave rise to "immunological cross priming", also known as "adjuvant effect".

This research provides clear evidence that a gene may behave differently when transferred from one organism to another, even if the two organisms are biologically very closely related.

All these aspects have to be taken into account in the assessments of all the traits, no matter whether they are disease resistance, phyto-remediation, lignin modification, tree restoration, herbicide tolerance or others.

2.3 Pleiotropic & other unintended effects

A pleiotropic effect is the phenomenon in which a single gene influences two or more seemingly independent characteristics (phenotypic traits) of an organism. Therefore a change in the one gene can impact on several traits. Transgenes are no exception to this, yet a pleiotropic effect is not always easily recognised. Pleiotropic effects have for example been described for the architecture of apple trees (Kenis & Keulemans 2007).

> Lignin content has been linked with pleiotropic effects. They were for example observed in GE tobacco plants with reduced lignin content and raised as a concern for GE lignin reduced trees as early as 1998.
>
> Here an enzyme was down-regulated (phenylalanine-ammonia-lyase, PAL). This enzyme regulates lignin content, but is also key enzyme in two other metabolic pathways (shikimate and phenylpropanoid pathways). The GE tobacco plants "exhibited pleiotropic effects (e.g. stunted growth and altered flower morphology and pigmentation)" (quoted in Tzfira *et al.* 1998.).

Unpredicted effects often cannot easily be explained or categorised, due to lack of information and understanding; to do so would require substantial further investigation.

> Bergelson from Chicago University, for example, described unexpected side effects when transferring a gene within the same species. Working with *Arabidopsis thaliana* (Thale cress), her group transferred a gene for herbicide resistance (derived via mutagenesis) from one *A. thaliana* to another via genetic engineering. The result was not only an herbicide-tolerant plant, but the transgenic *A. thaliana* became, among other things, also 20 times more likely to cross-pollinate (Bergelson *et al.* 1998).

2.4 Transformation induced mutations

It is well documented that the processes of plant transformation give rise to many mutations throughout the plant genome as well as at the insertion site of the transgene (reviewed in Wilson *et al.* 2004, 2006; Latham *et al.* 2006). Whilst not systematically studied, evidence so far shows that both *Agrobacterium* mediated and particle bombardment gene transfer give rise to numerous **insertion-site mutations**, including small and large insertions, deletions and/or rearrangements and scrambles of genomic and inserted DNA (Forsbach *et al.* 2003; Kohli *et al.* 2003; Makarevitch *et al.* 2003; Windels *et al.* 2001).

Gene transfer procedures as well as tissue culture also give rise to **genome-wide mutations**. Whilst not many studies have been carried out, DNA polymorphism analysis to date has shown a high number of mutations present throughout the genome. Indeed, results suggest that many hundreds or thousands of such genome-wide mutations are likely to be present in plants transformed using typical plant transformation methods, especially those involving the use on plant tissue culture techniques (reviewed in Sala *et al.* 2000; Labra *et al.* 2001). The nature of these mutations is not been investigated though, such as whether they are small scale or large-scale genomic changes and whether they occur in functional regions of the genome. Genome-wide mutations have been found in all transformed plants examined and such mutations have been shown to be heritable (Sala *et al.* 2000).

> Of the two types of tissue culture, one is used in genetic engineering processes, the other in standard clonal and vegetative propagation techniques. Both give rise to somaclonal mutations and thus to somaclonal variations, but the impact is vastly enhanced in tissue culture techniques as part of genetic engineering (Wilson *et al.* 2004).

Whilst mutations are not intrinsically good or bad, they constitute a change and risks of unknown qualities. They thus require further investigations and risk assessment. Genome-wide mutations will further require intensive back-crossing of the host plant to reduce the newly introduced mutations.

2.5 Risk assessment under stress

The ability to respond to biotic and abiotic stresses may be compromised by the performance of the transgene, its product(s) and the processes of genetic engineering. Vice versa, such stresses may also interfere with the performance of the transgene.

Testing for any impacts on tree performance will require a long time and exposure to all different stresses.

It has been reported that environmental stresses for example can trigger or enhance the onset of transgene silencing.

Since its discovery in 1992, the phenomenon of gene silencing is now frequently observed in genetically engineered plants, and especially under stress conditions (Broer 1996, Meza 2001) Gene silencing can, for example, be evoked by: the insertion of DNA that is recognised by the plant as foreign (such as viral DNA), by multi-copy inserts of a transgene, or by a homology (sameness) between sequences of the transgene and the plant's own DNA. The onset of transgene silencing is often not immediate but can occur after a few generations of unaffected growth. It is heritable, but can also be revoked after generations.

2.6 Risk assessment over time

Depending on tree species, seed production may start as early as at age of four years or as late as 30 years. Pollen and seed production increase with age.

> Onset of seed production of 4 years has observed in some poplars, e.g. *P. deltoids*; while Silver Birch and Eastern White Pine take 5-10 years, Poplar and White Spruce 10-15 years, Sitka Spruce and oak 20-25 years. The Norway Spruce even has an onset of seed production only after 20-30 years.

Any robust risk assessment study needs to take several generations into account, for example to assess the stability and heritability of the transgene, unintended side effects and changes due to transformation impact. With long juvenile stages before the first seed are produced, such risk assessment studies will take a considerable number of years. The same is true for studies of effects that can take place at different development stages or environmental stresses have to be repeated as part of a robust risk assessment

2.7 Socio-economic effects concerned in risk assessment

Any risk assessment needs to consider socio-economic effects of GE trees and their cultivation. The range of these issues cannot be covered in this report, but examples could include the competition for water between GM trees and regular field crops in the wider vicinity, land use or impacts on indigenous communities.

3. Propagative plant material will travel and cross national borders

Most trees and their genes will spread not only through sexual reproduction (pollen and seed) but also by asexual (vegetative) reproduction, such as roots, shoots, twigs that can set root. These propagules can be dispersed by wind, water, pollinators (insects), animals and humans. To assess possible contamination a wide range of factors need to be taken into account, ranging from normal weather conditions in which pollen and seeds already travel long distances (depending on direction, speed and uplift of the wind), to extreme conditions like storms and floodings in which broken branches are swept along and can set root somewhere else. Animals and humans also attribute to the spread of seeds when they either take fruits, nuts, cones along (such as squirrels), or even when they consume fruits, thereby passing the seeds through their body and depositing them somewhere else.

> *"In any event, as we deploy vast plantations of transgene-bearing forest trees, we can expect the transgenes to escape into the wild population and to persist there for a long time.* ***In conclusion, we can probably take the view that 'propagules will travel'.****"* (Smouse *et al.* 2007)

The issue is not only contamination, but also invasiveness, especially where pioneer species such as GE poplar or birch are modified such that they gain an advantage over wild trees of the same or of other species. An example of a transgenic trait that can confer an advantage is cold tolerance (developed in eucalyptus), allowing trees to be cultivated in colder regions and thereby potentially enabling them to get established in ecosystems where this tree species previously did not grow or maybe where trees in general did not grow. Other examples are trees producing insecticidal protein (e.g. Bt toxins) and therefore possibly (more) resistant to specific pest insects, and trees with faster growth or bigger leaves who can out-compete other tree seedlings competing for light and space in forest settings.

> *"Transgenes which provide a large fitness advantage, perhaps by protecting from herbivores or disease, may enhance invasiveness." "Transgenes which enhance fitness are most likely to increase invasiveness and frequency of recipient species outside cropping system."* (James 1998, see also Andow & Zwahlen 2006).

3.1 Pollen dispersal

Tree pollen is dispersed mainly via wind or insect pollinators. Forest trees are largely wind-pollinated, with pollen highly adapted to be transported by wind, often over large distances.

> Travel distances of 1000 km have been reported for spruce (*Picea*) (Gregory 1973[2]) and 100s of kilometres for birch.
>
> For white spruce (*Picea glauca*), the vast majority of pollen was found to cross-pollinate within a range of 250-3000m (O'Connell *et al.* 2007).
>
> *"As an evidence of long distance pollen transport, Betula pollen concentrations in Fennoscandia can be relatively high before the local flowering period. The pollen is transported by south-eastern air-masses from central Europe and the Baltic countries with travelling times for pollen grains in the range of 9-20 hours (Hjelmroos 1991)."* [3]

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[2] in OECD consensus document vol 2, p.208

[3] (in OECD)

A differentiation is generally made between short-distance dispersal (SDD) and long-distance dispersal (LDD), with long-distance dispersal pattern and range often poorly understood or documented as they are very difficult to investigate. Different methods are needed to investigate SDD and LDD. For risk assessment purposes, pollen dispersal rates cannot be taken into account for individual years only, but have to be looked at cumulatively over time. Furthermore, pollen production increases with age.

> Smouse *et al.* (2007) point out that a single-year LDD (long-distance dispersal) rate of 1% would amount to 9.6% over the period of a decade. They further state:
>
> *"Viewing LDD as 'escape', the long-term prospects for escape are sobering. Inasmuch as LDD dominates the evolutionary fate of any particular gene over any extended geographic scale (Petit et al. 2002a,b; Nathan et al. 2003; Austerlitz and Garnier-Géré 2003; Williams et al. 2006), it becomes clear that we have to 'think longer-term and larger scale' than is traditional in gene flow and dispersal studies."* (Smouse *et al.* 2007)

Pollen dispersal is only of concern if there are recipient trees within the range of dispersal, but as domestication of (forest) trees has only been taken place on a low level, pollen from transgenic tree plantings and plantations will easily cross-pollinate related trees in natural forests as well as in managed forests, plantings and plantations. Such transgene escape and contamination also cross the species boundaries.

> *"About 85% of the applications for field testing of transgenic tree plantations involve Populus, Pinus, Liquidambar and Eucalyptus […]. Several of these commercially important species hybridize with congeners under natural conditions (e.g., Pinus taeda with other southern pines, such as P. palustris or P. echinata, Schmidtling 2001). Transgenes can be expected to cross taxonomic boundaries with non-trivial probability, and we may well have to extend our tracking system to these congeners, which complicates matters."* (Smouse *et al.* 2007)

3.2 Seed dispersal

Trees have developed a multitude of strategies to have their seeds dispersed either by abiotic means, such as wind or water, or by biotic means, mostly animals including humans.

Trees, especially forest trees, produce large quantities of seeds often well adapted to wind dispersal (abiotic seed dispersal).

> **Popla**r seed for example is well adapted to transport via wind and water.
>
> *"A typical 12m Populus deltoides specimen was estimated to produce almost 28 million seeds in one season, and estimates for P. tremula have ranged as high as 54 million seeds."* (OECD p.105)
>
> *"..in addition to being wind-pollinated [poplar], the long white, silky hairs attached to the short stalks of the seeds promote wind dispersal over great distances (Schreiner 1974), resulting in high rates of migration."* (OECD p.105)
>
> **Birch** seed fall mainly 40-50 meters of the source, with a probable "secondary dispersal of seed over the surface of snow by wind" for *Betula pendula*. Seeds can also be "further dispersed by melting water in the spring", e.g. for *B. lenta*. (OECD 2006 p.51)
>
> **Spruce** seeds are winged and wind-dispersed. One study showed that 5% of seeds travel further than 100m and can reach several hundred meters. (OECD) Cones containing the seed are also dispersed by animals, such as squirrels or birds, and by people.

Seeds are also dispersed biotically when they (either as seeds, fruits, nuts etc) are consumed by animals, including humans. They can be dispersed when they are carried away (e.g. acorns taken and buried by squirrels, rowan berries taken by birds), as well as when fruits and seeds are consumed, carried along in the gut and then dispersed by faeces. In this way they can travel large distances undetected.

3.3 Vegetative propagule dispersal

Vegetative (asexual) reproduction is common in trees. Trees can reproduce through shoots from roots and stumps, through layering and through broken twigs or branches setting root.

Twigs and branches can get carried away by animals, wind and water and can set root at new locations. Many tree varieties also sprout again from the stump or the root collar once felled.

> *"Studies have also demonstrated that both natural and vegetative propagation occur in nature, for example with Populus nigra (Legionnet et al. 1997)."* OECD p.106

In some tree species like Birch (*Betula*) sprouting from special basal buds enables the tree to react to [major] incidents such as fire, damage by grazing, or felling (OECD 2006)

> *"Except for members of section Populus, all poplars sprout vigorously from the stump and root collar. Coppicing occurs occasionally on young aspen (Zsuffa 1975). Reproduction from adventitious shoots on roots (root suckers) is common in many species, although less frequent in those in the Aigeiros and Leucoides section."* (OECD p. 106)

Broken twigs and branches can be carried away by birds as nesting material or by humans as building material. During floods and storms, broken branches can be carried away over larges distances.

> *"For example, some species within the genus* ***Populus*** *are propagated via broken branches and twigs, circumventing the sexual process (Rood et al. 2003)."* (Farnum *et al.* 2007; p.128)

Stands of trees might in fact not be different individuals, but clonal groups where new trees grow from shoots from the roots of the existing tree.

> Such clonal groups are for example common for poplar (e.g. *Populus tremuloides* in North America), where they are generally less then 0.1 ha in size. However groups as large as 80 ha have been observed in the US (Kempermann and Barnes 1976)" (OECD 2006, p.106)

4. Sterility is no solution

Sterility is often proposed as a solution to the problems of outcrossing and contamination and the risks linked to the wider uncontrolled spread of GE trees. Yet reality is more complex for two reasons. Firstly, sterility cannot prevent vegetative propagation, which many trees are capable of. Second, there is no functional GE sterility system in place that would offer the reliability and effectivity required for biosafety purposes.

As described above, trees regularly reproduce vegetatively, and sterility only addresses sexual reproduction.

> *"Vegetative reproduction by transgenic trees also contributes to the risk of transgene spread (Hoenicka and Fladung 2006). For example, some species within the genus* ***Populus*** *are propagated via broken branches and twigs, circumventing the sexual process (Rood et al. 2003). Viable propagules can be transported considerable distances in streams. The primary concerns are suckers, which can proliferate on the lateral roots of certain species, as well as the formation of adventitious roots on branches shed from some of those same species. Given this, it may become necessary to develop methods for controlling vegetative reproduction. To reduce the number of root suckers, scions from commercially important genotypes could be grafted onto rootstocks that are much less prone to producing suckers. With regard to rooting, considerable progress has been made in our understanding of the genes regulating the development of adventitious and lateral roots in both angiosperms and gymnosperms (e.g., Casimiro et al. 2003; Ermel et al. 2000; Fu and Harberd 2003; Goldfarb et al. 2003; Lindroth et al. 2001; Xie et al. 2002). This information suggests several promising approaches for* ***engineering solutions*** *to vegetative spread, but their efficacy will have to be verified empirically."* (Farnum *et al.* 2007)

Thus far, there is no experience with sterility as form of risk management. Indeed, no robust risk assessment for transgenic (genetically engineered) sterility has been developed. Furthermore, there is considerable doubt that transgenic sterility would perform reliably (over long periods of time) as to prevent sexual reproduction. It is not known for example whether sterility is a stable trait or whether the transgene(s) might get silenced, for example under stress conditions.

> *“It has often been said that plant sterility should be an easy trait to engineer; after all, there are dozens of ways to damage a motor so it does not work. Unfortunately, motors do not have the redundancy and resilience of biological systems that have evolved to reproduce “at all costs” […]”* (Brunner *et al.* 2007)
>
> *“Engineering trees to remain sterile throughout their lives is technically difficult because of their long life spans and the large number of meristems that may potentially become reproductive.”* (Farnum *et al.* 2007)

Even scientists who consider containment of GE trees a social rather than an environmental goal, are of the view that containment of GE trees is not possible, at least not for the foreseeable future.

> *“Indeed, because of the long-known propensity for long distance movement of pollen and/or seed from most tree species, if complete containment is the social goal, there is unlikely to be any place for GE trees in forestry plantation or horticulture—at least not for many decades.”* (Brunner *et al.* 2007)

Even thought it is known that male sterility occurs spontaneous, the mechanisms involved and responsible are still not fully understood and are ‘under investigation’ (Hosoo *et al.* 2005 in Farnum *et al*. 2007). Whilst several genetic engineering approaches for gender-specific sterility are being tested, “definite results have not yet been reported”. Research is also hampered by long juvenile period of trees (Farnum 2007).

> Options under discussion are male sterility, female sterility, prevention of flowering and seed sterility. Methods considered for this purpose are (1) the destruction of tissue using genes for cell toxins, such as destruction of floral tissue (ablation), sexual organs, seed embryo; (2) gene suppression, including RNAi methods and (3) repression of mature phase to prevent the onset of flowering by altering the expression of genes to achieve extended juvenile phase.

To prevent gene escape via pollen and/or seed, the transgenic sterility trait would need to last for the full duration of a tree's life, no matter what biotic or abiotic stresses.

In order to rely on sterility as a risk management strategy, gene-silencing would have to be prevented. Indeed, genetically engineered sterility would have to rely, as a minimum, on the stable expression of the inserted transgene(s). However, so far no transgenic sterility has been developed, nor tested over an appropriate time scale (i.e. the entire lifespan of a tree), to meet the rigorous criteria for sterility as risk management.

Indeed, long-term field trials would be required for ‘evaluating the durability of various sterility systems’. Furthermore, the ecological viability of permanently or transiently sterile trees will need to be demonstrated (Farnum *et al.* 2007).

> “A **significant ecological concern** about flowering control is that it could deny forest-dwelling animals important sources of sustenance (pollen, flowers, and seeds). Continuing with our loblolly pine example, we might consider the possible effects of complete sterility on the brownheaded nuthatch (Sitta pusilla Latham), whose diet is 56% pine seeds (Martin *et al.* 1951). According to the Cornell Lab of Ornithology, this nuthatch “is restricted to the pine forests of the southeastern states” and is “decreasing throughout [its] range because of habitat degradation.” (Farnum *et al.* 2007)

Several authors have stressed that due to the potential instability of transgenes, total sterility of every single tree in plantations is highly unlikely even if the stability of the transgenic trait would be high. Especially where the GE trait confers an increased fitness, a few escaped seedlings can cause significant impacts (Richardson & Petit 2006, Lee & Nathan 2006, Williams & Davis 2005).

However, sterile trees in themselves are likely to cause adverse effects on biodiversity because pollen, seeds and fruits of plantations and plantings are also part of food webs. Sterile trees would deprive birds, insects and mammals of this source of food, leaving the plantations and plantings devoid of these animals and possibly causing cascading environmental effects that could impact on neighbouring natural ecosystems (Mayer 2004, Hayes 2001, Johnson & Kirby 2001). This concern is recognized by many.

5. Conclusions

- GE trees differ strongly from GE agricultural crops, especially in terms of
  - longevity,
  - complexity of the plant as organism as well as in their interaction with the biotic and abiotic environment.
- Consequently experience with GE field crops cannot sufficiently describe the range of possible impacts and effects of GE trees.
- Risk management developed for GE field crops will be insufficient to protect biodiversity and forest ecosystems.
- Currently any risk assessment can only be insufficient and inappropriate. Because too many issues cannot be accessed, especially those concerning effects on other organisms that use trees as source of food and as habitat, especially in a natural forest context. Socio-economic impacts on indigenous and local communities and on foresters will require further attention and deliberation, with examples including competition for water and land between fast growing trees and regular field crops.
- In addition, strict national regulations will likely be insufficient to protect the national biodiversity of forests because GE trees and their propagules can and will travel across national borders unnoticed.

Taking all this into consideration, a moratorium on the release of GE trees is key to protecting biodiversity in general and forest biodiversity in particular.

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