

## **Part 2**

# **ETHICAL AND SOCIO-ECONOMIC DIMENSIONS**

## 7. Theoretical and practical considerations of gene flow

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Gene flow, defined as the incorporation of genes from one gene pool into another, is at the core of the transgenic plant debate. In particular, a widespread societal perception of genetically modified plants is that of a hazardous material with high ‘pollution’ potential for the environment. The transfer of engineered genetic sequences (transgenes) from genetically modified plantations into natural populations of wild relatives via propagule dispersal is the natural vehicle for the feared ‘pollution’. From a scientific risk assessment perspective, proper evaluation of the environmental implications of genetically modified plants involves both hazard and exposure assessments (Johnson *et al.*, 2006). Hazard assessment targets the identification and quantification of potential adverse effects of transgenic plants for the environment. Exposure assessment evaluates the probability of the environment being exposed to the hazards. Gauging the probability of transgenic incorporation into natural plant populations is the key step of exposure assessment.

It must be stressed that the detection of transgene flow into natural populations is not a demonstration of the risk of genetically modified organisms, which would require evidence of the transgenes being hazardous for the environment. This chapter deals solely with the role of gene flow in the genetically modified-plantation debate, without additional consideration of hazard assessment. The chapter is structured along four lines, describing the main contributions of gene flow researchers to exposure assessment of genetically modified trees:

- characterization of propagule dispersal patterns in non-genetically modified tree populations, which provides general insights into transgene flow potential and quantitative measurements for model parameterization;
- elaboration of theoretical models of gene flow from genetically modified tree plantations into natural populations, essential for predictive inference over large spatial and temporal scales;
- detection of transgene flow into natural populations, necessary for real-time monitoring, decision-making and management;
- formulation of transgene flow limitation practices.

There are several specific features of trees that are relevant in the genetically modified forest risk assessment context, which will be reiterated throughout this chapter. First, trees are long-lived perennials, a fact that has three important consequences:

- propagules will be dispersed from genetically modified plantations recurrently for many years before harvesting;

- it is very difficult to establish empirically the multiple-generation fate of these propagules in natural ecosystems;
- induced-sterility containment measures have increased chances of failing, due to temporal instability.

Second, trees disperse pollen and seed over broad spatial scales, increasing the probability of long-distance transgene movement and hampering its effective containment and accurate monitoring. Third, trees have typically very high fecundities, translating into large numbers of dispersed propagules, which are expected to increase the longest realized dispersal distance, particularly for fat-tailed dispersal distributions (Clark, Lewis and Horvath, 2001; Klein, Lavigne and Gouyon, 2006). Fourth, genotypes used for genetic modification are often taken from undomesticated tree stands and grown in similar locations, so cross-mating with natural populations of the same species (or close relatives) is likely to be common (González-Martínez, Robledo-Arnuncio and Smouse, 2005). Lastly, trees are the dominant life form of many terrestrial ecosystems, so introgression of transgenes into natural tree populations might have long-term and large-scale impacts on ecosystem function.

### **DISPERSAL PATTERNS IN NON-GENETICALLY MODIFIED TREE POPULATIONS**

Given the absence of dispersal data for genetically modified trees and the legal and social restrictions on genetically modified-tree field trials, dispersal studies in non-genetically modified tree populations provide a necessary surrogate to investigate transgene flow potential. Assuming that no particular containment measures are taken and that genetic transformation for the target trait does not significantly alter the dispersal function, the available data on propagule dispersal patterns in natural tree populations, seed orchards and commercial plantations should reflect the potential scale of propagule flow from genetically modified plantations. Note that this section refers to the arrival of transgenes via pollen and seed dispersal into natural stands, and not to the long-term persistence of transgenes once they have arrived in the wild, which is discussed in the next section, on predictive models.

There are several statistical methods that have been developed for estimating gene movement within and among populations. Some of these methods provide historical estimates of gene flow, under various assumptions about evolutionary equilibrium, based on the spatial genetic structure of populations (Wright, 1931; Slatkin, 1985; Rousset, 1997; Beerli and Felsenstein, 1999) or individuals (Hardy and Vekemans, 1999; Rousset, 2000). Other methods yield contemporary gene flow estimates, inferred either from parentage analysis (Meagher, 1986; Devlin, Roeder and Ellstrand, 1988; Adams, Griffin and Moran, 1992; Smouse, Meagher and Kobak, 1999; Burczyk *et al.*, 2006) or from the spatial genetic structure of propagules (Smouse *et al.*, 2001; Austerlitz and Smouse, 2001; Robledo-Arnuncio, Austerlitz and Smouse, 2006). Several reviews on gene flow and transgenic trees have already extensively reported the main assumptions, statistical properties, pros and cons of each of these different estimation procedures (Ellstrand, 2003; Slavov,

DiFazio and Strauss, 2004; DiFazio *et al.*, 2004; Smouse, Robledo-Arnuncio and González-Martínez, 2007). The reader should refer to these previous works for detailed technical reference. Here, some results that are particularly relevant for genetically modified flow are summarized:

- Within-population mean dispersal distance estimates range from a few tens to several hundred metres (most frequently <1000 m in temperate forest trees), both for pollen (Dow and Ashley, 1998; Streiff *et al.*, 1999; Lian, Miwa and Hogetsu, 2001; Schuster and Mitton, 2000; Sork *et al.*, 2002; Robledo-Arnuncio and Gil, 2005; Goto *et al.*, 2006; Hardy *et al.*, 2006; Hardesty, Hubbell and Bermingham, 2006) and seeds (Clark *et al.*, 1999; Jones *et al.*, 2005; Goto *et al.*, 2006; Hardesty, Hubbell and Bermingham, 2006; González-Martínez *et al.*, 2006; Robledo-Arnuncio and García, 2007; Hardy *et al.*, 2006; Jordano *et al.*, 2007). Both insect- and wind-pollinated tree species show a similar range of mean dispersal distances in published studies, although there are large differences among species. It is noteworthy that estimates of the mean dispersal distance based on parentage analyses are likely to be downwardly biased, since the distribution of observed dispersal distances is usually truncated by the sampling plot boundaries, and propagules immigrating into the plot are usually discarded to compute this quantity.
- Yearly pollen immigration rates into forest fragments or stands are typically very high (>30%), and remain high (>5%) even with isolation distances of a few kilometres from the nearest conspecific stand (Kaufman, Smouse and Alvarez-Buylla, 1998; Adams and Burczyk, 2000; Schuster and Mitton 2000; Plomion *et al.*, 2001; Stoehr and Newton, 2002; Robledo-Arnuncio and Gil, 2005; Hanaoka *et al.*, 2007; O'Connell, Mosseler and Rajora, 2007).
- Seed immigration rates into sampling plots embedded within large forests (Jones *et al.*, 2005; González-Martínez *et al.*, 2006) and into isolated forest fragments (García, Jordano and Godoy, 2007) are both typically high (>10%). Secondary dispersal by fruit and seed predators, not always accounted for in seed migration estimates, is expected to increase the range of seed dispersal (Vander-Wall, 2001; Valbuena-Carabaña *et al.*, 2005).
- The estimated pattern of seed and pollen dispersal is very leptokurtic, i.e. there is a rapid decline in dispersal probability over short distances but non-negligible probability maintained beyond distances of several hundred metres (Clark *et al.*, 1999; Austerlitz *et al.*, 2004; Robledo-Arnuncio and Gil, 2005; Jones *et al.*, 2005; Goto *et al.*, 2006; Robledo-Arnuncio and García, 2007).
- Although empirical evidence for long-distance propagule dispersal in trees is abundant, its accurate probabilistic description remains a daunting challenge (Nathan, 2005). The usual procedure of parentage-based studies is to fit probability distributions to dispersal data collected on a small spatial scale and extrapolate the fit to the unobserved range of the distribution. Quantitative predictions established in this way should be considered with extreme caution, since functions with profoundly different tail-behaviour often fit observed data about equally well.

The general pattern is that while a substantial proportion of dispersal events occur over short distances, the potential for long-distance gene movement among tree populations or stands is quite high, though difficult to predict. The probability of seed or pollen from genetically modified tree plantations effectively reaching natural populations located even a few kilometres away should be considered non-negligible, *a priori*, especially when dispersal episodes accumulate over several years or decades. For instance, a low (say  $p = 0.01$ ) yearly probability of transgene dispersal from a genetically modified plantation can translate into a substantial ( $1 - 0.99^{20} = 0.18$ ) probability over a period of 20 years (Haygood, Ives and Andow, 2004; Smouse, Robledo-Arnuncio and González-Martínez, 2007). Similarly, a low probability of escape from a single genetically modified stand can translate into substantial risk of spread if there are multiple genetically modified plantations.

Observed dispersal patterns in natural populations provide a rough idea of the rate and spatial scale of transgene dispersal. Obtaining more precise estimates of transgene escape rate by direct extrapolation of these patterns, however, may not be adequate: most empirical studies report seed or pollen immigration rates into small study plots or small populations, surrounded by widespread conspecific forests, while source genetically modified tree stands (especially experimental plantations) may be small relative to wild recipient populations. This demographic scenario would result in transgene escape being less frequent than observed migration rates among natural stands, since increasing population size is expected to decrease immigration rates (Ellstrand and Ellam, 1993). But even if probably lower than reported immigration rates into small natural stands, potential rates of gene movement from small genetically modified tree stands into large wild populations may still be significant, as suggested by the observed low levels of gene flow from hybrid poplar plantations into wild populations of interfertile congeneric species (reviewed in Slavov, DiFazio and Strauss, 2004), and by the available estimates of transgene spread from genetically modified agricultural crops (Rieger *et al.*, 2002; Beckie *et al.*, 2003; Watrud *et al.*, 2004). Moreover, a very low rate of gene flow may be sufficient for eventual transgene fixation in the wild if it occurs recurrently or if it confers a selective advantage over conventional trees (Haygood, Ives and Andow, 2004; see next section).

Overall, considering an appropriately large temporal scale, the available evidence strongly suggests that the efficient dispersal systems of trees render the movement of transgenes from genetically modified plantations into conventional forests highly probable. But although it is reasonable to assume a very high likelihood of occurrence of a certain amount of transgene flow, predicting the rate at which it will happen, especially over very long distances, requires further empirical and theoretical analysis.

### Predictive models

Thoroughly assessing the long-term exposure of natural forests to genetically modified trees through gene flow can hardly be accomplished without theoretical

modelling. There are numerous challenging aspects of the problem for which field trials, though highly desirable, are not really feasible. The most difficult and critical factor is that the relevant spatial and temporal scales are very large, with serious implications for many aspects of the assessment of exposure through gene flow. One should be ready to imagine a mosaic landscape of genetically modified tree plantations and natural stands, in more or less close proximity, spreading over thousands of hectares of land, eventually across different properties or even national territories. One would like to be able to predict the expected rate of transgene movement into a particular natural population and the probability of long-term persistence and eventual fixation of the transgene in this population.

### Long distance dispersal models

A first consequence of the large spatial scale of the problem is the need to quantify the frequency and range of long-distance transgene dispersal, so that one can make predictions about the expected rate of transgene dispersal in particular spatial and demographic scenarios. Measuring rare long-distance dispersal events is very difficult in practice and, as mentioned above, extrapolating phenomenological functions beyond the experimental range of real data does not constitute a reliable approach to predicting long-distance dispersal. As pointed out earlier, phenomenological model predictions are quite sensitive to model selection, which in turn is highly dependent on sampling scale (Kuparinen *et al.*, 2007a). Moreover, the dispersal process is expected to be highly dependent on environmental variation, and thus extrapolating case-specific dispersal patterns to different environments may lead to misleading predictions (Kuparinen, 2006). Mechanistic dispersal models, by quantitatively describing the relationship between dispersal and the underlying physical factors causing particle movement (mainly propagule terminal velocity, release height, canopy structure and air flow statistics), may be more adequate to infer solutions outside the spatial and environmental domain for which observed data are collected, providing a wider range of predictive relevance. It must be noted, however, that mechanistic models are not so easily applicable to animal-dispersed species.

Mechanistic wind dispersal models are especially suitable to model long-distance propagule transport because they can emulate stochastic turbulent transport processes, such as updrafts above the forest canopy, considered a major determinant of long-distance seed and pollen transport (see Kuparinen, 2006 for a review of mechanistic wind dispersal models). For instance, in a study involving laboratory and field experiments with five tree species in a deciduous forest in North America, Nathan *et al.* (2002) fitted a Eulerian-Lagrangian model that was able to predict the proportion (1–5%) of seeds collected at different heights of a 45-m tower, a proportion considered as an upper bound on the probability of their long-distance transport. Given the typical high seed fecundity of wind-dispersed trees (roughly  $10^3$ – $10^5$  per tree per year; Clark *et al.*, 1999), this would represent substantial numbers of potential long-distance dispersal events. Using similar coupled Eulerian-Lagrangian simulations, parameterized for *Pinus taeda*, Williams

*et al.* (2006) predict 0.007% to 0.1% of seedlings establishing beyond 1 km from 16–25-year-old plantations, or about 40–60 seedlings per year, assuming a 10-ha genetically modified stand, a conservative annual fecundity of  $10^3$  seeds/ha, and a 6% germination rate.

More recently, Kuparinen *et al.* (2007b) have developed a specific mechanistic approach to airborne dispersal of propagules in forested areas that explicitly addresses long-distance transport by modelling complex turbulent flows in upper parts of the atmospheric boundary layer. Consistent with previous studies, their simulations suggest that large amounts of light pollen, and small but significant proportions of heavier particles like seeds, may easily disperse over several kilometres. Lower propagule terminal velocities, higher release heights and changing wind conditions significantly increased the predicted rate and range of long-distance transport. They also point out, however, that further work is needed for better understanding of implementing release and deposition processes and within-canopy turbulences, which are critical for effective seed and pollen dispersal.

### Population dynamics models

Once estimates of the frequency and spatial range of transgene escape are available, the next step is to investigate the long-term demographic dynamics of immigrant transgenes in natural populations, in competition with wild genotypes, and under a range of environmental conditions, including the presence or absence of the agent that the transgene may have been engineered to mitigate (Farnum, Lucier and Meilan, 2007). Only in this way will it be possible to predict the degree and duration of the exposure of natural forests to transgenes, which will range between fixation of the transgene in the recipient natural population or its quick elimination by natural selection. Given the long lifespan of trees, and taking into account that the relative fitness of transgenes may have multiple components expressed at different life stages, the necessity of theoretical models to examine multiple-generation transgene population dynamics becomes evident.

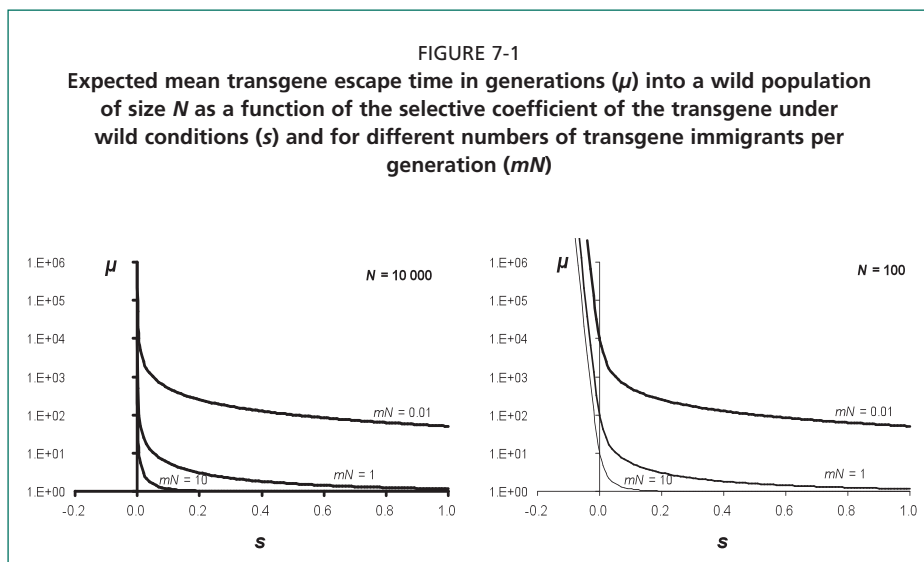
Simple demographically and spatially unstructured models can provide a first insight of transgene population dynamics. As an example, Williams and Davis (2005) use deterministic phenomenological simulations to investigate the fate of transgenes in a small escaped genetically modified-tree colony, with assumed initial transgene frequency of 50%, under different selective and demographic scenarios. Although their quantitative predictions are not easily interpretable, because of the absence of stochastic drift in the model and because of the artificial assumption that any immigrants arriving into the colony after its foundation had the same transgene frequency as colony residents, they illustrate the intuitive idea that both the relative abundance and relative fitness of the escaped genetically modified individuals are critical for transgene spread. Specifically, transgenic alleles in the escaped genetically modified-tree colony will tend to fixation if the transgene confers a net fitness advantage relative to wild-type alleles, but this process may be retarded if the genetically modified colony is embedded within a relatively large natural forest.

A more straightforward and formal description of the probability of transgene escape in a spatially and demographically unstructured model is provided by the analytical treatment of Haygood, Ives and Andow (2004). They define transgene escape into a wild population as the arrival of a transgene whose descendants will eventually take over the population, i.e. the descendant lineage of which will be destined for fixation, showing that the probability distribution of escape time (not time to fixation), defined in this way, is approximately geometric, with mean equal to the inverse of the probability of transgene escape.

Here, we derive the probability of transgene escape in a similar fashion to Haygood, Ives and Andow (2004), but considering a diploid transgenic locus and allowing for negative selective coefficients for the transgene, in order to illustrate the interplay between the probability of transgene escape, the transgene migration rate, the recipient population size and the adaptive value of the transgene. Let  $N$  be the number of mature individuals in the wild population,  $m$  the fraction of gametes in the wild population that flow from the genetically modified plantation per generation ( $m < 0 < 1$ ), and  $s$  the selection coefficient for the transgene under wild conditions. From standard population genetics theory (e.g. eqs. 3.31 and 5.47 in Ewens, 2004), the probability that a newly arrived transgenic lineage is destined for fixation is approximately  $\pi = (1 - e^{-s}) / (1 - e^{-2Ns})$ , assuming there is no dominance. The first generation after gene flow begins, we have  $Nm$  transgenes in the wild population, and the probability that at least one of them is destined for fixation, i.e. the probability of transgene escape, is given by  $p = 1 - (1 - \pi)^{Nm}$ . If the transgene does not escape in the first generation, we assume (following Haygood, Ives and Andow, 2004) that the situation is essentially the same in subsequent generations, until the transgene escapes or gene flow ends. That is, we assume that in these subsequent generations the amount of transgenes produced in the wild population and the number of individuals in transgenic lineages destined for extinction are small enough that  $p$  remains approximately the same until a transgene escape event occurs. That leads to a probability distribution of escape time, in generations, that is approximately geometric with mean  $\mu = 1/p$  (Haygood, Ives and Andow, 2004).

Using this model, we examined (Figure 7-1) the estimated value of the mean escape time ( $\mu$ ), in generations, for different values of wild population size ( $N = 100$  and  $10\,000$ ), transgene selective value ( $s = -0.1$  to  $1.0$ ) and number of transgene migrants per generation ( $Nm = 0.01, 1$  and  $10$ ). A first interesting result is that the escape time becomes virtually independent of  $N$  as soon as the transgene has a relatively small selective advantage ( $s > 0.01$  in our examples). If the transgene is neutral ( $s = 0$ ), by contrast, the mean escape time is greatly reduced for small population sizes, assuming a fixed number of transgene migrants per generation ( $Nm$ ). For instance, if  $Nm = 10$ , we have  $\mu \approx 10$  for  $N = 100$  and  $\mu \approx 1000$  for  $N = 10\,000$  (since, as expected under our model assumptions, the fixation probability of the transgene becomes approximately  $m$  for  $s = 0$ ). Now, if the transgene is maladaptive in the wild ( $s < 0$ ), escape time becomes enormous for large populations (the probability of transgene escape becomes negligible),





irrespective of the number of migrants, while it can be relatively short if the wild population is small and the number of migrants relatively large (e.g.  $\mu \approx 100$  for  $N = 100$ ,  $s = -0.02$ , and  $Nm = 10$ ) (Figure 7-1). This is because stochastic drift reduces the efficiency of selection in small populations. Finally, for any given value of the selective coefficient (with  $s \geq 0$ ), escape time increases as the number of transgene migrants decreases. Interestingly, however, escape time becomes fairly short ( $\mu < 100$ ) as soon as the number of migrants per generation is not too small ( $Nm \geq 1$ ) and the selection coefficient of the transgene is very slightly positive ( $s > 0.001$ ). We believe that the arrival of at least one transgene migrant per generation ( $Nm \geq 1$ ) can be considered a minimal working rate for genetically modified tree populations (given that this is a per generation rate and that trees may have a generation time of several decades), and thus that the probability of transgene escape will be generally very large for any transgene that is even slightly favoured by selection.

Spatial distribution, demographic structure and environmental variation may influence population dynamics in real systems, interacting with population genetic processes. Therefore, predictive models for the spatial and temporal dynamics of escaped transgenes need to be spatially, ecologically and demographically realistic. An early example of long-term spatial simulation modelling of transgene spread is provided by the STEVE model (DiFazio *et al.*, 2004), aimed at investigating potential invasiveness of transgenic poplars in the northwest United States of America. This stochastic model tracks transgenic and conventional genotypes in a virtual landscape that includes topographical and ecological information, with population dynamics being governed by modules simulating growth, reproduction, seed and pollen dispersal, and competition. The authors performed sensitivity analyses to study the consequences of different dispersal and selective conditions, several deployment and flowering control scenarios, and contrasting

selective values for the transgene. The main results highlighted by the authors (Slavov, DiFazio and Strauss, 2004) are:

- transgenic introgression into conventional stands was insensitive to the slope of local dispersal curves, but highly sensitive to changes in the proportion of long-distance dispersal;
- an imperfect, but tightly linked, sterility gene could dramatically slow the spread of a transgene that provided even a strong selective advantage;
- the spread of neutral transgenes could be greatly reduced by sterility levels whose effectiveness was of the order of 95%.

Perhaps the most elaborated and realistic spatial simulation model of transgene escape to date is that of Kuparinen and Schurr (2007). The model, which can be run in deterministic or stochastic form, includes: modules for seed dormancy; seedling establishment; tree growth; individual mortality; ovule, pollen and seed production; and pollen and seed dispersal. Many of the relevant demographic and reproductive processes are density-, genotype- and size-dependent. Seed and pollen dispersal are simulated using a mechanistic Lagrangian stochastic model especially configured to account for long-distance dispersal events. As an application, the authors examined the sensitivity of transgene escape to demographic differences between genetically modified and conventional trees, to the expression (dominant or recessive) of the transgene, and to the initial genotype of the genetically modified plants at the engineered loci (homozygous or heterozygous). After 100 years, a neutral transgene had diffused through short distance dispersal from the plantation into a contiguous conventional stand, with declining frequency with distance. Additionally, small numbers of transgenes had escaped the plantation via long-distance dispersal to distances beyond 1000 m. Decreased density-dependent mortality and increased growth, relative to conventional trees, were the two demographic factors of transgenes that resulted in a higher increase of escape rate into natural populations. The expression of transgenes only affected the probability of escape when they had demographic effects, with markedly reduced escape for recessive transgenes. Escape rate was also reduced for dominant transgenes if the initial genetically modified population consisted of heterozygous individuals.

Despite the utility of modelling, it must be kept in mind that theoretical models lacking realistic calibration will only provide qualitative insights on the sensitivity of transgene escape to particular factor effects. Quantitative predictions will require adequate parameterization, requiring experimental data, which should be pursued to the extent that model factors are legally amenable to empirical testing. That necessary caveat translates into a pair of serious challenges facing forest geneticists. One is the need to validate long-distance dispersal models empirically, including mechanistic models. The other, most critical, is to quantify the relative fitness of transgenes under different ecological conditions. As has been pointed out (Lee and Natesan, 2006), predictive models will not be really useful for transgene risk assessment if the uncertainty surrounding transgenic fitness impacts is not reduced.

## REAL-TIME TRANSGENE FLOW ASSESSMENT

### What do we need?

Another front where gene flow researchers can contribute to exposure assessment of genetically modified trees is the development of methods for real-time detection of transgenes. Although field release of genetically modified trees is still uncommon (Van Frankenhuyzen and Beardmore, 2004), there will soon be high demand for tools for field assessment for transgenic presence in natural forests. Many of the available methods for gene flow analysis are not adequate for this purpose. Genetic methods for assigning individuals to populations (Manel, Gaggiotti and Waples, 2005), for instance, require a thorough characterization of the recipient and the genetically modified donor populations, and will be of little help unless there is very strong divergence within the allele frequency spectra of the populations, since otherwise assignment error rates are likely to be larger than the presumably very low transgenic frequency to be estimated. Parentage assignment, in contrast, requires exhaustive genotyping of all potential parents within the study area, which becomes unfeasible over the spatial scales that are relevant for transgene flow detection, being moreover subject to a level of statistical uncertainty that may be unacceptable for decision-making. In fact, parental designation is not necessary for detecting transgenes, which only requires a categorical diagnostic criterion to conclude whether an individual is carrying the engineered sequence or not, for which several more powerful monitoring methods are available (Stewart, 2005).

### Transgene monitoring methodologies

The most straightforward detection method is laboratory screening of the transgenic sequence directly. This will require tissue collection and DNA extraction from potentially escaped genetically modified individuals in conventional populations for the examination of a diagnostic DNA segment at the modified region. This can typically be achieved by means of PCR amplification, followed by automated sequencing or by single nucleotide polymorphism (SNP) analysis. European regulatory schemes are already demanding all sequence information of transgenes in applications for authorization for release of genetically modified organisms, including the location of primers used for detection (EFSA, 2006). Ideally, the proposed 'biobarcode' technology (Gressel and Ehrlich, 2002) would permit a standardized procedure for transgene detection. This technology would consist of the inclusion of a non-coding DNA segment in the engineered DNA sequence, flanked by universal PCR primers, which would contain a variable region encoding information on transgene identity and origin.

More elaborated screening procedures, using nanotechnologies, would allow faster and *in vivo* monitoring of transgenes in the field. These techniques, still not implemented for commercial transgene detection in plants, involve developing nucleic acids that are complementary to the target transgenic transcript and that carry a fluorescent label that can be seen by shining an ultraviolet light on the plant (see Stewart [2005] for a detailed description of different methods). There are, however, several barriers to the use of this kind of approach, that may

eventually prevent its implementation, such as safety concerns about fluorescence-based technologies, the additional investment for genetically modified tree re-engineering, and, most importantly, legal restrictions on and social rejection of further transgenic engineering (Stewart, 2005).

An alternative approach for transgene screening is testing for diagnostic phenotypic traits expressed by the transgene, such as herbicide and pest resistance, or some easily detectable protein. This procedure can allow an intensive, low-cost screening, prior to more direct assessment using DNA-PCR analysis. Watrud *et al.* (2004), for instance, used two cycles of herbicide spraying to detect the presence of herbicide-resistance transgenes in progenies collected from conventional populations of creeping bentgrass. Survivors of the second cycle were then tested for the presence of a transgene-encoded protein using commercial test strips. Finally, DNA from herbicide resistant and protein-positive plants was extracted and sequenced for final confirmation of transgene presence. Similar screening protocols might also prove useful for forest trees, as long as the engineered traits are expressed at an early life stage (Smouse, Robledo-Arnuncio and González-Martínez, 2007), which may not be the case for altered fibre quality or growth. Testing for herbicide or pest resistance by spraying progenies collected from seed trees could be feasible for detection of transgene flow via pollen, but similar tests on naturally regenerated seedlings in the wild might be ecologically unacceptable.

### Challenges related to sampling

The challenge of categorically detecting the early stages of transgene spread in the wild can be intimidating. Assume that a transgene is present in the natural regeneration of a conventional forest at a frequency of  $q = 10^{-3}$ . Then, if we wanted to reduce the probability of not detecting the transgene below  $\alpha = 0.01$ , we would need to screen at least  $n = 4600$  seedlings (ensuring that  $\alpha = (1 - q)^n < 0.01$ ). If the introgression rate were as low as  $q = 10^{-4}$ , we would then need over 46 000 samples to ensure  $\alpha = < 0.01$ . Given the additional advisability of sampling over large spatial and temporal scales, the problem becomes such that some have simply concluded the impossibility of proving that transgenes are absent from a given region (Ortiz-García *et al.*, 2005). Of course, if a transgene were ultimately to reach fixation, its frequency would eventually have to reach levels much easier to detect, but this may only happen after a minimal initial frequency (as low as  $1/2N$ ,  $N$  being the recipient population size) and several generations of random drift or positive selection, which probably means several centuries for forest tree species. Nevertheless, early detection is critical if we are to intervene. That being the case, strongly replicated sampling over large spatial scales seems unavoidable, which, if legally enforced, might have implications for the economic payoff associated with genetically modified tree plantations.

The intricacy of accurate early detection of transgenes is illustrated by the intense and publicized scientific debate about the presence or absence of transgenic flow into maize landraces in Mexico, with more than ten studies conducted since

2001 and several replies and counter-replies disputing statistical and sampling issues (see Mercer and Wainwright, 2008 for review and discussion). In fact, it has been argued that too much emphasis is being placed on the rate of transgene flow, when the parameter of greater concern should be the relative fitness of the transgenes (Hails and Morley, 2005; Lee and Natesan, 2006; Chapman and Burke 2006). The reasons for this argument can be summarized as follows:

- it is reasonable to assume that occasional transgene flow into natural populations is unavoidable in practice, even if at very low rates;
- the magnitude of the transgene migration rate may be very difficult to estimate;
- the relative fitness of transgenes is the primary force governing their spread.

One agrees with this view, and stresses the need for a shift towards further empirical research on life-time fitness costs and benefits of transgenes under contrasting ecological conditions, a challenging task for long-living forest trees. It is also likely, however, that any scientific risk assessment protocol and, perhaps more importantly, any political or social debate on the risks of genetically modified trees, will hardly pass without convincing transgene flow estimates.

### Transgene flow avoidance

The exposure of natural ecosystems to genetically modified trees could be essentially avoided if effective gene flow from transgenic plantations were interrupted. Since, as discussed above, spatial isolation does not provide an efficient barrier to transgene flow, alternative transgenic containment and mitigation strategies are being developed. Specifically, containment methods use different forms of genetic engineering to prevent transgenes from leaving genetically modified plants, either by inducing sterility or by removing the transgene from gametes before their release (excision techniques). Mitigation procedures intend to reduce the fitness of transgenes by tightly linking it to an engineered gene that is maladaptive in the wild, hence providing a useful complement to the expected leakages in containment strategies. Technical and practical details concerning the development, implementation and efficiency of different containment and mitigation strategies were extensively dealt with in an earlier chapter. Here, it is simply asserted that fully safe transgene containment methods are yet to be developed and thoroughly tested on a case-by-case basis. A recent study reports promising results along this line, with some excision techniques achieving 100% deletion of functional transgenes from pollen and/or seed, as tested on more than 25 000 progeny of tobacco plants for each transgenic event (Luo *et al.*, 2007). Further research is needed, however, to test the temporal and environmental stability of this technique for tree species and different transgenes. Due to the long life cycle of forest trees and the diverse ecological conditions they experience, the stability of any genetically engineered transgene containment strategy remains a matter of concern. It must be kept in mind that containment failure rates much lower than  $10^{-3}$  may be necessary to reduce transgene escape probabilities to acceptable levels (Haygood, Ives and Andow, 2004).

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## 8. Ethical considerations regarding genetically modified trees

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### NON-TECHNICAL LIMITS TO BIOTECHNOLOGY

Until recently, the main limits to modern biotechnology were of a technical type: “What is it possible to do?” However, as the technical difficulties began to be resolved, and as practical applications came within reach, the question increasingly became one of “What is it acceptable to do?” Today, scientists and the biotechnology industry face a growing number of ethical issues and questions relating to the social context in which biotechnology is used. This may mean a growing discrepancy between expert and public views. Public apprehension about gene technology is triggered by a range of concerns: about environmental risks; the patenting of genetically modified organisms; labelling of products; and the possibility of exerting democratic control on the development and application of biotechnology (Holland and Pratt, 1995; Thompson, 2001).

When it comes to genetically engineered trees, systematic silvicultural improvement measures such as selective breeding are, compared with agricultural plant breeding, very much in their infancy (Campbell *et al.*, 2003). The science underlying the genetic engineering of forest trees, i.e. tree and plant genomics, is limited (Adams *et al.*, 2002). Moreover, the first large-scale commercial applications of transgenic trees are only just beginning to appear (Sedjo, 2004). It is clear that ethical discussion of the complex issues raised by the genetic engineering of forest trees needs to be appropriately directed.

Successful adoption of genetically engineered trees will depend not only on the soundness of the technology and science, but also on how these trees are perceived by the public. In public debate, the terms ‘genetically modified’, ‘transgenic’ or ‘genetically engineered’ are used interchangeably for those trees that have been modified using recombinant DNA and asexual gene transfer methods, regardless of the source of DNA employed (Brunner *et al.*, 2007). Potential use of gene technology with forest trees has raised concerns around the world. These concerns are serious in Japan and in Europe; they have also emerged in North America (Owusu, 1999; Strauss, 2004a). The results of silvicultural genetic engineering have been disapprovingly dubbed ‘Frankenstein forests’ (Warwick, 1999), ‘Designer trees’ (Rautner, 2001) and ‘Frankentrees’ (Native Forest Network, 2000) – with clear reference to the term ‘frankenfoods’ used in the genetic modification food debate. A number of protests, sometimes involving the destruction or vandalism of field trials, have occurred – for example in the United Kingdom in 1999, where

two genetically modified poplar trials owned by AstraZeneca were ruined; in the United States of America in 2001, where a laboratory of the University of Washington was firebombed (cf. Strauss, 2004b); and in Finland in 2004, where Finland's only field study on genetically modified trees was attacked, destroying 400 trees (Hall, 2007). Concern has also been manifested in non-violent protests and campaigns by high-profile environmental organizations. At the same time, genetically engineered trees have been characterized as 'superior' (Merkle and Dean, 2000) and 'highly green new tool[s]' (Valenzuela and Strauss, 2005) by proponents of the use of genetic engineering in forestry. Indeed it has been argued that genetically engineered trees are part of, if not the key to, sustainable silvicultural development (Salwasser, 2001; Doering, 2004).

In the context of the aim for greater sustainability, forests can be viewed according to their underlying management philosophy. Genetic engineering in forestry relates in an interesting way to two currently recognizable and opposing trends in forest management. One trend is technological. It involves an efficient system of tree cropping, advances in tree breeding and the continued use of exotic species. Characteristically, followers of this trend respond to, and control, the prevailing ecological, environmental and economic conditions by employing artificial seeding, planting, breeding and so forth. The other recognizable trend is the 'ecological' or 'back to nature' trend, which in some respects parallels the organic trend in agriculture. Here the aim is not one of exploiting natural forests, but rather of controlled, sustainable harvesting of semi-natural forests. It is also considered important to plant forests and silviculturally treat them so that they resemble the structures and processes of comparable naturally wooded areas (Gamborg and Larsen, 2003). The 'back to nature' approach is gaining a foothold in Europe and elsewhere, as problems are now recognized associated with intensively managed plantations: problems of ecological stability and flexibility, of biodiversity, and of an aesthetic and recreational nature. When it comes to new technology, it is not just a question of the technology itself but of the attitude to the technology and the underlying management philosophy in which the technology is embedded.

The forestry sector can learn lessons from the development and introduction of genetically engineered (food) crops – although, evidently, there are significant differences between genetically engineered forest trees (by which we mean trees without edible fruits) and genetically engineered food crops (cf. Hall, 2007). Some important differences are set out in Table 8-1.

Forestry is essentially different from agricultural plant production, not only in respect of biological factors such as rotation age, but even more so in socio-economic and cultural factors. These differences should be borne in mind when one assesses gene technology in forestry.

The distinctive features of forestry are biological and socio-economic as well as cultural. Strikingly, forest trees for timber, pulp or fuel production are not part of the human food chain. Hence, the use of genetically engineered trees will not be part of the massive food safety discussion that surrounds genetically engineered

TABLE 8-1

**Parameters of genetic engineering: non-food forest trees versus agronomic food crops**

Biological factors	Socio-economic and cultural factors
Forest trees are far less improved through selective breeding than agricultural crops	Forests are more accessible to the public than agricultural fields
Forest trees evidently have a much longer life than agricultural, even perennial, crops, and the forest persists much longer; rotations may span more than a hundred years	Forests, unlike agricultural production units (fields), encompass everything from natural or semi-natural woodland to tree plantations
As ecological systems, forests are much more structurally and functionally complex than their agricultural counterparts	Forests produce several recognized goods and services at the same time
Forest trees (by definition) do not produce edible goods (but timber, pulpwood, woodfuel and so on)	Forests have conferred upon them a diversity of social, cultural, symbolic and other values

food crops. Initially, genetically engineered trees should have an advantage, as the food discussion has many cultural and ethical connotations. However, some concerns are aggravated, such as environmental concerns and concerns about biodiversity, as uncertainty mounts with ecological complexity and time. Some concerns are specific to forests and forest trees, including concerns about the special cultural and symbolic values attaching to forests, and forest as an important component of the landscape (O'Brien and Claridge, 2002). Nevertheless, many of the features listed above do not alter the fundamental mechanisms underlying our attitudes to, and concern about, genetic engineering.

In examining the potential concerns related to genetically engineered trees, and in assessing the underlying ethical issues, we need to consider two sets of issues. First, what should be on the agenda, and what is considered an ethically relevant concern? Second, how should we address and discuss these concerns, how should we handle conflicts of interest, and how should we take into account the differing opinions? It is to be hoped that early addressal of these issues will help to avoid the problems and controversies connected with the introduction of gene technology in agriculture.

## GENETIC ENGINEERING AND RISK ASSESSMENT

Like most new technologies, gene technology gave rise to both huge expectations and widespread worries when it emerged in the 1980s. In Europe, so-called Eurobarometer surveys (CEC, 1992, 1993, 1997, 2000, 2003) have consistently shown that among the general public the use of gene technology in agriculture and other areas of food production has a low level of support relative to other applications. In general, studies have shown a more positive perception of biotechnology among the United States of America public than the European public (Eyck, Thompson and Priest, 2001). Since 1999, approximately 60% of the respondents were of the opinion that biotechnology would provide them benefits within a five-year time frame. However, it is interesting that concern about genetically engineered crops does not involve total opposition to gene technology but instead specifically relates to the application of such technology to food. For example, in a series of qualitative nationwide interviews conducted in the Eurobarometer surveys, it was apparent that most people welcomed medical

progress brought about by genetic engineering (Lassen, Holm and Sandøe, 2003). Consequently, the antagonism is not created by the process of genetic engineering but its application to modern food production. A reasonable assumption is then that other factors must be at stake, and that these factors have to be included in any analysis (Madsen and Sandøe, 2001).

The conceptual framework for dealing with the worries was risk analysis: before the release of genetically engineered organisms a scientific risk assessment should be undertaken to identify and evaluate any potential adverse effects on human health and the environment. The results of the assessment should form the basis for management of the risks by public authorities; it should also provide input to risk-communication efforts directed at the broader public. In some cases this strategy seemed to be a successful model for introducing gene technology in ways acceptable to the public. In Denmark, for example, after an intense public debate, the industrial use of gene technology for the production of enzymes and pharmaceuticals was accepted by the public. In other cases, however, the introduction of gene technology and other forms of modern biotechnology has led to controversies that seem to have no end.

Some of the time, then, rather than putting an end to controversies, risk assessments have appeared to fuel new controversy. This is paradoxical. Considerable sums have been spent on the risk assessment of genetically modified crops. Broadly speaking, no major scandals have occurred, and a number of the plants have been deemed safe both for human health and for the environment. Nevertheless, after more than ten years of, at times, intense debate the crops are as controversial as ever, and in particular, it seems, to the European public. Is there any reason to believe that the same will happen with genetically engineered forest trees?

### **KEY ETHICAL CONCERNS ABOUT MODERN FOREST BIOTECHNOLOGY: GLOBAL PERSPECTIVES**

When it comes to new technology, it is not just a question of the technology itself but of the management philosophy in which the technology is embedded and the underlying values. Here, concerns specific to forests and forest trees play an important role. Using genetically modified trees in silviculture is not exclusively a technical issue. Ethical assumptions relate to what kind of nature is wanted and the means considered acceptable (List, 2000). In answering these questions, we should be looking at what are the likely consequences of genetic engineering. Should we be trying to improve on nature? (Reiss and Straughan, 1996). 'Ethics', as the term is here understood, has as its main function to reflect and clarify. Reflection may for example concern the complex trade-offs between conservation and the consumption of renewable resources. The output may be a better understanding of various ways of looking at such trade-offs and thereby making room for dialogue about the goal of forest management. In general, ethical reflection may help to formulate and discuss the relative importance of potentially conflicting concerns and values.

Modern forest biotechnology has brought the techniques of silviculture and plant development before the public eye in a way that is unprecedented in recent times (Thompson, 2001). Yet, in forestry, biotechnology has not (yet) been subject to anything like the intensity of debate it has received where agricultural products, and in particular genetically engineered crops such as soybean, are concerned. There are several reasons for this.

One reason is that, at present, no large-scale commercial production is taking place. For gene technology in agriculture, serious debate first started when genetically modified crops were produced commercially. In forestry, commercialization has begun in China and is imminent in South America (Strauss, 2004a). In Europe, genetically engineered trees are unlikely to appear for the time being. Despite the fact that the number of trees tested has risen substantially in recent years, timber trees still make up only a small proportion of the total number of field trials with plants. Thus, between 1987 and 2001, timber trees were involved in just 1.2% of field tests (Sedjo, 2004). Consequently, data on the ecological effects, and of any unintended potential side-effects, of genetic modification in this field are currently scarce. Moreover, we cannot necessarily transfer the abundant number of more available results from studies of genetically modified plants used in agriculture because trees are essentially undomesticated, have intrinsically long life spans and host a wide variety of organisms.

A second reason, as already mentioned, is that trees are not food crops, and therefore strongly held beliefs about genetically modified foods do not carry over to them. The worry about genetically engineered trees is often portrayed as relating to the natural environment. This worry seems to be intensified by the longevity of trees (as compared with agricultural crops), since this makes it harder to anticipate potential implications.

In general, the concerns we are dealing with here have to be seen in connection with the ways in which forests are perceived by the public. Forests have an emotive value for many people, which does not apply to agricultural crops like wheat. Trees have a place in history, mythology and identity. And as a North American study reported by Hall (2007) claims, genetically modified trees could “come into conflict with a socio-psychological need found throughout Western history, for the forest to remain apart from civilization, uncultivated and untamed”. Forests, unlike agricultural fields, are seen as ‘uncultivated’ – even though they are, in fact, in many cases both cultivated and intensively managed. So concerns about genetic modification may be rooted in an unacknowledged disapproval of the management of forests as such. Three main sets of concern about genetic engineering may be distinguished and separately discussed: risk-centred, socio-economic and cultural. These are set out in Table 8-2.

According to Lassen and Jamison (2006), it is a characteristic of the concerns of the public that these concerns are framed in ways that go beyond risk to the environment and (in the case of genetically engineered food crops) health. Socio-economic concerns deal with gene technology as a way of achieving economic development while looking at the socio-economic costs and benefits, and the

TABLE 8-2

**Matrix of public apprehensions concerning genetic engineering in the context of risk-centred, socio-economic and cultural concerns**

Concerns for discussion	Central themes	Key concepts
Risk-centred	Environment, health	Risk, uncertainty
Socio-economic	Profitability, production	Cost/benefit, power
Cultural	Moral, religious aspects	Ethics, rights, integrity

Source: After Lassen and Jamison, 2006.

power determining the distribution of these costs and benefits. They also examine intellectual property rights in relation not only to economic profits, but also to democratization. And, finally, cultural discussion of gene technology changes our understanding of ourselves and our capabilities, and indeed the borders between the natural and the unnatural.

Across large sections of the general public, there is limited understanding of biotechnology and its requirements (BEPCAG, 1997). A major problem is, though, that greater knowledge does not *per se* lead to less scepticism towards biotechnology; in some cases, indeed, quite the contrary occurs. Where information about biotechnology is provided, both the overall proportion of people with a more positive attitude towards biotechnology and the proportion of sceptics increase, but the net result is that the number of sceptics rises. Improved understanding and knowledge puts one in a better position to take a stance, but it does not necessarily lead one to sympathize with the technology.

## **PUBLIC ACCEPTANCE? LESSONS FROM GENETICALLY MODIFIED AGRICULTURE**

In reality, the attitudes of the general public and other stakeholders to genetically engineered forest trees are far from as well known, as demonstrated by the worries that agricultural crops provoke (cf. Hall, 2007). Results from studies on perception of plantation forestry have been used to gain a greater understanding of how the public reacts to land-use changes (Neumann, Krogman and Thomas, 2007). Studies from Asia (Yap, 2004) and Australia (Barlow and Cocklin, 2003) find that the development of plantation forestry may be accompanied by controversy. A recent study of public perception of hybrid poplar plantations in Canada – although the techniques used to create the trees are different from genetic engineering, the social impacts of going from traditional management, for example, to more intensive tree production may be similar – suggests that landscapes are closely linked to the values and identities of the people living there.

One of the main lessons from genetic engineering in agriculture shows that if modern biotechnology is to stand a chance, three main conditions for public acceptance must be met: utility, low risk, and an assurance that biotechnology is used in a ‘decent’ way. These three conditions are somewhat interrelated. Many people would accept a certain risk (depending on how risk-averse they generally are) as long as potential utility attaches to the application of the biotechnology (BEPCAG, 1997). Nevertheless, something more seems to be at stake. In surveys, this has sometimes been labelled ‘moral doubt’, but it has no clear definition.

Earlier results from the United States of America suggest that there moral acceptability is a better predictor of encouragement than risk or usefulness (Eyck, Thompson and Priest, 2001). The important thing about ‘moral’ is that it seems to override what would otherwise be seen as an acceptable technology in terms of risk and utility.

### **Utility**

A technology or innovation can possess utility in several ways. From an economic perspective a technology is useful if it is competitive in commercial conditions, e.g. through increased productivity. However, more than this will be required if the public are to consider a certain forest tree biotechnology useful: the technology in question has to contribute significantly to mitigation of key human or societal problems. Evidently, what is considered ‘significant’, and what is a ‘key’ problem, are debatable issues, but examples could be positive environmental impact or helping to alleviate poverty in developing countries. Moreover, usefulness is gauged not only relative to existing conditions but also in relation to alternative methods of reaching the same level of utility (e.g. insect damage to trees might be reduced through increased insect resistance obtained by conventional breeding practices or altered silvicultural practice).

### **Risk**

Another key factor in the acceptance or rejection of modern biotechnology is risk. A number of studies have consistently shown that the majority of people are willing to run a risk provided there is a proportionate gain. For example, most of us drive a car although this specific activity has a high, well-documented risk. Clearly, people may evaluate risks in incompatible ways and make conflicting proposals for mitigating risks (Thompson and Dean, 1996). In the domain of genetically engineered crops, several risk assessments have failed to show conclusively that there is a (‘substantial’) danger to the environment or to health. Nonetheless the public, especially in Europe, does not feel comfortable with the use of the technology. The discomfort is partly grounded in a public scepticism about science’s ability to judge the long-term, accumulated consequences of applying a new technology. Risk assessments that try to identify hazards and quantify risks will not help in this sense, as they are part of the scientific research that is being questioned.

If risk assessments are to help reduce public apprehension, the public must have more trust in experts and authorities: something currently lacking, especially in Europe. Experience from the introduction of gene technology in agriculture and food production suggests that, to regain or strengthen this trust, it would help to ensure that risk assessments are not seen as a way to relieve decision-makers of their part of the responsibility. Moreover, a fair account of the limitations of the risk assessments should be given, and there should be openness about when and where more than scientific reasoning and assessment are needed (i.e. about when we will accept that we must live with the remaining uncertainty).



### Moral doubt

The third condition on the use of modern biotechnology requires us to rebut ‘moral doubts’ by applying the technologies in a ‘right’ way. To many people, the whole idea of meddling with the genes in living beings, whether they are animals or plants, is ethically problematic. The challenge here is to formulate these concerns about (broadly speaking) respectful use of nature. An important point in this connection is that the public at large do not necessarily share the biological scientists’ conception of nature. Many, for example, see species as stable entities that change only as a result of our technical manipulations. However, for the ecologist, stability is a relative concept and species are constantly changing.

Two types of argument often appear in surveys of attitudes to modern biotechnology (Madsen *et al.*, 2002). Roughly, one – ‘nature as a safety mechanism’ – is that, by relying exclusively on ‘natural’ processes (here understood as not using genetic engineering and not crossing natural species barriers), we obtain greater control. The second – ‘natural order’ – succinctly avoids scientific considerations about such matters as the risks of genetic engineering and presents a fundamental ethical criticism. In brief, the position is that we should not ‘tamper with nature’, implying that genetic engineering is ‘unnatural’ and inconsistent with the ‘balance of nature’. A traditional scientific rebuttal is that we are already, in conventional breeding practices, changing the make-up of nature, and the use of gene technology is merely an extension of currently known tree breeding practices (Kellison *et al.*, 2007). This type of answer may, however, lead to people drawing a conclusion opposite to what was intended. Instead of encouraging acceptance of gene technology, it may lead to a more critical attitude towards existing breeding practices and methods.

### ADDRESSING LOCAL AND GLOBAL CONCERNS: TRANSPARENCY AND STAKEHOLDER PARTICIPATION

Some environmental concerns that seem to cause apprehension in the public, such as biodiversity, soil and water effects, can be assessed through comprehensive risk assessments giving detailed information and recommendations to the best of our knowledge. As pointed out by Strauss *et al.* (2004), given the cold reception of the first generation of genetically engineered crops in many parts of the world, a record of usefulness and safety may well be needed for the acceptance of genetically engineered forest trees. Evidently, the type of knowledge required in the latter case will differ from that needed where agriculture and food are concerned because of the elements of uncertainty in forestry, which result from the lengthy time span between establishment and harvesting and the complex interplay between organisms and the natural environment (Tømmerås *et al.*, 1996).

Environmental risk assessments are based on scientific and technical data. But these data must fit into a normative framework that is *not* scientific in nature. This framework stems from the decision problem of whether or not a given application to release and market a particular genetically engineered tree should be approved. The questions the risk assessment is required to answer depend on the criteria

for approval. These criteria involve assumptions about what kinds of risk need to be assessed. Many of these assumptions rest on value judgements. By 'value judgements' we mean judgements implying that, under certain circumstances, something ought to be the case, or one thing or course of action is preferable to another. Only when these judgements are made explicit will it be possible to conduct an effective debate about the broader issues involved in the approval of genetically engineered trees. Hence, an environmental risk assessment views the world through a 'risk window', and this window only makes visible that which has been predefined as a relevant risk. The size and structure of the window is determined by value judgements about what is considered to be an adverse effect within what is considered to be the appropriate horizon of time and space (Jensen *et al.*, 2003). These points are not new. They have been argued for many years by philosophers and social scientists, and they are clearly acknowledged by important scientific bodies like the United States of America National Research Council and the World Health Organization. Nonetheless, they appear not to have diffused into the field of genetic engineering of plants.

Risk assessments are based on current science. Unfortunately they do not reflect the uncertainties inherent in that science. Problematic aspects of new technologies are understood by the public in ways that are essentially different from the risk approach of the scientist, who focuses on risk as the product of effect and probability. This is a lesson which could have been learned already from earlier debates such as the nuclear power debate, but it has either never been learned or has been forgotten. The process of deliberation about genetically engineered forest trees would benefit from its recollection.

Moreover, as was indicated previously, the wider public does not view risk in isolation from potential benefits and other issues. Therefore, to satisfy the concerns of the public, risks should be discussed and dealt with in connection with an assessment of potential benefits to society and other ethical issues. Forests are often associated with naturalness, wilderness, integrity and authenticity. They may also be culturally important (DEFRA, 2002). For example, individual trees and woodlands may represent ways of marking history, contribute to a sense of place, express intergenerational contrasts, or be symbols that represent a 'raw' and 'immediate' bond between 'man and nature'. These aspects cannot be a meaningful part of a risk or impact assessment. In addition, to meet the worries of the general public, some kind of technology assessment is called for that addresses the broader social and ethical issues and goes beyond ordinary risk assessment. Politicians and the authorities must understand the general need for thorough public debate before new technological methods are introduced in order to avoid public frustration arising from the feeling that things are out of control, or beyond the individual's democratic control (Madsen and Sandøe, 2001). A critical issue in this context may be the patenting of crops that biotechnology's critics find so troubling (Cayford, 2003). In view of all this, there is a very reasonable case for the claim that decision-making concerning risk-prone activities should better cohere with societal views and needs.

The participation of the main stakeholders is important. When decisions are made (e.g. about industrial roundwood plantations or reserves designed to protect biodiversity), the subsequent establishment and management routines should take into account the local people. They can do this through social contracts that have been negotiated through discussion and voluntary agreements, as well as through international, national and local policies (Friedman and Charnley, 2004).

Transparency, although now something of a buzz word, is also important. Transparent decision-making can be defined as “decision-making in which the decision-maker clearly presents to others the normative and factual premises behind his conclusions and explains the reasoning leading him from these premises to the conclusion” (Rasmussen and Jensen, 2005). What transparency involves is the uncovering, describing, documenting and communicating of all the steps of the reasoning and evidential assessment that underlies any decision taken. To do this properly, it is necessary to take into account “limitations, weaknesses and uncertainties, as well as pointing at issues which – even though they might be considered relevant from the perspective of some stakeholders – are not addressed by the decision process”. It is clear that, to take account of such factors, new efforts from policy-makers, as well as from the scientific community, will be needed: both parties will need to make the value premises of any given risk assessment known, say what is considered a hazard, what constitutes harm, what are the acceptance criteria, and so on. At the same time, it is worth noting that increased transparency may not come easy, as it leaves the authorities and science as a whole more vulnerable to public scrutiny. Decision-makers may also fear that greater transparency about the limitations of the processes through which decisions are made may lead to more public concern instead of increasing trustworthiness.

## **CONCLUSIONS**

Use of gene technology in forestry has been referred to as a help towards producing more efficient forms of plantation forestry, to generate cost-efficient renewable energy and to solve major environmental problems. However, the very same technology has also been met with initial distrust in several parts of the world, a distrust especially pronounced in Europe and Japan (Herrera, 2005), and a distrust that is already discussed as being a sign of the same resistance and type of polarized debate that occurred regarding genetic modification technology in agriculture (Mayer, 2001); a debate which Merkle and Dean (2000) warn that the research community ignores at its own peril. The question remains whether genetically engineered forest trees will make a difference and contribute to more sustainable silvicultural practices. That is, will the public benefit or will the utility of engineered trees – something which, given the time lag between first proof and commercial application, may be hard to establish – be seen as adequate compensation for the environmental and ecological risks? Will genetically engineered forest trees be considered ‘morally’ acceptable?

The issue of risks and benefits is viewed by many people as something that should be handled by proper scientific evaluation (cf. Strauss, Raffa and List, 2000)

and, to some extent, regulation. The assessments here have to be developed to suit the specific conditions of forest trees. The appropriate way to prepare regulation to address public concerns is currently unresolved. And it might be that evaluation on a crop-by-crop basis or a trait-by-trait basis would not come without a cost because it can be conceived as something that lends credibility to the idea that all genetically engineered products are more dangerous than conventionally bred crops (Strauss, 2003).

One way of dealing with the question of using technology in the 'right' way, i.e. the question of 'moral' acceptability, is to embark upon more public debate. Stakeholder discussions suggest a call for increased public consultation, and for a more participatory decision-making process (Simosi and Allen, 1998). However, it is important to stress the obvious point that dialogue is no guarantee of consensus. Decision-making and regulation in an area where there is no clear consensus may benefit from transparency of the kind discussed above, namely from clear statements of the choices that are to be, or have been, made and the values upon which they rest (Lassen, Holm and Sandøe, 2003). This way, decision-making and regulation stand a better chance of being respected by all parties and ongoing trench warfare may come to a halt.

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## 9. Genetically modified trees and associated environmental concerns

*M. Fladung, H.-L. Pasonen and C. Walter*

Over the past 15 years many techniques such as tissue culture, transformation (gene technology) and genome analysis have been developed for various tree species, including both broad-leaved trees and conifers (Groover, 2007; Henderson and Walter, 2006; Merkle and Nairn, 2005; Giri, Shyamkumar and Anjaneyulu, 2004; Campbell *et al.*, 2003). A very powerful tool in forest tree breeding programmes is gene technology, which can be used to transfer genes of interest into tree genomes. Tree species that have been genetically modified belong to genera like *Populus* (poplars), *Betula* (birches), *Picea* (spruces), *Pinus* (pines) and *Eucalyptus* (eucalypts), and many transgenic lines carrying a variety of gene constructs have been produced and tested in the laboratory and the greenhouse. In addition, a few transgenic tree lines have been tested in the field under natural environmental conditions.

In trees, a range of traits are of general interest as target traits for genetic engineering in trees, such as lignin and/or cellulose modification, disease and pest resistance, tolerance to abiotic stresses, male or female sterility and modification of developmental processes. More recently, traits that make trees more suitable for a bio-ethanol or biomaterials economy are also being considered. Transgenic trees carrying transferred or engineered genes and expressing novel traits may have implications for environmental parameters when grown in scientific field trials or for commercial purposes. Comparative risk analysis is required, considering both direct and indirect environmental effects, including possible gene transfer to wild relatives, weediness, effects on non-target species and other unintended effects of genetically modified or transgenic trees. Any risk identified needs to be compared with accepted practice to achieve the same outcome (the production of wood) and the magnitude of risk evaluated in this context. These risks may result from the deployment of transgenic trees, but they may be similar to risks associated with introduced, non-native trees as well as trees bred by conventional tree breeding methods (Hoenicka and Fladung, 2006).

Major concerns have been raised regarding the introduction of transgenic trees into natural environments. These include potential risks related to the functional stability of the transferred genes in long-lived tree species (operational safety), as well as possible flow of recombinant DNA into the environment through a range of different pathways (Strauss *et al.*, 1995; Strauss, DiFazio and Meilan, 2001; Hoenicka and Fladung, 2006). Direct or indirect environmental effects of

transgene instability or spreading of transgenes are mainly still unknown. For instance, insect-resistant trees have direct effects on the target insect species, but insecticidal GMO pollen may indirectly affect non-target organisms, such as butterflies. A herbicide-resistant tree may have direct effects on ecosystem biodiversity because of fewer weeds following herbicide treatment, but at the same time it may have indirect effects on invertebrates because of lower plant diversity. The possible increased sensitivity of 'low-lignin' trees against fungal and bacterial pathogens is considered as a direct effect, while the escape of genes from lignin-modified transgenic trees into natural ecosystems is considered as an indirect long-term effect. However, it is important for any discussion on risk related to the deployment of genetically modified trees to keep the risk in perspective. This means that risk must be compared with the risk inherent in accepted practice currently used to achieve the same outcome, such as the production of wood. This can, for example, be achieved by using insect-resistant trees or alternatively spraying against insects. Both methods carry a certain amount of risk for the environment, and informed decision-making must take both into account.

On a worldwide scale, several field trials have been established during the past few years to study transgenic trees under natural environmental conditions. Since many genetically modified tree lines are close to or even ready for commercialization, environmental concerns should be discussed and monitoring programmes developed before the release of genetically modified trees to the market. The focus on risk assessment should be on the trait introduced rather than the technology used to achieve genetic gain. This chapter summarizes available information on direct and indirect environment concerns following the release of transgenic trees into natural environments. The focus will be on resistance evolution, vertical and horizontal gene transfer, effects on non-target species, transgene stability, weediness and invasion, and other unintended effects.

### **FIELD TRIALS WITH TRANSGENIC TREES TO STUDY ENVIRONMENTAL CONCERNS**

To date, more than 200 field trials with genetically modified forest trees have been documented worldwide (Robischon, 2006). The majority of those were carried out to test herbicide and insect resistance, lignin reduction or developmental processes, and only few to investigate biosafety issues such as sterility, transgene stability, or vertical and horizontal gene transfer (Valenzuela, Balocchi and Rodriguez, 2006; Robischon, 2006).

In North America, transgenic trees were tested mainly in relation to improvement of plantation forestry (Robischon, 2006). Also, an increasing number of studies in recent years have focused on sterility or altered fertility of forest trees. Reduced fertility can increase the productivity of a tree by redirecting energy and resources to growth rather than production of reproductive structures (El-Kassaby and Barclay, 1992). Further, research in this area could lead to an increasing contribution towards lowering potential environmental risks related to gene flow to interfertile species. It has been demonstrated that sterility strategies



developed for annual plants can be transferred to forest tree species (Wei *et al.*, 2007; Brunner *et al.*, 2007).

In Germany, four field release experiments with transgenic forest trees were established between 1996 and 2002. The initiative of the first field trial was a first step toward the evaluation of the possible risks versus benefits of genetically modified trees (Fladung *et al.*, 2004). Transgenic trees carrying a screenable morphological marker based on the *rolC* gene of *Agrobacterium rhizogenes* were planted. Transgenic trees carrying the 35S::*rolC* gene are characterized by dwarf growth and smaller leaves. This type of marker system has some advantages compared with biochemical markers. First, morphological markers can be detected phenotypically during every stage of the life cycle of the plant, or at least at specific developmental stages, whereas marker genes such as *npt-II* (antibiotic resistance) or *uidA* (detected using a histochemical staining procedure) provide results only at the time of evaluation and not during the lifetime of the organism. Second, it is advantageous to use a cell-specific marker that does not diffuse to adjacent cells and hence is detectable at the cell level. Thus, plants transgenic for the *rolC* gene from *A. rhizogenes* offer an appropriate model system meeting the requirements of a morphological marker.

In this field trial, four research projects related to biosafety issues were carried out. The first project was related to the integration pattern of the foreign gene construct into the genome. Originally it was thought that integration patterns are important for stable transgene expression under changing environmental conditions and during the long life span of trees. Analysis of *rolC*-transgenic poplar revealed that expression of the transgene may vary over time (Kumar and Fladung, 2001). At the same time, data from transgenic radiata pine indicate that once the trees are a couple of months old and still express the transgene, this will not change later on, i.e. they will continue to express (Walter, unpublished results). In an associated project, the mycorrhizal status of the roots in the transgenic and non-transgenic trees, and the conditions for a putative transfer of the foreign gene(s) from the tree roots into the mycorrhizal fungal symbiont (horizontal gene transfer), were analysed. In the two remaining projects, the status of phytopathogenic fungi on leaves was investigated, and correlated with parameters of the carbohydrate and hormonal metabolism of transgenic versus non-transgenic trees.

In a second field trial with genetically modified aspen the horizontal gene transfer to mycorrhiza fungi was investigated. Transgenic aspen trees carrying a fungal-specific promoter controlling the bar gene were planted out in the field. The hypothesis was that following horizontal gene transfer the mycorrhiza fungi living in association with these transgenic trees should become BASTA resistant. Subsequently, large screening programmes were initiated to identify putative BASTA-resistant mycorrhiza fungi. The two remaining field trials with genetically modified aspen were initiated by the University of Freiburg (Germany). Transgenic poplars were produced, modified for higher glutathione content. The trees were more tolerant of increased heavy metal concentrations in soil. In the field trials in Germany and in the Russian Federation, the capacity of the uptake of heavy

metals by the transgenic plants was tested, and the possible pleiotropic effects on morphology, growth parameters and mycorrhization were studied.

In New Zealand, several field trials with transgenic *Pinus radiata* and Norway spruce have been conducted. The trees have been mainly genetically engineered with selection and reporter genes in order to collect basic information on the patterns of gene expression. Some of the trees also contain genes that may have an effect on the reproductive capacity of the trees. The aim of the studies was to gain a better understanding of the operational and environmental risks involved and how to manage them in a plantation forestry context. The public has full access to the data generated and that will enable society to assess the risks of genetic engineering and compare them with the risks of techniques currently in practice, leading to informed decision-making. Researchers are investigating the expression of foreign genes in genetically modified conifers by quantifying reporter protein levels in the GMO greenhouse and field trials. Researchers in New Zealand also generate data on the impact of genetically modified needles on selected native insect species, and effects of roots from modified trees on micro-organism populations, in particular mycorrhizae.

In Finland and elsewhere in northern Europe, silver birch (*Betula pendula* Roth) is the most important deciduous tree species and is used commercially, for example, in plywood, pulp and furniture production. Various genetic modifications have been tested in silver birch by several research groups, either in the lab or under greenhouse conditions (e.g. Keinonen-Mettala, Pappinen and von Weissenberg, 1998; Lemmetyinen *et al.*, 1998, Lemmetyinen, Keinonen and Sopenan, 2004.; Valjakka *et al.*, 2000; Pappinen *et al.*, 2002; Tiimonen *et al.*, 2005). Also, three field trials with genetically modified silver birch have been established in Finland. One of the central aims in the establishment of the field trials has been the evaluation of environmental effects of transgenic birch. The first two field trials were established in 2000. The field trial established by the University of Helsinki included silver birch lines genetically modified for fungal disease resistance. So far, most published research results are available on birch lines carrying a chitinase IV gene from sugar beet. The interactions of chitinase transgenic birch with other organisms, e.g. pathogenic and mycorrhizal fungi, soil micro-organisms and herbivorous insects, have been widely studied. This field trial was harvested in autumn 2003, after three growing seasons.

Another field trial was established at the Punkaharju Research Station by the Finnish Forest Research Institute and contained transgenic silver birch lines altered for carbon and nitrogen metabolism (sense-RbcS and NR lines). Unfortunately, this field trial was destroyed in 2004. The third field trial was established by the University of Joensuu in 2005 with the aim of studying the environmental effects associated with the potential establishment of plantations of silver birch genetically modified for the prevention of flowering, using silver birch lines carrying the BpFUL1::Barnase gene construct. This trial was still in progress at the time of writing.

## TRANSGENIC TRAITS AND ENVIRONMENTAL CONCERNS

### Resistance evolution

Genetically modified trees have been made tolerant to a broad spectrum of herbicides. These herbicides are used to kill all plants considered as weeds growing alongside the tolerant transgenic tree. These herbicides can also be harmful to animal species, including both vertebrates and invertebrates. Spraying of herbicides on large-scale herbicide resistant tree plantations can have negative effects on nearby natural ecosystems, such as forests and grassland, due to wind distribution.

The primary concern related to herbicide-tolerant trees, however, is the development of plant populations that are resistant to particular herbicides. These wild populations may acquire invasive potential and thus can become 'weeds'. The resistance may develop via gene flow from herbicide tolerant trees to wild interfertile relatives. Also the species mix and population structure of known weed communities may change: weed populations may develop tolerance to certain herbicides, which under selective conditions (continued and regular use of herbicides) may enable them to out-compete weed species or populations without that tolerance. Once such use of herbicide has selected for resistant individuals, continued use of herbicide (i.e. continued selection pressure) favours resistant plants over their susceptible counterparts. Over time, the frequency of resistant plants in a weed population increases, representing a potentially serious long-term weed management problem. This observation has typically been associated with reliance on a single herbicide active ingredient over time, i.e. a high level of herbicide selection intensity (Volenberg, Stoltenberg and Boerboom, 2001; Stoltenberg and Wiederholt, 1995). It is important to consider, however, that in plantation forestry, herbicides will only be used prior to and during the first two to three years of establishment of a plantation. Subsequently the plantation will not need further spraying for protection and hence the selective pressure will no longer be present.

Current commercial transgenic insect-resistant trees are grown in China (Ewald, Hu and Yang, 2006) carrying the gene from *Bacillus thuringiensis* (Bt-gene). Concerns raised relate to insect populations potentially adapting rapidly to this pest-protection mechanism. In the event of establishment of Bt-resistant insect populations, the use of higher toxicity pesticide will become necessary. Also, the Bt-resistant insects can move to other tree stands where classically Bt-toxin is sprayed as a pest control mechanism. Thus severe environmental impacts could be the consequence. Regional or interregional scale plans, rather than local, are needed because insects are highly mobile.

However, a number of studies are already available to discuss this aspect of risk in context. These studies have investigated Bt-transgenic maize and cotton and the development of resistance mechanisms in associated insects. It is documented that Bt-resistant insects have been developed in a Bt-transgenic maize field with a frequency of about 3% (Tabashnik *et al.*, 2000). At the same time, it was reported that field outbreaks of resistance to Bt have not been observed so far (Morin *et al.*,

2003). Following new insect resistance strategies that involve gene stacking, the chance of development of insect resistance with two or three stacked Bt genes is infinitesimally small. Further, it has been described that the Bt protein can be used as supplementary food source that may account for faster development rate of Bt-resistant insects (Sayyed, Cerda and Wright, 2003). However, in contrast, Tabashnik and Carrière (2004) state that Bt crops had adverse effects on resistant insects.

Unfortunately, no information is available for Bt-transgenic tree plantations regarding resistance breaks and ecological implications. The only study available so far regarding insect community structure has been published by Gao *et al.* (2003, cited in Ewald, Hu and Yang, 2006). The authors mention that the presence of Bt-transgenic poplar can reduce the density of individuals of defoliating insects and shift the dominance of individual species. At the same time, the insect diversity was enhanced (Gao *et al.*, 2003, cited in Ewald, Hu and Yang, 2006). In general, complete risk assessment must also consider alternative practices used to protect plants from insects. This may show that the use of transgenic trees may actually be more benign to the environment than the conventional and accepted practice, which might, for example, involve the spraying of Bt protein. Further, Bt transgenics controlled by an inducible promoter that triggers the development of Bt protein only where and when insect damage occurs may have even greater benefit and much reduced risk to the environment.

The possibilities of improving fungal disease resistance in a deciduous tree species by genetic engineering have been tested in silver birch by producing birch lines carrying a sugar beet chitinase IV gene. In many crop plants, the introduction of a transgenic chitinase gene has led to improved disease resistance against the fungal pathogens studied (e.g. Grison *et al.*, 1996; Emani *et al.*, 2003; Vellice *et al.*, 2006). Improved resistance against the leaf spot fungus (*Pyrenopeziza betulicola*) was detected in chitinase transgenic silver birch in a greenhouse experiment (Pappinen *et al.*, 2002) but the improvement in disease resistance could not be confirmed in a field trial after natural infection with *P. betulicola*. However, some transgenic lines showed improved resistance against birch rust caused by the fungus *Melampsorium betulinum* in the field (Pasonen *et al.*, 2004). The contradictory results from the greenhouse and the field trial in the resistance of chitinase transgenic birch to birch leaf spot disease may be due to the fact that only one isolate of *P. betulicola* was used to infect the plants in the greenhouse, while natural infection in the field is likely to consist of more than one genetically distinct individual of the same pathogen (Paavolainen *et al.*, 2001). Also several biotic and abiotic factors, to which the plants were exposed in the field trial but not in the greenhouse, may have influenced the fungal disease resistance reaction of the birch lines studied. These results actually demonstrate the importance of field studies with genetically modified trees, where conditions are very similar to a commercial plantation situation.

## INVASIVENESS OF TRANSGENIC TREES

### Weediness

Genetic modification may cause unpredictable change in the fitness of a tree species. Thus, it is important to determine whether newly introduced traits have a potential to make genetically modified trees more likely to be invasive in natural habitats. More invasive means increased weediness that is based on many different characters, and weediness of a plant species plays a more important role than isolated genes used for genetic transformation (Luby and McNichol, 1995). At the same time, Fitter, Perrins and Williamson (1990) and Williamson, Perrins and Fitter (1990) propose that small genetic changes can cause large ecological alterations. The potential impacts of individual transgenes should be determined by evaluating their phenotypic effects (Hancock, 2003). Although current information may be insufficient to rank the relative risk of many transgenes, they can be grouped by the type of impact they have on reproductive fitness. Genes, such as mercuric ion reductase in the absence of heavy metal contamination (Bizili, Rugh and Meagher, 2000) or *rolC* from *Agrobacterium rhizogenes* (Fladung, 1990; Fladung, Muhs and Ahuja, 1996) should be considered detrimental because they reduce plant fitness. In general, genes with detrimental effects will be selected against in the natural environment and will not spread (Hancock, 2003).

Genes improving stress tolerance to detrimental biotic or abiotic factors fall into a group whose incorporation into natural populations could increase fitness. Transgenes already deployed that fall into this category include Bt or chitinase genes for insect or fungal resistance (Genissel, Viard and Bourguet, 2000; Pasonen *et al.*, 2005) or those conferring tolerance against drought, salinity or high temperature (Wang, Vinocur and Altman, 2004). In general, however, it must be considered that conventional tree breeding practice, which can include crossing the species barrier (forced hybridization, embryo-rescue), introduces far greater genetic change than the transfer of a single or a few genes into a species. Further, forest tree breeders frequently breed for increased resistance against specific pathogens or other environmental challenges and the weedy potential of those new genotypes has never been evaluated, nor any risk considered in the context of environmental impacts. Consequently, the consideration of weedy potential of transgenics must take place in the context of accepted breeding and selection practice. This will lead to informed and better decision-making that takes all aspects of a specific practice into account, and ultimately will reduce the environmental impact of forestry practice.

### Vegetative spreading

Spread by vegetative means, through root suckering, that is known for a number of tree species is also a very important factor in risk assessment for both transgenic and non-transgenic poplars (Fladung *et al.*, 2003). Root suckers arise from adventitious buds on the extensive lateral root system. Large numbers of suckers from a single tree can quickly develop into a dense colony. Strategies for controlling vegetative reproduction may be necessary for containment of modified trees.

In a field trial using 35S::*rolC* and *rbcS*::*rolC* transgenic aspen, the appearance of a root sucker was first observed after four years from planting (Fladung *et al.*, 2003). In the following year (i.e. fifth year), a total of 226 root suckers were found within the field trial, and their positions were determined. The determination of the exact origin of root suckers was not possible, because root length of individual trees was found to be up to 10 m (Kaldorf, personal communication). All root suckers derived were phenotypically wild type and hybrid aspen (*P. tremula* × *P. tremuloides*) but not pure aspen (two clones of *P. tremula*) or 35S-*rolC* transgenic (Fladung *et al.*, 2003). To confirm the absence of any 35S::*rolC* gene construct and to determine the portion of the *rbcS*::*rolC* transgenic plants, PCR analysis was performed to determine the presence of the 35S::*rolC* chimeric construct as well as the single *rolC* gene.

The results clearly indicate that in 124 plants the *rolC* gene was present but in no case in combination with the 35S promoter, and 97 root suckers showed no *rolC* but genomic control amplification (Fladung *et al.*, 2003). From these results it is suggested that more than half of the root suckers analysed originated from *rbcS*::*rolC* transgenic trees. In the same assessment year (five years from planting), 15 root suckers with wild type phenotype were also observed outside the field trial's borders, reaching 5 m to the margin of the field. From 13 plants investigated, nine plants revealed the presence of the *rolC* gene. Only four plants were characterized as non-transgenic. The results indicate that possible vegetative propagation should also be included in risk assessment research studies.

## VERTICAL AND HORIZONTAL TRANSFER OF GENES

### Gene flow via pollen and seeds

For good management practices of transgenic tree plantations, knowledge of relevant gene flow parameters is required. Gene flow from transgenic plants to interfertile wild or weedy relatives is often cited as a potential risk in the commercialization of transgenic crops. In a poplar plantation, DiFazio (2002) studied gene flow and its implications for transgenic risk assessment. A combination of large-scale field studies, genetic analysis and simulation modelling was used. Field studies demonstrated low levels of gene flow from existing hybrid poplar plantations (*Populus trichocarpa* × *P. deltoides*) in three settings. Using sensitivity analysis, it was demonstrated that competitiveness and fertility of transgenic trees are important factors determining the extent of modelled gene flow, and that these factors interacted such that effects of enhanced competitiveness appeared to be obviated by cultivation of low-fertility transgenic trees. Disturbance regime, plantation silviculture and characteristics of the landscape surrounding plantations also had a strong influence on the rate of gene flow. It has, however, not been demonstrated so far that gene flow from genetically modified trees presents more risk than that from conventionally bred trees. However, as a precaution, the development of sterility strategies provides a favourable solution to limit gene flow to native species and non-native species or bred taxa. If the production of pollen and seeds is reduced, gene flow can be minimized or even prevented (DiFazio, 2002).

In another study, the percentage and flow distance of reproductively effective poplar pollen was estimated. Seeds were harvested from two female trees growing in the Arboretum of the Institute of Forest Genetics (Grosshansdorf, Germany). By microsatellite-based parental analysis germinated seedlings were investigated with respect to pollen origin (Fladung, unpublished results). It could clearly be demonstrated that only two to three trees from the close neighbourhood contributed as the main pollen donors, and approximately 70% of reproductively effective pollen originated from trees growing in the vicinity of the mother trees. The latter result is surprising, in particular in light of the fact that poplar is a wind-pollinated species. However, the results indicate that gene flow might be a problem when dealing with transgenic trees.

To reduce or even avoid gene flow of transgenes into non-transgenic relatives, incorporation of sterility genes into transgenic trees has been proposed (Strauss *et al.*, 1995). A number of sterility gene constructs have successfully been tested in crop plants, e.g. by expression of deleterious genes, such as barnase (Mariani *et al.*, 1990), stilbene synthase (Fisher, Budde and Hain, 1997), the gene for ribosome inactivating protein (Palmiter *et al.*, 1987), use of dominant negative mutations (Mitzukami *et al.*, 1996), and gene suppression strategies such as antisense suppression, co-suppression and RNA interference (Skinner *et al.*, 2003). Sterility conferring genes, however, need specific floral regulatory promoters (e.g. TA29 promoter from tobacco) to direct expression of genes in reproductive structures (Koltunow *et al.*, 1990; Mariani *et al.*, 1990). Few investigations have been reported for induction of sterility in transgenic *Populus* (Meilan *et al.*, 2001; Skinner *et al.*, 2003), but the effectiveness of the transgenic sterility systems still needs to be demonstrated.

The first poplars transformed with sterility genes showed a lower growth performance compared with control plants (Meilan *et al.*, 2001). Here, the use of heterologous promoters seems to direct the activity of cytotoxic gene expression in non-target, vegetative tissues ('leaky' expression; Meilan *et al.*, 2001). However, when these cytotoxic genes are controlled by other, more specific, promoters, e.g. in optimal case under forest trees floral promoters (Skinner *et al.*, 2003) or other genes are used, 'leaky' expression may be avoided. The expression of stilbene synthase under control of radiata pine male cone promoters or the C-GPDHC from *Cuphea lanceolata* revealed no effects on plant performance (Hoefig *et al.*, 2003; Hoenicka and Fladung, 2006).

### Horizontal gene transfer

The exchange of genes between organisms that are sexually non-compatible is called horizontal gene transfer (HGT) and it is a common evolutionary mechanism, mainly found in micro-organisms. The possibility of transfer of a transgene from a transgenic plant into other organisms (mainly bacteria, fungi and viruses) has become an important argument against genetically-modified plants (Stirn, 2000; Peerenboom, 2000). Natural HGT has been detected between bacteria and plants (Brown, 2003), where the gene transfer system by *Agrobacterium* species is one of

the best characterized examples of HGT (Chilton *et al.*, 1977; Schell *et al.*, 1979). So-called *Ngr* genes that are similar in sequence to genes in the left transferred DNA (TL-DNA) of *Agrobacterium rhizogenes* have been found in the genome of untransformed plants of *Nicotiana glauca* (Aoki and Syono, 1999). This implies that this HGT has occurred very early in the evolution of the genus *Nicotiana* (Aoki and Syono, 1999).

Sequence homologies between plant genes and the respective genes in bacteria have indicated that HGT is also possible from plants into bacteria. For instance the glucose-6-phosphate-isomerase gene in *Clarkia unguolata* is similar to the one in *E. coli* (Schlüter and Potrykus, 1996). The mechanisms underlying such prokaryote-eukaryote gene transfer or vice versa, excluding that between *Agrobacterium* and angiosperms, as well as conditions by which HGT takes place, are broadly unknown (Kondo *et al.*, 2002; Won and Renner, 2003). So far, researchers have been able to demonstrate HGT from genetically modified plants to micro-organisms like plant-associated fungi (Hoffman, Golz and Schieder, 1994) or bacteria (Nielsen, van Elsas and Smalla, 2000), but only under optimized laboratory conditions or in soil microcosms. Several experimental studies have failed to demonstrate HGT from transgenic plants to bacteria (Bertolla and Simonet, 1999; Gebhard and Smalla, 1999; Nielsen *et al.*, 1997, 1998) and, to our knowledge, HGT from transgenic plants to other organisms has not been detected in field conditions. In the light of present knowledge, HGT can occur but at such low frequencies that detecting it is extremely difficult, mainly due to the huge sampling efforts needed and the non-cultivable nature of most bacteria (Heinemann and Traavik, 2004; Nielsen and Townsend, 2004).

Since the availability of free DNA in soil is a limiting factor for HGT (Gebhard and Smalla, 1999), and because some fungi grow in intimate contact with trees (ectophytic fungi) or even within plants (endophytic fungi), uptake of plant DNA by these fungi might be more likely. Mycorrhizae are highly evolved, mutualistic associations between soil fungi and plant roots. Many forest tree species are largely dependent on ectomycorrhizal fungi for the uptake of mineral nutrients (Smith and Read, 1997). Up to now, two different approaches have been used to study HGT from trees to fungal hyphae in ectomycorrhizas. In the study of Kaldorf *et al.* (2004), transgenic aspen lines, containing the *rolC* gene from *Agrobacterium rhizogenes* under the control of the light-inducible plant *rbcS* promoter (Fladung, Großmann and Ahuja, 1997.), were used. The occurrence of HGT was analysed by the amplification of the fungal DNA with nested *rolC* gene primers. No single *rolC* signal was detected in any of the samples analysed (Kaldorf *et al.*, 2004). Unfortunately, only a few replicates were tested in this study.

In a second approach, transgenic aspen containing the *Streptococcus hygroscopicus* bar gene conferring herbicide (BASTA) resistance under the control of a fungal GPD promoter were field tested (Nehls *et al.*, 2006). Mycorrhizae were formed under axenic conditions between transgenic aspen and wild type hyphae of *Amanita muscaria* using a Petri dish system. To detect HGT events, a total of 35 000 ectomycorrhizas were dissected and tested for BASTA resistance.



From these, 102 fungal colonies were formed under BASTA selective conditions. However, since these fungal isolates stopped growth when transferred to fresh selection plates, and no bar gene could be amplified from fungal DNA by PCR, these fungal colonies were characterized as false positives (Nehls *et al.*, 2006).

Another method to determine the frequency of HGT from a tree species to associated micro-organisms may become applicable with increased genome sequence information available for an increasing number of tree species and associated organisms. Any historical HGT of a tree gene into such associated organisms could easily be detected simply by *in silico* analysis and comparison of the genomes. Such study might actually confirm the view of many authors that the frequency of HGT, if it exists between higher eukaryotes and prokaryotes, is infinitesimally small. Further, discussion of risk related to HGT from transgenic plants to other organisms tends to ignore the fact that genes used for transgenic plant production mostly originate from the natural environment, and hence have been available for transfer to other organisms over evolutionary time frames. It is hard to imagine why the HGT of a particular gene from a transgenic plant into a micro-organism should be of higher risk potential than the HGT of the same gene from its natural source into a new organism.

### IMPACTS ON NON-TARGET SPECIES

All living organisms including trees are part of the ecological food chain, and thus many non-target species are in contact with transgenic ones expressing the foreign gene and synthesizing its product (Mullin and Bertrand, 1998). Genetically modified trees transformed with the intent of conveying greater resistance to pathogens have been of particular concern because ecotoxic effects on other organisms such as insects or soil organisms have been assumed (Myhr and Traavik, 2003). The expression of broad-spectrum antimicrobial components by genetically modified plants may not only suppress target pathogens, but may also affect plant symbionts such as mycorrhizae and rhizobia, as well as other micro-organisms involved in decomposition and nutrient cycling (Morra, 1994; Glandorf, Bakker and Van Loon, 1997). We need to consider, however, that this is not intrinsic to genetically modified trees and that trees bred and selected for increased resistance to insects or pathogens may have similar effects.

#### Mycorrhiza fungi

In boreal soil ecosystems, forest trees form symbiotic associations with a number of ectomycorrhizal fungi that facilitate nutrient supply and provide protection against pathogens (Smith and Read, 1997). Mycorrhizal and saprophytic fungi contain chitin in their cell walls and may be highly sensitive to transgenic chitinases or to overexpression of a plant's own chitinases. In a preliminary study, the ability of eight chitinase transgenic birch lines showing varying levels of sugar beet chitinase IV expression to form ectomycorrhizae with the common ectomycorrhizal fungus, *Paxillus involutus* (Batsch) Fr., was tested *in vitro*. All tested transgenic birch lines were able to form normal ectomycorrhizae containing

distinctive mantles and Hartig nets, and the level of sugar beet chitinase IV expression was not detected to have an influence on mycorrhizal colonization. Two transgenic lines showing high chitinase expression had a lower percentage of mycorrhizal root tips than the other transgenic lines or the control plants, but the difference was not statistically significant (Pasonen *et al.*, 2005).

Root samples were also collected from chitinase transgenic and wild-type plants grown in the field, and mycorrhizal colonization as well as mycorrhizal species diversity of the roots of different types of the plants were studied. The roots of all the chitinase transgenic and control plants were well colonized by ectomycorrhizas expressed as the percentage of mycorrhizal root tips. Seven lines showing varying levels of sugar beet chitinase IV expression and total endochitinase activity were selected for detailed analysis of fungal species diversity. Fungal species were separated in denaturing gradient gel electrophoresis (DGGE), and sequencing of the DGGE bands have so far revealed that all the plants were colonized by a variety of fungal genera (Pasonen *et al.*, 2008). Although the transgenic lines were slightly less colonized by mycorrhizae than the control plants, the differences were so minor that the ecological consequences are difficult to estimate. In particular, the influence of environment will most probably be stronger and lead to more variation compared with the results of this experiment. This has been shown in a field trial experiment studying the mycorrhizal populations around genetically modified versus non-genetically modified radiata pine roots in a field test. Population differences between genetically modified and non-genetically modified trees could be detected; however, they were smaller than seasonal differences, and also smaller than differences between non-genetically modified individual trees (Walter *et al.*, in prep.).

The mycorrhizal colonization was also investigated in field-released 35S::*rolC* and *rbcS*::*rolC* transgenic aspen trees over a 15-month period (Kaldorf *et al.*, 2000, 2002). Arbuscular mycorrhizae were unambiguously identified in root samples from all aspen lines investigated. Arbuscular mycorrhizae formation was rare, with an average of less than 10% of the root length colonized. Quantitative differences between the transgenic and non-transgenic aspen trees were small and not significant. However, the majority of the fine roots were colonized with ectomycorrhizae. Taking all types of ectomycorrhizae together, again no significant differences in the quantity between the different aspen lines could be detected, including all transgenic and non-transgenic lines.

Within the release area of the transgenic aspen in Grosshansdorf, Germany, four fungal species were found to be dominating the ectomycorrhizal community. These four species formed more than 90% of all mycorrhizae, but a further eleven ectomycorrhizal types were found occasionally. The average of different ectomycorrhizal types found in each single sample was 5.1 for Esch5 (untransformed control), 4.9 for E14-4 (*rbcS*::*rolC* transgenic line) and 4.7 for E2-5 (35S::*rolC* transgenic line). These small differences were not statistically significant and indicate similar ectomycorrhizal diversity between transgenic and non-transgenic aspen (Kaldorf *et al.*, 2000). When investigating the structure of the mycorrhizal

community, a significant difference was found only for one transgenic line. In roots of the transgenic aspen line E2-5 one of the mentioned four common ectomycorrhizal morphotypes was rare and poorly developed when compared with other transgenic lines and with non-transgenic controls (Kaldorf *et al.*, 2002). It is suggested that this effect is clone specific, as the formation of this ectomycorrhizal type was not affected by the transgene expression in the other transgenic line carrying the same construct.

### Soil micro-organisms and decomposition rates

The decomposition process of the leaf litter derived from sugar beet chitinase IV transgenic silver birch and the effects on the decomposer populations were studied in a field trial by Vauramo *et al.* (2006). It was hypothesized that the expression of the chitinase gene in transgenic birch would influence chitin-containing saprophytic fungi and fungal-feeding microfauna, thereby affecting the decomposition rate of the litter. The influence of the transgenic leaf litter on the decomposer community was studied by analysing the living fungal biomass and the nematode community structure. Of the soil fauna, nematodes are considered as potential indicators of the function of the decomposer food web because of their high abundance, diversity and close relationship to soil processes via their food specificity. Functional (trophic) group analysis on the abundance of different feeding groups – bacteriovores, fungivores, omnivores and predators – can provide a quick source of information of the available resources, since nematodes can respond rapidly to environmental changes (e.g. Ritz and Trudgill, 1999).

An indication of negative effects of chitinase transgenic leaf litter on the number of nematodes was previously obtained in a microcosm experiment (Kotilainen *et al.*, 2005). The decomposition experiment was established in a field close to the field trial of transgenic birch trees. The experiment included leaves from chitinase transgenic birch lines that showed low, intermediate or high transgene expression. Only the highly expressing transgenic lines and the control plants were included in the nematode and microflora assays. The leaves were collected from birch trees, placed into litter bags and buried in the soil at a depth of 5 cm. Half of the leaves were allowed to decay in the field for eight months and the other half for 11 months. The decomposition rate of the litters was expressed as litter mass loss, fungal biomass as litter ergosterol content, and total microbial biomass as substrate induced respiration, which is a measure of respiratory response of soil microbes to the addition of glucose (Vauramo *et al.*, 2006).

The decomposition rate of any of the leaf litters from the chitinase transgenic plants did not differ from that of the control plants. Also, no differences in fungal biomass, total microbial biomass or activity (basal respiration) were detected between the litters. In the nematode assay, the total number of nematodes and the abundance of bacterial feeding nematodes varied significantly with the decomposition time, but the litter type had no influence on the number of nematodes or on the relative abundance of the different feeding groups. However, pair-wise comparisons revealed that after eight months of decomposition the

transgenic litter from one line showing high chitinase IV expression contained significantly more nematodes than the control litter, while after 11 months the situation was reversed. No differences in the community structure of the nematodes between the transgenic lines and the control plants were detected (Vauramo *et al.*, 2006). These results indicate that the chitinase transgene *per se* had no influence on the decomposition of the transgenic litter or the microbial content of the litter. The negative effect of the transgenic litter on the number of nematodes in the microcosm experiment (Kotilainen *et al.*, 2005) and in the field trial between one transgenic line and the control plants after 11 months of decomposition (Vauramo *et al.*, 2006) may indicate sensitivity of nematodes to the transgenic chitinase or to some other chemical change occurring in the transgenic line(s). The experiment also shows that an effect is not necessarily a negative effect: it may in fact be positive.

### Phytopathogenic fungi

The phytopathological status of the leaves and stems of 35S::*rolC* and *rbcS*::*rolC* transgenic aspen trees were studied in a field trial and infection studies were initiated under controlled conditions. The objective was to determine the influence of the *rolC* gene on infection as well as on the spectrum of fungal pathogens. First, the diseased foliage of the aspen crown was assessed. The assessment of leaf spot disease (*Pollaccia radiosa*) and poplar rust (*Melampsora* spp.) was made by iterative estimation of the symptoms (Gieffers and Fladung, 2000). The infestation patterns of both fungi can be identified with the different transgenic lines. Resistance reactions were not found. Former infection studies showed similar results for 35S::*rolC* transgenic potatoes (Fladung and Gieffers, 1993). The infestation level of the poplar rust was higher than that of *Pollaccia radiosa*. Both fungi showed a similar infection, which is confirmed by a high correlation coefficient ( $r > 0.9$ ).

In addition, infection levels of phytopathogenic fungi and, simultaneously, the content of important metabolites (sucrose, glucose, fructose, starch) were determined. Positive correlations were found between the level of diseased foliage of the aspen crowns and the contents of glucose and fructose in the leaves. Measurements of carbohydrate contents were made on the same dates as phytopathological investigations were done. Again, similar results were obtained with 35S::*rolC* transgenic potatoes.

### Insects

The most extensively studied examples of engineered resistance are based on the use of delta-endotoxins of the bacterium *Bacillus thuringiensis*. *B. thuringiensis* is a naturally occurring ubiquitous soil bacterium that produces a toxin (Bt toxin) lethal to certain insects (Dale, Clarke and Fontes, 2002). There is no doubt that Bt-transgenic plants will kill the target pest species, but there is no serious scientific report available describing that non-target pest species are affected as well. The evaluation of possible environmental damage due to insect-resistant Bt trees should take also into account the environmental damage caused by the use of

pesticides. It is argued that millions of birds and billions of insects are killed each year in the United States of America alone as a result of pesticide use (Dale, Clarke and Fontes, 2002). Advantages and disadvantages of Bt trees, therefore, should be carefully considered in the context of accepted practice.

It is also noteworthy that sprays containing living *B. thuringiensis* are broadly accepted as an alternative for pest management, even in organic farming. Release of these living bacteria or proteins into the environment may represent a similar or probably higher risk than genetically modified plants (Brimner and Boland, 2003; Boland and Brimner, 2004), particularly when transgenic plants express the Bt gene only when and where insect damage occurs. However, conventional Bt use has not been raised as a concern with similar implications to those discussed for Bt-toxin-carrying transgenic plants (Bt trees). Further, these conventional Bt pesticides are freely available in many countries and are used frequently in all types of agricultural practice and in forest protection against lepidopteran defoliators, and have been for many years (Bauce *et al.*, 2004; Kouassi *et al.*, 2001; Cadogan and Scharbach, 2003).

In the Finnish field trial using chitinase IV transgenic silver birch, the composition and density of insect populations and leaf damage caused by insects were monitored three times during one growing season, and compared between transgenic lines and wild-type birch clones. The composition of insect populations was studied at order level, and temporal, horizontal and vertical variations in insect density and species composition were recorded. Different types of leaf damage were classified as leaf chewing, leaf mining, gall, leaf roll, web formation, leaves glued together and sucking damage. The level of the leaf damage was expressed as the proportion of the branches studied in which any type of leaf damage was observed. No clear differences between the transgenic and control trees were found in the species composition, but the total insect densities were generally higher among the chitinase transgenic plants than among the corresponding control plants. Also only minor differences in the composition of different types of leaf damage were found between the transgenic and control plants. The results indicated that the expression of the transgenic chitinase gene in birch did not have clear harmful effects on insects (Vihervuori *et al.*, unpublished results).

## TRANSGENE STABILITY AND EXPRESSION

Stable integration of foreign genes into plant genomes and predictable transgene expression are important, in particular when transgenic plants are considered as basic material for plant breeding programmes. Depending on the introduced trait, transgene stability may be required for the whole life cycle of plants, including their vegetative growth (mitotic cell divisions in somatic tissues) as well as during the formation of generative cells following meiosis. However, many investigations using annual crops have shown that expression of transgenes is less stable than had originally been thought.

Most of these events reported are homology-dependent gene silencing phenomena that function at the level of transgene transcription, or post-

transcriptionally (reviewed in Paszkowski, 1994; Meyer, 1995). In transgenic trees, gene silencing has been reported in those transformed with the *rolC* gene (Fladung, 1999; Kumar and Fladung, 2001). Plants transgenic for the 35S::*rolC* gene construct show an altered plant phenotype (Fladung, Muhs and Ahuja, 1996.) that was used for morphological screenings of transgene instability under *in vitro* cultivation, in the greenhouse as well as under field conditions (Kumar and Fladung 2001).

Under *in vitro* conditions, long-term (five to six years) morphological observations for *rolC* expression have so far revealed a stable *rolC* phenotype in 15 hybrid aspen (*Populus tremula* × *P. tremuloides*) transgenic lines analysed. Out of the 16 wild aspen (*P. tremula*) lines obtained, however, only seven lines survived the long-term *in vitro* culturing. Among these seven lines, variable morphological expression of the *rolC* gene was detected in three lines (Kumar and Fladung, 2001). More lines revealing alterations in *rolC* expression were observed after transfer from *in vitro* conditions to the greenhouse or field (Kumar and Fladung, 2001). Out of the 15 Esch5-based transgenic lines transferred to the greenhouse, incomplete suppression of the transgene expression was observed in three lines (Fladung, 1999; Kumar and Fladung, 2001). Among five wild aspen transgenic lines transferred to the greenhouse, three lines were observed with altered or reverted transgene expression.

Reversion of leaves or single shoots of a *rolC*-transgenic aspen plant to wild type was observed for the line Esch5:35S::*rolC*#1 (Fladung, 1999; Kumar and Fladung, 2000). In two other hybrid aspen-based transgenic lines (Esch5:35S::*rolC*#2 and Esch5:35S::*rolC*#12), the phenotypically visible *rolC* expression decreased gradually over a period of three to four years of cultivation in the greenhouse (Kumar and Fladung, 2001). The loss of the *rolC* expression seems stable in these lines, thus the plants, once reverted, maintain the changed features in the following years. Similar stable complete *rolC* suppression was observed in two wild aspen transgenic lines (W52:35S::*rolC*#9 and W52:35S::*rolC*#3; Fladung and Kumar, 2002).

Compared with the lines showing completely suppressed *rolC* phenotypes, the alterations in morphological expression of transgene were more complex and variable in other wild aspen-based transgenic lines planted in the field. The altered plants from Brauna11:35S::*rolC*#2 showed morphological features different from both the control and *rolC* phenotype (Kumar and Fladung, 2001). The length and width of the leaves collected from the reverted plants were intermediate between the control and 35S::*rolC* phenotypes. The reverted morphological expression was confirmed by northern experiments, which clearly showed very weak *rolC*-specific transcripts from the leaves of reverted plants grown under field conditions. The *rolC*-specific transcript was, however, present in leaves collected from the plants maintained in the greenhouse, or from *rolC*-expressing plants in the field.

However, other reports for transgenic trees claim that there is no evidence that expression of transgenes under vegetative propagation is more variable than expression of most endogenes (Strauss *et al.*, 2004). Analysis of GUS expression of 35S::*uidA* transgenic poplar grown in a field trial in France revealed that all transgenic

plant lines showed stable expression of the transgene (Pilate, Ellis and Hawkins, 1997). Hawkins *et al.* (2003) evaluated the transgene expression in a hybrid poplar (*Populus tremula* × *P. alba*) clone transformed with constructs carrying the *uidA* reporter gene under the control of either a constitutive or a vascular-specific promoter. While important variations in expression levels occurred, the transgene appeared to be stably expressed throughout a six-year period. Similar results were reported for hundreds of different poplar transformants carrying various gene constructs and tested under field conditions (Strauss *et al.*, 2004). Even when 35S::*uidA* and *rbcS*::*uidA* transgenic trees are treated with stress conditions (high temperature, UV-light) no stress-related transgene silencing could be observed for poplar, larch or fir (InfoNet-Umwelt SH, 2004). During a field trial of transgenic radiata pine in New Zealand, expression stability of the introduced and non-selected *nptII* gene could be demonstrated. It was observed that, while transgenic radiata pine tissue shows frequent silencing, those transclones that still express reliably when trees are regenerated usually continue expressing the new gene (Walter, in prep.).

Silencing in 35S::*uidA* transgenic poplar was detected only for lines that were probably silenced from the beginning, i.e. shortly after the transformation process (Hawkins *et al.*, 2003; Strauss *et al.*, 2004; InfoNet-Umwelt SH, 2004). However, due to the destructive nature of the GUS activity test or other enzyme measurement procedures, only a small part of the plant at a given time can be screened with respect to transgene stability. As shown by Kumar and Fladung (2000) and Fladung and Kumar (2002), inactivation of the phenotypic marker gene construct 35S::*rolC* is a very rare event and occurs in an unpredictable manner. Thus, transgene silencing can happen at a single branch of a single plant among a high number of clonal ramets, and in the next year disappear in the same shoot (Fladung and Kumar, 2002). Such silencing events remain undetectable with destructive reporter genes and can only be monitored when non-destructive reporter gene assays are being used.

The occurrence of a transgene repeat is often accompanied by methylation of the promoter and/or the transgene (Kumar and Fladung, 2000). However, not every transgenic line harbouring two T-DNA copies in repeat form is consequently silenced from the beginning. Two 35S::*uidA* transgenic poplar lines, characterized by the presence of T-DNA repeats, that were cultivated either under greenhouse or *in vitro* conditions, had at the time of writing revealed GUS expression over a period of seven years in plants. It remains unknown whether these lines are 'insensitive' to repeat-related transgene inactivation, or silencing has occurred but was not detected so far, or silencing of the transgene may happen sometime in the future. It is also interesting to note that transgenic radiata pine was still expressing a transgene reliably at age nine years of the trees that were originally transformed using biolistic techniques, leading to highly complex integration patterns.

Taken together, the fact that silencing is possible but may happen sometime in future is in particular important when the efficiency of strategies for biological confinement of transgenic plants is discussed, e.g. use of genes leading to male

and/or female sterility. Gene silencing of these genes would allow crossings of transgenic woody plants with their natural relatives even when a low rate of instability is assumed. The question is whether the out-crossed transgene can 'survive' in the gene pool of the natural population or will disappear (DiFazio *et al.*, 2004).

### GROWTH PARAMETERS AND UNINTENDED EFFECTS

Few reports are available on the performance of transgenic trees under natural environmental conditions in long-term field trials. In a recent report, transgenic poplars carrying antisense transgenes of lignin biosynthesis key enzymes were field tested for growth indicators, interactions with insects and paper-making characteristics. It was concluded that transgene expression did not interfere with tree growth or fitness under field conditions (Pilate *et al.*, 2002).

Also during the field trial with *rolC* transgenic trees in Germany, results on growth and other parameters were obtained in different risk assessment-related scientific projects (summarized in Fladung *et al.*, 2004). Measurements of height as well as stem diameter were made every year during the field experiment. Tree height revealed higher values for the controls and the *rbcS::rolC* transgenic aspen compared with the *35S::rolC* transgenics. Further, the dynamics of growth as well as stem diameters at 10 cm height of transgenic and control aspen trees were significantly different. In 1999 and 2000, the stem diameter in the control trees was double that of the transgenic aspen trees of equivalent maturity (Gruenwald, Ruel and Fladung, 2001). However, a higher annual increase in stem diameter was found in the *35S::rolC* transgenic plants than in the control aspen trees, which showed constant increase. Further, the leaf size of the *35S::rolC* transgenic aspen was much smaller than the controls (Gruenwald, Ruel and Fladung, 2001), and also the length-to-width ratio was different (Fladung, Muhs and Ahuja, 1996). The effect of the *35S::rolC* gene construct on flushing of greenhouse-grown plants has already been described earlier (Fladung, Muhs and Ahuja, 1996; Fladung, Großmann and Ahuja, 1997). In spring, the *35S::rolC* transgenics started to flush at least two weeks earlier than the controls and transgenic plants carrying different gene constructs. A similar effect was observed in spring of every year in the *35S::rolC* transgenic aspen grown in the field.

The effects of the expression of the sugar beet chitinase IV gene on growth and growing habit, and the quality and leaf phenology of the chitinase transgenic silver birch lines, were monitored during three growing seasons in the Finnish field trial. The traits monitored are important for adaptation as well as for birch breeding. The attractiveness of chitinase transgenic birch to larvae of the cambium miner (*Phytobia betulae* Kang, Diptera: Agromyzidae), causing an aesthetic defect to birch wood, was also studied. Three lines out of fifteen were frequently different from the control plants in growth and leaf phenology, and these differences are suggested to be due to position effect of the transgene. The level of the transgene expression was not detected to have an influence on the growth parameters studied nor leaf phenology. In a field trial with transgenic and non-transgenic radiata pine



in New Zealand, transgenic trees were indistinguishable from controls with regard to growth characteristics (Walter, in prep).

The level of transgene expression, however, correlated with parameters related to stress status of a tree indicated by the increased amount of red colour in the leaves and lowered general condition of the transgenic trees. The stress status of the tree was described by the amount of red colour in the leaves because the ecophysiological function of foliar anthocyanins has been suggested to be related to the protection of the photosynthetic apparatus in the plants experiencing environmental stress (Hoch, Zeldin and McCown, 2001; Close and Beadle, 2003). The variation in the occurrence of *Phytobia* spp. was explained mainly by the differences in plant size, not by the level of transgene expression (Pasonen *et al.*, 2008). The expression of the sugar beet chitinase IV gene in transgenic birch *per se* did not cause significant changes in plant morphology, but was presumed to influence the stress status of the transgenic plants, which is expected to make the transgenic plants less fit than the wild-type plants.

## CONCLUSIONS

Based on the authors' experience with field-released transgenic trees, support and encouragement is given to:

- the adoption of a case-by-case assessment process, with a focus on scientifically informed decision-making, with regards to deployment of genetically modified trees in plantation forestry;
- an informed and evidence-based decision-making process on GMO deployment by government authorities, ensuring that any potential risk is evaluated in the context of accepted practice;
- the continued development of environmental risk assessment technologies for genetically modified trees, in the context of currently accepted forestry practice;
- the adoption of a precautionary approach where there is either a scientifically substantiated and quantifiable risk of GMOs becoming invasive weeds, or of introducing foreign genes into native forest with potential adverse impacts on biodiversity values or plant growth characteristics;
- the active development of risk mitigation strategies, where a risk is identified. For example, where the spread of genetically modified material through pollen or seed dispersal is considered a risk, sterility techniques should be evaluated and deployed to prevent the formation of seed or pollen, or both;
- encouraging the development of GMO trees that are unable to spread genetically modified material.

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## 10. Social, legal and regulatory issues related to transgenic trees

*R.A. Sedjo*

Genetic engineering has already had a huge worldwide effect on agriculture. Genetic engineering is the use of recombinant DNA and asexual gene transfer methods to modify organisms (Strauss *et al.*, 2001) and produce so-called genetically modified organisms (GMOs) or transgenics. Although the first commercial genetically modified crops (tomatoes) were planted in 1994, 1996 was the first year in which a significant area (1.66 million hectares) of crops was planted containing genetically modified traits. Since then there has been a dramatic increase in plantings, and by 2005–06, the global planted area reached almost 87.2 million hectares. This is equal to five times the total agricultural area or nineteen times the total arable cropping area of the United Kingdom.

Almost all of the global genetically modified crop area comprises the four main crops in which genetically modified traits have been commercialized, namely soybean, maize, cotton and canola. In 2005, genetically modified traits accounted for 29% of the global plantings of these four crops: genetically modified soybeans accounted for the largest share (62%), followed by maize (22%), cotton (11%) and canola (5%). In terms of the share of total global plantings to these four crops, genetically modified traits accounted for a majority of soybean plantings (59%) in 2005. For the other three main crops, the genetically modified shares in 2005 were 13% for maize, 27% for cotton and 18% for canola (ISAAA, 2006).

Much of the biotechnology already developed for agriculture has direct applications in forestry, and many of the biotechnological innovations being introduced to forestry are being adapted directly from agriculture. Innovations such as the introduction of the herbicide-tolerant gene into tree seed stock follow directly from the success of the same herbicide-tolerant gene in agricultural crops. Research similar to that in agriculture is also being undertaken with disease- and pest-resistant genes, as well as other gene-altering modifications. It is anticipated that these innovations could result in substantially reduced wood costs through increased wood yields, the reduction of plantation establishment costs and reduced tree losses through the growing cycle. Also, biotechnological research in forestry is moving in the direction whereby genetic alteration would enhance wood quality by producing desired modifications in fibre characteristics, lignin content or limb thickness in a manner that would reduce processing costs. All of these modifications have the potential to generate financial benefits through reduced production costs and enhanced productivity. Additionally, conservation benefits could be achieved from the restoration of certain species ravaged by disease, such as the American chestnut (Bailey, 1997).



Internationally it is recognized, both through the Cartagena Biosafety Protocol and the United Nations Industrial Development Organization, that there is a need to form national regulatory systems to control the release of genetically modified organisms into the environment (Pachico, 2003; Sedjo, 2005). However, many countries have a variety of legislative and regulatory processes involving transgenics that predate the various international initiatives.

While there is a general agreement that existing procedures in some countries provide the basic process for deregulation, specific procedures and protocols may need to be worked out for trees, both industrial wood and orchard. This understanding was reflected in the meetings held in July 2003 outside Washington, DC, organized by USDA APHIS, which discussed some of the regulatory problems unique to transgenic trees. For example, there remains the question as to whether regulation ought to focus on the process of transgenics or on the character and attributes of the plant, irrespective of the process. In the United States of America, discussions continue and some regulatory changes are expected. In addition, developers are looking to devise field trials that will provide more efficient, low-cost testing procedures, including adequate testing in relatively short periods, with procedures to test a number of genes in one trial. These approaches differ from the current concept that each gene needs separate testing and that complete testing is required *de novo*. Also, there is re-consideration regarding allowing contingent deregulation, which might provide for continuing testing and monitoring for some time after partial deregulation.

Although the existing provisions are designed to provide for deregulation given that the requisite criteria are met, the paucity of transgenic tree deregulation over the past decade raises questions about how the law and regulations are being applied. The absence of successful transgenic tree deregulation appears to be having an important effect, not only in that transgenic trees are not deployed, but also upon the vigour of the science and on the scientists involved in transgenic tree research (Bradford *et al.*, 2005).

## LEGAL AND REGULATORY ISSUES

The general regulatory framework for transgenic trees, which is similar in many respects to that for crops, deals with two major areas of concern: food safety, and consequences for the environment. However, since food safety is rarely a problem, the focus of transgenic tree regulation is the environment. While regulatory systems vary by country, the usual case is for transgenic trees to fall under the same general set of regulations as crops and other plants. However, deregulation protocols may be modified to recognize the longer lifespan of trees and the associated longer-term deregulation problems.

In all regulated situations in the United States of America, and most countries globally, transgenic plants are automatically regulated and therefore require a deregulatory process before they can become commercial. In some countries, however, the criterion is based on the novelty of the plant, and thus regulation can also apply to non-transgenic genetically modified plants.

The long lives of trees make monitoring for potential problems more difficult than for annual plants. Most tree improvement programmes try to identify superior trees early in the cycle. This allows utilization of the superior-performing trees quickly, although with only a limited amount of information. Such an approach requires continual refining and adaptation of the genetic stock, and may contain surprises in tree performance. There is a fair degree of support in the United States of America industry for a conditional deregulation, whereby distribution would be limited and monitoring would continue for a specified period of time or until outstanding uncertainties were resolved.

### CONCERNS REGARDING TRANSGENIC TREES

As the regulatory structure suggests, the primary reason for regulation of transgenics is the concern that there may be health, safety or environmental risks. The problem areas for trees are largely environmental (e.g. see Mullin and Bertrand, 1998). The regulators must behave as if the introduction of transgenics may pose new risks of environmental damage. In the United States of America the existence of concerns about the extent to which transgenics could become weed pests is clearly reflected in the Federal Plant Pest Act. More broadly, there are concerns that damage due to gene flow could occur or that transgenics could in other ways disrupt the environment (DiFazio *et al.*, 1999). Some have likened the introduction of a transgenic into the environment as providing a similar risk to the introduction of an exotic, some of which have become invasive. However, many ecologists have argued that the risks of a transgenic are generally lower and more predictable than for an exotic, since the transgenic has only a few introduced genes and the general expression of these is known. Thus, the gene expression associated with transgenics should be more predictable than with an exotic, in which the full expression of most of the genes is unknown, and any problems arising with a transgenic would be easier to identify and manage.

In any event, the primary concern with transgenic trees continues to be environmental risks, and that remains the focus of their regulation. Trees, being perennials, differ from the annual plants common in agriculture because of their long life and delayed flowering. We should note, however, that trees are not the only long-lived plants considered for genetic engineering. Other long-lived plants importantly include many of the grasses. Delayed flowering generally makes the examination of the impacts of the introduced genes over generations more difficult, but not impossible, since certain tissue-culture approaches may be helpful in mitigating the intergenerational delays. Nevertheless, regulatory complexities are likely to persist.

Thus far, only a few trees have been deregulated. In the United States of America, papaya has been deregulated and a plum tree appears about to be deregulated. In China, a transgenic poplar appears to have been commercialized (Xu *et al.*, 2004), although the extent to which it is deregulated remains unclear.

## **RISK AND COVERAGE**

There are at least two major issues when determining the nature of regulation. First are the types of plants that are covered. Second is the level of acceptable risk. An issue in the development of the appropriate criteria for determining whether plants, including trees, are to be regulated centres around whether the regulation should apply to the transgenic process itself or to the attributes of the plant or product, such as whether it may generate concerns about weediness or other adverse risks.

Some biologists have argued that regulation would better be applied to plants on the basis of the plant attributes, rather than simply on the basis of the process of genetic engineering. The decision would be based on the novelty of the plant independent of the process used in its development. This criterion would be applied, in principle, to all novel plants, including genetically modified plants, whether the modification occurred by traditional breeding or genetic engineering.

The argument of those suggesting novelty as the critical criterion is that the transgenic process itself does not inherently lead to more risky products. Rather, it is argued, the regulatory process should focus on the changes and the attributes, whether generated by traditional or transgenic approaches, that could provide a social or environmental risk. The risks associated with the attributes of the products ought to be regulated and hence the products themselves, regardless of the process used in their development.

## **RISK REGULATION IN SELECTED COUNTRIES**

Countries vary in their approach to risk. The formal United States of America decision criteria are that the product has “no significant or unreasonable adverse risks”. Note that some “reasonable risk” is allowed. Reasonable is sometimes equated to allowing no more risk than would be expected from plants developed through traditional breeding. As currently practised, regulation in the United States of America is applied only to transgenic plants. Using this approach, all transgenic plants and trees are automatically classified as regulated articles that must go through the deregulation process to be eligible for commercialization. Alternatively articulated, any plant that involves the insert of a gene using a non-sexual approach is defined as a transgenic and is automatically a regulated plant. The European Union’s decision criteria are particularly adverse to risk and require that all genetically modified plants do not present any additional or increased risks. Thus, the European Union calls for zero-risk criteria. This is a more severe standard than that of the United States of America or Canada, which accept some risks. In general, deregulation procedures are the same for all transgenics. More generally, although most countries agree on the need for some types of risk assessments for plants, there is as yet no consensus as to the degree of potential harm that will be tolerated, that is, the degree of severity of the risk (Pachico, 2003).

The question of what to regulate is also answered differently in various countries. While most countries automatically regulate transgenics, Canada

applies the criterion of novelty for regulation of both traditional and genetically engineered GMOs. However, no tree modified by traditional sexual processes has yet been required to go through formal deregulation procedures, whereas in almost all cases transgenic plants and trees require deregulation in Canada (personal communication, Phil MacDonald, Canadian Food Inspection Agency, Quebec City, 23 October 2003). Thus, in practice, the initial selection of the transgenic process may be an initial proxy for novelty.

Another question has been whether all regulated plants should be subjected to the same procedures in order to achieve a deregulated status. Some have maintained that a different deregulation channel should be adopted depending upon an initial assessment of the level of risk of a plant. China, for example, has a risk scale running from ‘no risk’ to low, medium and high risk, with the stringency of the deregulation procedures reflecting the category. A preliminary appraisal gives the plants a risk rating in one of these categories. Those in the no- or low-risk range have a relatively easy deregulation process, while those given a higher initial risk rating are required to go through a more extensive deregulation protocol. Many have argued that such a system might be appropriate to the United States of America (Strauss, 2003, 2007).

However, in some countries the law and regulatory structure remain unsettled. Chile, for example, allows field testing of certain transgenics, but does not allow or have a procedure to commercialize transgenics. Brazil had a prohibition against certain transgenic crops, which has been widely violated. However, recently some of this prohibition has been lifted ([www.isaaa.org/kc](http://www.isaaa.org/kc)).

The countries discussed above are not the only countries involved in deregulation and field trials of trees. While it is estimated that in recent years about 61% of worldwide tree trials have been in the United States of America, a host of other countries are undertaking tree field trials, including Australia, Canada, Chile, France, Italy, Japan, New Zealand and South Africa.

## **THE UNITED STATES OF AMERICA AS A CASE STUDY**

An example of the regulation of transgenic trees is found in the experience of the United States of America (see Sedjo, 2004a, b).

### **An overview: law and regulations**

The Federal Plant Protection Act 2000 gives the Secretary of the USDA the authority to adopt regulations preventing the introduction and dissemination of plant pests. Pursuant to this authority the USDA, through APHIS, regulates “organisms and products altered or produced through genetic engineering that are plant pests or are believed to be plant pests”. Such products are known as “regulated articles”. It is unlawful for any person to introduce a regulated article into production without first obtaining permission from APHIS. However, any person can submit a petition to deregulate, seeking a determination that a regulated article does not present a plant-pest risk and therefore should not be regulated (Section 12.7.3).

Additionally, the National Environmental Policy Act (NEPA) “requires a federal agency such as APHIS to prepare detailed EIS [environmental impact statements] for all ‘major Federal action significantly affecting the quality of the human environment’”. NEPA’s responsibility is to ensure that APHIS will have available detailed information concerning significant environmental impacts and will have carefully considered the information. It also guarantees that the relevant information will be made available to the public. If a proposed project will significantly affect the environment, then an EIS is required. If an EIS is not required, the agency must prepare an environmental assessment to determine whether the environmental impact is sufficient to warrant an EIS. An environmental assessment is a concise public document that briefly provides sufficient evidence and analysis for determining whether to prepare an EIS or, alternatively, a finding of no significant impact.

### Agencies and responsibilities

In the United States of America, three main agencies are involved in regulating transgenics: APHIS; the Food and Drug Administration (FDA) of the USDA; and the Environmental Protection Agency (USEPA). The FDA is involved with food safety, and the USEPA with pesticides and toxic substances under various legislation and overall environmental safety (NEPA).

The legal responsibility for protecting agriculture from pests and diseases from all sources resides with APHIS, and under the Federal Plant Pest Act, which mandates monitoring of plants that offer potential pest risks. The Plant Protection Act (Title 7 U.S.C. Sections 7701 *et seq.*) provides additional legal authority to APHIS, which, drawing from these two acts, has the authority and responsibility to determine whether a genetically altered plant, crop or tree is likely to become a plant pest or provide unacceptable risks to the environment. While APHIS has considerable experience with crop plants, it has only limited experience with trees.

Products of biotechnology, however, do not always fit comfortably within the lines the law has drawn based on historic function and intended use of products. In 1986, the Coordinated Framework for the Regulation of Biotechnology was adopted by federal agencies (see 51 Fed. Reg. 23302; 26 June 1986) to provide a coordinated regulatory approach. Products of biotechnology are regulated according to their intended use, with some products being regulated under more than one agency.

### Deregulation process: some details

Transgenic plants are automatically defined as a ‘regulated article’. The general deregulatory process for trees is essentially the same as for crops and other plants and is designed to assess a transgenic plant to determine if it provides increased risks of harm over that of traditional breeding. If it is found not to provide an unacceptable level of risk, it can be deregulated. The regulatory approach of APHIS requires three steps: permitting, notification and petition to deregulate.

For regulated articles, a permit must be obtained for the importation, interstate movement or release of the article into the environment. Deregulation requires field testing, which provides information as to the characteristics of the regulated article. Next, the deregulation process requires that a petition for deregulation be submitted to APHIS. Upon receipt and evaluation of the petition, APHIS, utilizing a scientific committee and a public participation process, makes a determination of whether to deregulate. APHIS has three ultimate options: to deregulate fully, to reject the petition or to provide qualified deregulation, e.g. to deregulate for a specific geographic region. Once a determination of full deregulated status is made, the product and its offspring no longer require an APHIS authorization for transport, release or commercialization in the United States of America. If the regulation is qualified, the article is treated as fully deregulated within the specified region, but subject to all of the regulatory restriction outside that region. If the petition is rejected, then full regulation continues.

It should be noted that a regulated article can be commercialized without being deregulated. This is common in biopharmaceutical products where the article is utilized but never deregulated. In this case the regulation provisions on the article continue.

The implementation of the Plant Protection Act related to transgenic plants centres on assessing the safety and environmental implications of the modified plant. Field testing is one of the major sources of information and is typically undertaken by the developer and occurs under controlled conditions for most genetically engineered organisms, particularly new or genetically modified plants. Field testing is designed to ensure that new plants are as safe to use as those generated by traditional breeding. The tests are also designed to prevent controlled items from escaping into the natural environment while being tested. Thus, strong containment measures are required. The developer is authorized by APHIS to gather information through field trials as well as through laboratory tests, literature reviews and other approaches, to confirm that the product has the new intended property and to determine that it is as safe to the environment as traditional varieties.

The final step of the deregulation process requires that a petition for deregulation be submitted to APHIS that details the field test results (including the use of statistical analysis) and provides a literature review and any other relevant information and/or experience. When enough information is gathered, the developer can petition APHIS to make a "Determination of Nonregulated Status". When APHIS receives a petition, a team of agency scientists begins the review. The agency announces to the public that the petition has been received, and the completed petition is made available for public review and comment. In these reviews, the APHIS standard is that an organism must not directly or indirectly cause disease or damage to plant, plant parts or processed products of plants. Additionally, the environmental implications are examined. It is common for the scientific review committee neither to accept nor reject the petition initially, but to return it to the developers with requests for additional information.

Also, it should be stressed that the overall assessment by APHIS includes a consideration of the potential effects on the wider environment to ensure that any environmental impacts are not likely to be significant. Broader environmental considerations are mandated under the National Environmental Policy Act of 1969. Furthermore, if the plant has pesticide properties, such as the introduction of a Bt gene, USEPA becomes involved in the deregulation of such a transformation. USEPA would have responsibility since the plant would involve pesticides and/or toxic substances. In this case, two agencies would be actively involved in the deregulation process, which undoubtedly would raise the costs to the developer, perhaps substantially. Up to now, there have been few pesticide-resistant transgenic trees, and most of the current research and development in the United States of America appears to be of the type unlikely to fall directly under USEPA pesticide and toxic substance regulation.

Ultimately, APHIS has several possible responses to a petition: it can approve the petition in whole, approve in part or deny the petition. APHIS can also determine that the plant poses no significant risk in certain geographic areas, but significant risk in others, and therefore approve the petition only within a given geographical area.

### Tree deregulation

There are three types of trees that APHIS might consider deregulating: orchard, ornamental and wood trees. Over the period 1987–2001, wood trees were involved in only 1.2% of the total number of field tests of genetically modified plants in agriculture and forestry. Most of those, 91%, occurred in the latest reported period (1997–2001). A total of 90 wood-tree field tests were undertaken, representing four tree genera, between 1987 and 2001, with poplar being involved in well over one-half of the trials ([www.isb.vt.edu/cfdocs/fieldtests1.cfm](http://www.isb.vt.edu/cfdocs/fieldtests1.cfm)). Although trees make up only a small portion of the plants tested and about 57% of the trees are timber trees, the number of trees tested has increased dramatically in recent years (as has the total number of plants of all types).

The general approach to the petition process in APHIS appears to be to work cooperatively with the developer. Petitions are seldom rejected outright but they are often returned as being incomplete or providing insufficient information. Despite increasing field testing in recent years, only one tree has been deregulated by APHIS: a papaya fruit tree. This tree was experiencing severe disease problems (papaya ring spot virus) in Hawaii (AgBiotech Buzz, 2002). A GMO was developed to address the disease, and the transgenic papaya was deregulated and is now in widespread use in Hawaii. Despite this success, few other trees of any type appear ready for imminent deregulation. An exception is a plum tree that suffers from plum pox virus, a viral disease of stone fruit trees such as plums, peaches and apricots. Transgenic plants expressing viral genes have been shown to exhibit varying degrees of resistance to the virus (Levy *et al.*, 2000), and recent reports suggest that a transgenic plum tree may be nearing deregulation. Thus far, however, APHIS has received no petitions

for the deregulation of a transgenic forest tree. Worldwide, there is only one documented commercially released transgenic forest tree, in China, that has been deployed (Xu *et al.*, 2004).

### APHIS performance

Deregulation is based on assessment of the results of field testing, statistical analysis and literature review. APHIS reviews about 1 000 applications for field testing of transgenics each year. Only about 59 transgenics, representing 13 species, have been deregulated over a 15-year period. Examples of deregulated articles include salt- and drought-tolerant Bermuda grass, maize-expressing proteins with pharmaceutical applications, virus-resistant squash, soybean with altered oil profile, Bt maize, and herbicide-tolerant and insect-resistant cotton.

To date, however, APHIS has authorized thousands of field tests for more than 50 plant species, mostly related to agricultural crops. Many of these have achieved deregulated status. So far, however, only a relative few (124) field tests of genetically altered trees have been authorized (McLean and Charest, 2000), including transgenic spruce, pine, poplar, walnut, citrus, cherry, apple, pear, plum, papaya and persimmon.

### Recent court decisions

Recent court decisions in the United States of America appear to require the regulatory authorities to apply more stringent standards than they had, in fact, been applying. Although the decisions apply to perennial grasses, the inferences suggest that similar standards will probably apply to trees. In the alfalfa seed decision in the United States of America District Court for the Northern District of California (Case 3:06-cv-01075-CRB Document 83, Filed 02/13/07), the court ruled that APHIS erred in applying an exception and not undertaking an EIS, as sometimes called for by NEPA (Geertson v. USDA 2006). An EIS requires a substantial increase in time and costs for APHIS and also imposes large additional costs on the developer. This EIS process allows opponents to raise hypothetical and conjectural negative environmental impacts for detailed scrutiny. A similar opinion came from the District of Columbia District Court (Civil action 03-00020 [HHK]) regarding the Scott Company's genetically engineered creeping bent grass (ICTA v. USDA/Scotts 2006). Both of these cases involved the introduction of pesticide-resistant genes to seed grasses, and the issues appear likely to be applicable to the transfer of certain types of genes to trees. While pesticide-resistant genes in trees are apparently not imminent, the fact that the APHIS procedures were deemed by the courts as "arbitrary", and therefore inadequate, necessitates the revision and complication of APHIS deregulatory procedures, at least for certain types of transgenic innovations.

### SOCIAL ISSUES: POSITIONS OF USERS AND MARKETS

This section characterizes the attitudes of various groups towards transgenic trees and the regulatory structure. These characterizations are not based on scientific



sampling procedures but rather reflect general impressions based on documentation from various groups and conversations with some of their members.

### **Attitudes towards transgenic trees and regulations**

Numerous groups have an interest in transgenic trees. These include tree growers, tree processors, tree developers, direct and indirect consumers of forest products, as well as environmentalists. Not surprisingly, attitudes towards transgenic trees vary substantially among these groups. Additionally, as has been shown in various surveys of attitudes towards transgenic foods, attitudes towards transgenics generally tend to vary considerably across countries.

### **Tree breeders and developers**

Not surprisingly, among transgenic tree developers, whether in the private sector or with universities, the attitude towards transgenics is basically positive. These groups generally believe that there is a place for some type of regulation. There is common criticism, however, of the United States of America approach of requiring all transgenics to go through the same deregulation process. As noted earlier, a common view among transgenic biologists is that certain types of transgenic changes are predictable so that a formal deregulation approach is not required. However, such an approach would obviously require some preliminary assessment to determine which transgenics require a more comprehensive assessment.

### **Tree planters and growers**

While many tree planting firms engage in tree improvement, and some are involved in research to improve the ability to clone trees, especially pine, few forest industry firms are directly engaged in tree genetic engineering research and development. The industry structure that has emerged in the past decade in North America has seen the work on transgenics being undertaken largely by universities and specialized research firms. This differs from an earlier period when individual forest firms often included work on transgenics as part of their overall tree improvement programmes. An explanation of this restructuring apparently is, at least in part, the desire of forest firms to distance themselves from the activity of genetic engineering during a period of questionable public acceptance. Additionally, there are almost surely economies of scale in concentrating research efforts in a few places rather than fragmenting the efforts.

In general, tree planters and growers are looking for opportunities to reduce costs and increase productivity. Transgenics offer both possibilities and thus, in concept, are attractive to tree growers. However, tree growers are very sensitive to actual and expected market behaviour and thus, given some of the current controversies over transgenic products, are somewhat wary.

### **Environmentalists**

A systematic inquiry at the booths at the World Forestry Congress in Quebec City (September 2003) found the attitude of environmentalists towards transgenic

trees tends to run from extremely hostile to quite sceptical. Strongly ‘green’ organizations, such as Greenpeace, exhibited great hostility, with ominous predictions of how transgenic trees would damage the natural environment. The guidelines of the Forest Stewardship Council, a certifier of acceptable forestry practices, specifically prohibit the certification of transgenic trees. At the other end of the spectrum are organizations (e.g. The Nature Conservancy) that have no institutional position on transgenics. These organizations have some staff professionals who agree that transgenic trees may have a role in forestry’s future. However, they point out that this issue is generally out of the mainstream of their organization’s direct concerns.

### **Consumers**

Two groups of consumers might have attitudes on transgenic wood. Consumers of wood as an input to other production, such as a pulp mill, find that transgenic trees with certain characteristics are desirable for their production needs. Trees with more fibre, less juvenile wood, and less or more easily removable lignin, for example, have characteristics that reduce processing costs and are therefore, in principle, desirable. A concern of these producers is whether such products will be acceptable to consumers.

The second group is consumers of final products (paper, lumber, panels, etc.) that are made from transgenic wood. From a product-performance perspective, there is little reason to believe that the final products from transgenic wood would be less suitable to their needs. In fact, in some cases the transgenic wood might produce a better final product. If processing costs were reduced, the lower price of the product would be a desirable feature. Also, there are generally no food safety issues involved with wood, although cellulose is sometimes used as a filler in foods. Thus, except for any philosophical concerns about transgenics, the products made from the wood ought to be acceptable to final consumers. The extent to which final consumers might actually resist transgenic wood products remains problematic. Some insights might be gained from the experience of certified wood and ecolabelled wood products. There is little evidence that consumers are willing to pay a price premium for certified wood. However, some firms may find it to their advantage to be certified, presumably because certification imparts a competitive advantage, even if not a price advantage. How these attitudes might translate to a transgenic wood market remains to be determined. It could be that consumers might prefer natural, non-transgenic wood, other things being equal. However, a modest price discount could overcome this tendency.

### **CONCLUSIONS**

As forestry makes a transition from foraging wild forests to tree cropping, the potential of plant improvements that will contribute to general social and economic benefits increases. Innovations that can be developed along the lines of those in crops, such as herbicide and pest resistance, and innovations involving the form and fibre characteristics of trees, offer promise. Although the life cycle of tree

improvement often means long delays between innovation and the realization of financial benefits, a number of potential transgenic innovations offer possibilities of the early capture of benefits.

Although transgenics appear to offer substantial potential for increasing productivity in forestry, there are concerns about risks that might be involved, particularly environmental risks. The purpose of regulation and the deregulation process is to ensure that these transgenic innovations are safe. Nevertheless, consumers are sensitive to these situations, and these concerns could be translated into the performance of markets for wood-based products.

Thus far, no country has publicly approved the deregulation, and hence commercialization, of a transgenic forest tree. Only one tree – the papaya – has been deregulated and is now commercialized in Hawaii. A transgenic plum tree resistant to pox appears about to be deregulated. In China, a transgenic poplar has been released on a scale that is not entirely known, but it appears to be approaching deregulatory status, if it is not already there. Finally, many forest trees are currently in field trials in several countries, and it appears further deregulation is likely to occur in some countries in the relatively near future.

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# 11. Forest biotechnology: more than wood production

*R. Kellison*

The announcement in 2007 by ArborGen LLC ([www.arborgen.com](http://www.arborgen.com)) of their acquisition of the seed orchards and nurseries, inclusive of the advanced breeding programmes and materials, of International Paper Co., MeadWestvaco Corp. and Rubicon Limited's Horizon2, emphasized that the joint venture would increase wood production from planted forests while alleviating the drain on native forests. The conclusion is that transgenic trees will grow faster than their non-transgenic counterparts, and that they will be more resistant to insects and diseases and more tolerant of environmental extremes such as cold and drought. The claim has validity as exemplified by the results from the earlier chapters of this book. But there is more to transgenics than just trees that grow bigger and faster, have more resistance to pests and have greater adaptability than run-of-the-mill trees or those from advanced tree breeding programmes. There are benefits from forest biotechnology aside from tree growth and plantation yield. The multitude of the 'aside' benefits will probably have greater value in the long run than growth and yield. The 'aside' benefits will include phytoremediation, species restoration, afforestation, biofuels and bioprocessing. The list does not necessarily stop there. The future products from bio-engineered trees are limited only by one's imagination.

## PHYTOREMEDIATION

Toxic wastes are of two types: those that escape, either by accident or design, from their intended use, and those that are residues from an approved use. A prime example of the former type is trichloroethylene (TCE), a solvent that is used worldwide to remove clothing stains and as an industrial degreaser. The second type is exemplified by chloroform, which is the by-product of chlorine that is used for purification of drinking water.

Bioremediation, the forerunner of phytoremediation, gained attention when it was observed that the plumes from oil spills contracted in the presence of underground water. Research showed that the contraction was caused by microbes in the water that, through denitrification, turned the contaminants into CO<sub>2</sub>. Further research showed that the denitrification process was effective against pollutants of oil, chlorinated solvents, pesticides, agricultural chemicals, creosote and gasoline (Chapelle, 1985).

The positive results from the denitrification process led to the discovery that some plants can detoxify contaminated soils. Such plants produce enzymes that can break down trichloroethylene (C<sub>2</sub>HCl<sub>3</sub>) into chloride ions, which is a harmless

salt that the plant sheds, and recombines the carbon and hydrogen with oxygen to produce water and carbon dioxide. One of the plant groups found effective in detoxifying contaminated soils is poplar (*Populus* spp.) (Newman *et al.*, 1997). Poplars are indigenous to the northern hemisphere, with numerous species of similar phenology that hybridize with one another under controlled conditions. The hybrids find common use in commercial forestry because of fast growth, pest resistance, adaptability, wood properties and ability to be vegetatively propagated.

The enzymes in poplars that metabolize the contaminants are from a group of cytochromes called P450, which are common to both plants and animals. In research trials, unaltered poplar plants can metabolize the TCE into salt while recombining the carbon and hydrogen with oxygen to produce water and CO<sub>2</sub>. The limitation of this process is that it is very slow. To speed the process, a gene from P450 in mammalian livers of rabbits has been inserted into the plant. That gene causes the P450 genes of poplars to overexpress the enzymes, which causes the pollution degradation process to be speeded up manyfold in comparison with P450 of the non-engineered plant. Research is continuing by Dr Sharon Doty and colleagues (Doty *et al.*, 2000) at the University of Washington (United States of America) on the use of promoters to enhance the production of the inherent P450 in poplars to have the same effect as those with the transgene from rabbit livers (<http://uwnews.org/article.asp?Search=p450&articleid=37313>).

The research just described is confined to the laboratory, or to very limited and highly controlled research trials. The potential value of the technology has such tremendous application to the polluted sites around the world that it will be only a matter of time before it finds common usage.

## AFFORESTATION

Afforestation is the occupancy with trees of landscapes that are barren of forest cover. Some of those landscapes have never borne forests during the modern era and others have been denuded of trees by humans for alternative uses of the land. Within the latter category, large areas of land are barren because the soils have been depleted of nutrients and moisture holding capacity, have become water saturated in low lying areas or have become subject to invasive insects and diseases. Other areas have reverted from being highly productive for agronomic cropping to wasteland because of salt intrusion. The intrusion is very often the result of inadequate irrigation where the minerals are not flushed from the rooting zone of the plants or, in other situations, displacement of fresh water by salt water from the sea or impounded waters.

With the advent of forest biotechnology, trees will be genetically engineered to occupy adverse sites, such as those with ambient temperatures too hot or too cold for normal tree growth. Other lands, whether arid or water-saturated, will be candidates for afforestation or reforestation, and still others with soil nutrients in limited supply or oversupply will, one day, be supporting thriving forests. In addition to additional wood production for anthropogenic uses, such forests will serve as windbreaks, wildlife refuges, recreational areas and, most importantly, for carbon sequestration.

Examples exist where plants other than forest trees have been genetically engineered to tolerate high levels of salinity, drought, cold and high temperatures. Working with the wine grape (*Vitis vinifera*) in northern Nevada (United States of America) tolerance has been shown for extremes in temperature and adverse soil conditions by manipulating cell length of the roots (Cramer *et al.*, 2005). The process involves the selection of mutants of *Arabidopsis thaliana* for salt tolerance, which is then genetically engineered into the grape plants. Interestingly enough, tolerance to salinity conveys added tolerance to drought, and cold and high temperatures. That technology is suitable for transfer to forest trees, the results of which will occupy some of the most adverse sites for tree growth in the world.

Care will have to be exercised to assure that the extension of forests to lands of marginal productivity does not create a problem of equal or greater intensity. An example of such a travesty would be the additional drawdown of water on which a community or municipality might be dependent. Conditions already exist in some parts of the world, such as in South Africa, where plantation forestry is restricted at the local level because of an inadequate water supply. Used judiciously, however, the benefits of forest biotechnology will help solve more problems for the human population than it creates.

## SPECIES RESTORATION AND CONSERVATION

Heritage forest tree species are threatened and endangered throughout the world. The situation is exacerbated by transnational movement of goods from continent to continent. Along with those goods are hitchhikers of the insect and disease phyla. Such pests are often benign in their indigenous range, but become catastrophic when introduced to new environments in the absence of natural biological control agents. On top of that are the indigenous pests that create havoc for tree monocultures because of changes in climate. This section will deal with those two types of forest destruction.

### Heritage trees

Heritage trees are those that are threatened, endangered or have high social and economic value. The epitome of that category is American chestnut (*Castanea dentata*). That species comprised about 30 percent of the overstorey forest of the Appalachian Mountain range, with extensions into the Central and Lake States (United States of America), inclusive of southern Ontario (Canada). Its nut production had tremendous importance for wildlife as well as for Native Americans and European colonizers as a food supplement, and as a bartering commodity for essential goods and services. Additional values were for wood products that were essential for buildings, conveyances, fences, furniture and myriad other uses. In addition to the wood being easy to split, saw, form and assemble, it was durable. The tannins responsible for durability also found other uses such as in leather tanning. In short, chestnut was the all-American tree (Bolgiana, 2007).

Its prominence began to wane, probably in the late 1800s, because of a disease dieback syndrome. The decline, which killed the aboveground portion of the

tree but left the root system unaffected, was identified in 1904 by the New York Botanical Garden as *Endothia parasitica*, a pathogen from the Orient. The pathogen, subsequently named *Cryphonectria parasitica*, enters through wounds to form stem galls that girdle the tree. Trees of small size (10-cm range at breast height) are quickly killed, whereas those of larger size die at a progressively slower rate. Within a 40-year period, the pathogen had made its way to the ends of the range of the once-dominant tree species.

Efforts were initiated to select and breed for blight resistance within American chestnut, but the results were inadequate to justify continued funding. Research was also initiated with hybrids from the native species and Chinese (*Castanea mollissima*) and Japanese (*Castanea crenata*) chestnuts, both of which have higher resistance to chestnut blight than does the North American species. Even though some progress was made over a 40-year period, the results were sufficiently variable that the work by public agencies to find a cure was largely abandoned. The exception to abandonment by public agencies is the work being carried on by The Connecticut Agricultural Experiment Station under the guidance of Dr Sandra Anagnostakis (Anagnostakis, 2007).

In lieu of public funding, formation of The American Chestnut Foundation (TACF) and The American Chestnut Cooperators Foundation (TACCF) in the 1980s was initiated to continue the cause for restoration of American chestnut. The emphasis of TACF was to use a backcross breeding programme with Chinese chestnut to obtain disease resistance while maintaining the tree phenotype and nut production of American chestnut. TACCF, in contrast, concentrated its efforts on finding trees with partial resistance and escapes of pure American chestnut and hybridizing those to produce progeny with added resistance.

While both programmes have made progress, TACF is nearing completion of backcross breeding that is producing a tree of 15/16<sup>th</sup> American chestnut and 1/16<sup>th</sup> Chinese chestnut (Sisco, 2004). While high achievement is expected from the backcross progeny, the theory of quantitative genetics means that the product will not be one with complete Chinese blight resistance or one with complete American phenotype. Breeding experiments have also revealed that only two or, at most, three genes are responsible for disease susceptibility or resistance. To identify those genes, a project is under way with collaboration from the universities of North Carolina State, Clemson, Penn State, Syracuse and Georgia, in addition to the USDA Forest Service, The Connecticut Agricultural Experiment Station and TACF. Good progress is being made in this endeavour, with the welcome news that the results can be used to enhance the screening process in the backcross breeding programme. In addition, it lays the groundwork for direct insertion of the resistant genes from Chinese chestnut into American chestnut to engineer a blight-resistant tree.

### Application

The application of achieving disease resistance in American chestnut is fast approaching. It bodes well as the pioneer for other tree species that are threatened



or endangered by invasion of insects and diseases from abroad. Chief among the threats are sudden oak death caused by a root pathogen (*Phytophthora ramorum*), and the insect invasives of emerald-ash borer (*Arilus planipennis*) and sirex woodwasp (*Sirex noctilio*).

The common carriers of the sudden oak death pathogen are the landscaping plants of rhododendron (*Rhododendron* spp.) and azalea (*Azalea* spp.), but the oaks (*Quercus* spp.) of the Pacific Southwest are especially vulnerable (Barrett *et al.*, 2006) and, in laboratory tests, many of the oaks of the Eastern Deciduous Forest have also proven to be highly susceptible. The emerald ash borer of Asian origin has, within a decade, killed about 30 million trees of white ash (*Fraxinus alba*) in Illinois, Indiana, Ohio, Pennsylvania and Wisconsin, and has even been found in the Canadian province of Ontario ([www.emeraldashborer.info](http://www.emeraldashborer.info)). The sirex woodwasp of European origin has been a common pest in pine plantations in the southern hemisphere, specifically in Australia, New Zealand, Chile, Argentina and Brazil. Under plantation conditions, the pest can be reasonably controlled by good silvicultural practices and by biological means. In native stands of pines common to the northeast and north-central parts of the United States of America, however, control becomes extremely complex (Haugen and Hoebeke, 2005). Biological control, including both the genetic engineering of plants for resistance and biological manipulation of the insect, seems to be the only reasonable method of countering the pests.

In addition to the exotic pests are those of indigenous origin that are causing catastrophic losses, presumably as a result of global climate change. The one that is claiming international attention, especially in western Canada and southwestern United States of America, is the mountain pine beetle (*Dendroctonus ponderosae*). Within British Columbia, hundreds of square kilometres of the naturally occurring monoculture of lodgepole pine (*Pinus contorta*) have been killed, leaving a desolate landscape. On a smaller scale, in the Pacific Southwest of the United States of America, especially in Colorado, the insect has denuded the landscape of live trees of lodgepole and ponderosa (*P. ponderosa*) pines. The cause of these catastrophic events is purported to be the lack of prolonged freezing temperatures, which allows successive broods of the insect to continue unabated.

The effort to maintain tree cover and to colonize areas formerly occupied by a native tree species is becoming ever more important as plagues proliferate. American chestnut can be the pioneer species because of its appeal to a wide audience for restoration, even as a transgenic. At the same time, its recolonization of diverse sites will pave the way for dealing with other species that are beset with plagues, such as those of lodgepole and ponderosa pines.

## Biofuels

Global climate change is catching the attention of nations worldwide. Global warming is thought to be a major contributor to climate change because of the elevated load of CO<sub>2</sub>, which is presumed to be creating a greenhouse effect. CO<sub>2</sub> levels have increased from about 280 ppm in pre-industrial time to 381 ppm in

2007. Anyone doubting the incremental increase has only to look at the trend from 1958 through 2007 from readings made by the National Oceanic and Atmospheric Administration (NOAA) at Mauna Loa Observatory, Hawaii. During that 50-year period, every annual amount is higher than the year before.

Burning of fossil fuels is the primary cause of the increasing amount of atmospheric CO<sub>2</sub>. In the United States of America, for example, some estimates are that more than 80% of atmospheric CO<sub>2</sub> levels are from the burning of fossil fuels. Allocations of that total by user segment are: electricity generation (34%), transportation (28%), industrial use (19%), commercial use (6%), residential (5%), and agricultural use, including forestry (8%). The values change somewhat on a worldwide basis, with estimates that 20% of atmospheric deposition is due to deforestation, primarily in the tropics.

In addition to the adverse effects of greenhouse warming from the burning of fossil fuels, civil strife in the areas where the petroleum reserves are found have made the long-term availability of the resource questionable. That combination of limitations has caused governments in various parts of the world to look for alternatives sources of fuel. Even though solar power is a bountiful source of energy relatively little use has been made of it because of the expensive photovoltaic cells needed to convert light to energy (Cohen, 2007). Nuclear, wind, geothermal and water forms of energy generation hold potential, but they have been relegated to low priority because of initial cost, regulatory issues and real or perceived safety concerns.

Alternative fuels, including products like ethanol and methanol that can be made from biofuels, have been hyped by some countries for the past 40 years. As a result of the petroleum crises in the mid-1970s, Brazil decreed that 20% of its gasoline usage would be replaced by ethanol, the feedstock of which would be sugarcane (*Saccharum* spp.). The technology in automobile engine manufacture in that country has advanced so that cars of today are equipped to operate efficiently on ethanol of 80-percent grade. In conjunction with the improved manufacture of ethanol from sugar cane, scientists have been active in increasing the yield of the crop per unit area. Biomass yields were increased by 3.5% annually from 1978 to 2000, and the yields had yet to plateau. In combination with increased yields, the sugar content of the plants has increased proportionally. That, along with the added area for sugar cane production, which is projected to increase from 5.7 to 11 million hectares, makes Brazil the leading country in the world for the production of biomass fuels (Orellana and Neto, 2006).

Other biomass crops that are candidates for fossil fuel replacement are maize (*Zea mays*), switchgrass (*Panicum virgatum*) and wood cellulose. Relative to the cost of gasoline in 2005, ethanol from maize, switchgrass and wood cellulose were 29, 50 and 57% more expensive, respectively (Pimentel and Patzek, 2005). Those values are slightly less onerous than they were in 2005 because of the higher price of gasoline, but in some respects they have not changed greatly because of the higher prices for the feedstock, especially maize. The price of maize has roughly doubled during that time because of competition for the limited resource, but the

costs for production inclusive of equipment, seeds, chemicals for plant nutrition and weed control, and harvesting and transportation have increased similarly. More and more emphasis is being given to plant residues and, especially, plants grown specifically for energy production are gaining in priority. Woody biomass is gaining favour over switchgrass because of its ability to be stored 'on the stump' and to be harvested as needed. The harvesting of switchgrass is done at maturity, otherwise the energy content begins to decline slowly at first and rapidly with increasing age beyond maturity.

The prognosis is that woody biomass will be genetically engineered to increase the syringal type of lignin at the expense of guaiacyl. The genetically engineered plants will be grown within easy haul distance of the bioenergy plant. Portions of the southern United States of America are in a favourable position for such operations because of the option to convert abandoned pulp mills to ethanol production. Such facilities are already equipped for the processing of timber for pulp and the only remaining addition is the conversion of the cellulose to sugars and the sugars to ethanol. It is estimated that such a facility could be retrofitted for about 25% of the cost of building a new converting plant.

A limitation to conversion of cellulose to ethanol in today's world is the desired enzymes. Great progress is being made in the discovery of new enzymes and the creation of additional ones by biotechnology organizations. The prognosis is that a cornucopia of enzymes fit for rapid conversion of cellulose to ethanol, together with genetically engineered plants that are rapid growing and have a high syringal to guaiacyl ratio, will one day be offsetting as much as 25 percent of the fossil fuels needed for industrial society. As a case in point, the United States of America is set to enact into law an energy bill that boosts ethanol use to 36 billion gallons by 2022, up from 5.5 billion gallons in 2007. Of the 2022 total, 21 billion gallons was expected to be from raw materials other than maize. The prognosis is that trees will be a major contributor to that alternative fuel.

### **Paper manufacture**

With an expanding world population that today is at 6.5 billion people and is expected to peak at 9 to 10 billion people by 2050, the need for paper will continue to increase. The increase will come with the numbers of people while, at the same time, per capita consumption will decrease because of reliance on computer technology. Thus there will continue to be a huge market for paper and paper products.

In the same way that trees will be grown for conversion to fuel, trees will be genetically engineered for high cellulose content for the manufacture of paper and paper products. Alterations will be made in the pulping with reliance more on enzymatic action to separate the cellulose from the lignin, thus supplanting the costly steps now encountered in both chemical and mechanical pulping. Similarly, the caustic chemicals used for bleaching the pulps will be greatly reduced in favour of enzymatic bleaching. The processes of both pulping and bleaching will be greatly simplified and, as a result, greatly reduced in cost and environmental impacts.

The two methods for separation of cellulose from the lignin of woody biomass are chemical and mechanical pulping. The most common method of wood property separation is by chemical pulping, which is done in combination with causticizing chemicals and heat. The pulp yield of such an operation varies from about 45 to 55%, by weight, depending on species of the woody biomass. Mechanical pulping, on the other hand, produces pulp yields of 85 to 90%, which is accomplished by grinding at high and costly energy levels in the presence of heat. The major difference between pulps of the two methods is in the lignin removed. Mechanical pulps have limited use because of the retained lignin, which causes papers to yellow when exposed to ultraviolet light; they therefore find application in lower-grade products or in limited combination with chemical pulps.

Both types of pulp require bleaching to some degree to meet paper and paperboard specifications, but bleaching of chemical pulps is less intrusive in cost and in environmental impact than mechanical pulps. That scenario is likely to change as the result of biotechnology. With the use of fungi, such as the white-rot basidiomycete *Ceriporiopsis subvermispora*, the lignin between the cells (fibres and tracheids) as well as the lignin within the cell walls can be separated from the cellulose by mechanical means (Teeri, 2004). Such a process would limit the need for bleaching.

Even though the pulping and bleaching process with fungi is operational on an experimental scale, it has not yet achieved commercial application because of logistics and the lack of advanced-stage enzymes. The logistics deal with the inability to distribute the fungus equally through large piles of chips and to the time required for the fungus to chemically separate the lignin from the cellulose of the woody cells. The former limitation should be overcome with design alterations at pulp mills, and the latter will come about with the discovery and genetic engineering of enzymes that will speed the process with uniformity. Energy consumption alone with the envisioned process will be about 30% less than with pure mechanical pulping (Shukla, Rai and Subramanyam, 2004).

## CONCLUSIONS

Mention has been made of only a few of the benefits of biotechnology in the forestry sector: bioremediation, afforestation, conservation and restoration, and biochemical processing of wood for fuels and paper and paperboard. The list can go on to include pharmaceuticals and foodstuff from trees, carbon sequestration through extension of forest plantations to marginal sites as well as to genetically engineered trees that speed the process of sequestering carbon while sequestering larger amounts in the tree parts and in the soil (Kellison, 2007).

Concerns have been raised about the negative ecological impacts that forest trees might have on ecosystems where escapes might occur. Those concerns need to be studied, which will probably result in strict guidelines being applied for commercial application. We ought not, however, to be overly conservative because of the population increases in the world coupled with a limited land base. In fact, the land base is steadily diminishing due to human development, inclusive of expansion of industry, housing and land-use alternatives.

A case in point for keeping the options open for an expanded use of biotechnology for humans, medicines, domestic animals, agronomic crops and forest trees is the situation arising in the European Union. That suite of countries has been opposed to plant biotechnology, be it agronomic crops or forest trees, and has enacted legislation that bans the use of transgenic crops for human consumption, either directly or indirectly. The situation is now arising, however, where the demand for non-transgenic farm crops is exceeding domestic or international supply. The projection is that in one or, at most, two years some of the imported grain for animal feed will be of the genetically engineered variety (Mitchel, 2007). There will be no other option because the crops for animal feeds are progressively being used for ethanol production. The question then becomes “is it equally undesirable to consume meats from farm animals that have been fed transgenic crops as it is to directly consume the transgenic crops?” This question presumably answers itself in the long run. With population increase and arable land decline in the world, the populace will have to make use of its every resource if the human race is to survive.

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## 12. Regulation for genetically modified forest reproductive material moving in international trade

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When European countries started provenance research with the main indigenous forest tree species, including some exotic ones like Douglas fir, from the 1880s, it became obvious that populations from different origins and provenances of the same species react differently in growth and other characters. In the further historic development of modern forestry, IUFRO played a big role, especially in provenance research (Kriebel, 1992) and acted in many cases as a forum for discussion on how to put the results into practice. So it happened in the following century, as results of provenance research have formed the basis to develop recommendations and also rules for proper use of that material in reforestation and afforestation.

Since the 1950s, the whole legal system has been modernized in many countries. In the countries that later became the European Economic Community (EEC), property and the free use of it (despite the many restrictions existing) became very important rights. Thus, the rules for use of the material in reforestation were obsolete, because the owners themselves could decide what material to use (free choice of species and provenances). Consequently, in 1966 the EEC enforced the first regional regulation for the ‘marketing’ of forest reproductive material, rather than the ‘use’ of that material: Council Directive 66/404/EEC (EEC, 1966).

Meanwhile, that Council Directive has been revised, last in 1999 as Council Directive 1999/105/EC (EC, 1999) (EC = European Communities, later becoming the EU = European Union). The philosophy behind this was to establish rules for the production and marketing of reproductive material and give the user and consumer of that material all necessary information so as to enable them to make the best choice. In this respect, the Council Directive can be seen as a regulation to boost consumer protection.

In addition, the Council Directive will also enhance the production of forest reproductive material by setting standards for production, which includes activities such as: seed collecting and processing; vegetative propagation; producing clonal material; producing new types of basic material for the production of reproductive material; raising plants in the nursery; handling of the material at all stages from beginning to delivery to the consumer; and certification (see below). Wherever breeding is involved in these activities, it is wise to adhere to the regulation

otherwise it could happen that a new breeding product does not receive approval (see below) and as a consequence will be excluded from the EU market.

### **REGULATIONS FOR MARKETING OF REPRODUCTIVE MATERIAL**

There are two regional regulatory schemes, that of the EU (Council Directive 1999/105/EC; EC, 1999) as mentioned above, and that of the Organisation for Economic Co-operation and Development (OECD), namely the OECD Scheme for the Certification of Forest Reproductive Material Moving in International Trade (OECD, 2007). The OECD Scheme was established in 1974, thereafter amended several times and revised in 2007. The OECD member countries (of which 25 were participating in the Scheme at the time of writing) and the EU member states (currently 27 have full membership, of which ten are simultaneously participating in the OECD Scheme) agreed to harmonize their regulations.

When in 1987 the issue of genetic modification came into the picture and the first genetically modified (transgenic) poplar clone was tested in the field, it was necessary to consider whether this type of breeding product would automatically be included in the regulation. An expert group established by the OECD assembly of Designated Authorities participating in the OECD Forest Seed and Plant Scheme worked from 1993 to 1996 on a proposal to revise the Scheme. The expert group recommended a revised version, which does not regulate the procedure of genetic modification, because this was not necessary and out of its competence while ruled on the national level or on the EU level, but included some requirements for genetically modified reproductive material to easily facilitate marketing under the regulation with a view to providing full information to meet the demand for consumer protection.

The proposal was not adopted by OECD, because it did not achieve unanimous agreement, the reason being the inclusion of requirements for genetically modified reproductive material in the text, which could not be accepted by one member country.

The OECD then took another approach for revising the Scheme, because many other items still needed to be revised and harmonized with the EU regulations, which had been in conflict and hindered the trade between OECD countries and EU member states. This part of the text consists, for instance, of using the same definitions of terms, identical descriptions of the types of basic material and categories, the same specifications for the national register and the certificates and the same concepts for the selection and testing procedures. While the OECD Scheme should not contain any additional requirements for genetically modified material, those paragraphs regulating the two advanced categories “Qualified” and “Tested” were omitted, in which such requirements were incorporated. The result was the OECD Forest Seed and Plant Scheme (OECD, 2007), which includes only the first two categories “Source identified” and “Selected” instead of four, while the two advanced categories are under consideration for further extension. That is the reason why the OECD Scheme has no regulation concerning genetically modified material.



The proposal was adopted and harmonized by the EU member states. The result was Council Directive 1999/105/EC (EC, 1999), which consequently also contains the requirements for marketing of genetically modified material. In the following discussion, the Council Directive will be the only reference for regulations for marketing of genetically modified forest reproductive material at regional level.

For the release of genetically modified organisms into the environment in general, another Council Directive has competence. Therefore the genetically modified forest reproductive material needs to meet the requirements of two directives: Council Directive 90/220/EEC on the deliberate release into the environment of the genetically modified organisms, and Council Directive 1999/105/EC (EC, 1999) on the marketing of forest reproductive material. The following sections deal with requirements that genetically modified material must fulfil to get permission for release into the environment and at the same time to obtain approval to produce reproductive material for marketing.

## **METHOD OF OPERATION OF THE REGULATIONS**

Both sets of regulations operate according to the same principles. The government will designate the Authority to implement the Scheme or Directive in the country and to control all necessary operations. Where a country already has a national regulation, it would be advantageous to combine the authorities of the national and the international regulatory schemes. In case of the members of the EU, this is already practised.

The regulation comprises definitions and rules under which the forest reproductive material shall be certified. The procedure can briefly be described as follows: the main principles are approval and certification. The basic material will be approved, after that it can serve for the production of reproductive material. The basic material can consist of a seed source, stand, seed orchard, parents of family(ies), a clone or clonal mixture, of which all, except the seed source and stand, may be derived from genetically modified material. The procedure for approval starts with the declaration of what shall be approved (type of basic material), the exact location and delineation of the basic material so as to clearly identify it (unit of approval), and after having approved the basic material according to the rules (see below) each unit of approval shall be identified by a unique register reference.

The register reference will be listed in the National Register of approved basic material (see below). Each unit of approval is related to a category. There are four categories recognized in the Directive, namely “Source identified”, “Selected”, “Qualified” and “Tested”. Reproductive material derived from approved basic material will be certified according to its nature (either derived from seed or clonal material) and status (category). The Certificate of Identity will reproduce all relevant information on the basic material from the National Register and add the information related to the actual lot of reproductive material. Each certificate has a number and a member state code. All lots of forest reproductive material will be

accompanied by a label containing the certificate number and code together with other information relevant for the actual lot.

## **RULES FOR GENETICALLY MODIFIED FOREST MATERIAL**

### **Safety requirements**

The procedure for basic material that is genetically modified has to satisfy the requirements of the two regulations mentioned above. The regulations are implemented and controlled by two different Authorities in the member states of the EU. Council Directive 90/220/EEC regulates the procedure of genetic modification and sets up requirements for the material to be released into the environment. It is not the place here to outline Council Directive 90/220/EEC and the philosophy behind it. Here only the requirements to be satisfied for the release of reproductive material will be summarized, which are explicitly demanded in Council Directive 1999/105/EC (EC, 1999).

If the basic material consists of a genetically modified organism within the meaning of Directive 90/220/EEC, such material shall only be accepted if it is safe for human health and the environment (Art 5,1 of Directive 1999/105/EC). This is the fundamental requirement, which all genetically modified organisms have to fulfil. For forest basic material as well as for crops, an environmental risk assessment as laid down in Directive 90/220/EEC shall be carried out additionally. If all these requirements are met, the genetically modified basic material will be accepted for inclusion in the National Register (see below) after having been authorized in accordance with the Directive (Art 5,2b).

The meaning of the last sentence may not be clear for those who are not familiar with Directive 1999/105/EC. It actually means that the basic material, which has satisfied the requirements above, is not free for immediate commercialization. But the basic material is accepted for inclusion in the National Register of basic material. To get a full inclusion for the basic material, the other requirements set up in the Directive 1999/105/EC have also to be satisfied, which are necessary to get approval and thus permission to produce for commercialization reproductive material from the basic material.

### **Approval**

The unit of approval is the basic material, for instance a clone as noted above. A single gene construct cannot be approved, as it exists only in an organism and can only be expressed in an organism. Consequently, each clone of a group of clones, of which all are transformed by the same gene construct, must be tested separately. It is obvious that each transformation is unique, because the position in the genome and the composition of the flanking regions of the position are different. Further, each clone contains another genetic background and therefore transgene expression may vary.

What are the special requirements for genetically modified basic material set up in the Directive 1999/105/EC? Genetically modified material can only be marketed under the category Tested (Art. 6d and Annex V). After authorization

by the Authority responsible for release into the environment has been granted, the basic material must be tested in the field, because field testing is compulsory. (Early tests, which may be accepted for approval under certain conditions, are not feasible in the case of genetically modified material.) The basic material can be tested in two ways, either by genetic evaluation of its components or by comparative testing. If genetic evaluation is preferred, the identity, origin and pedigree of the evaluated components of the basic material, together with the crossing design used to produce the reproductive material, must be documented. Pedigree involves not only information about parents and their characteristics, but also the origin of a gene construct and other genes used for transformation that have been incorporated into the genome of that component. The evaluation must satisfy certain well described requirements and must be superior to standards. Test duration is not laid down in the rules, but it is understood from the philosophy of the regulation that half of the rotation age may be accepted. In certain cases the full rotation age may be necessary to judge whether results satisfy the requirements.

As the genetically modified material must also be field tested according to Directive 90/220/EEC for deliberate release, a question could be in which order the test should be put. Usually the field testing according to Directive 1999/105/EC has to be done after the material has fulfilled the requirements and received authorization for release. The reverse order is inefficient. Another question concerning whether the subsequent genetic modification of approved basic material, which is already on the market, is possible without field testing once again, can be negated. The genetic modification leads to a severe change of the target trait and possibly also of non-target traits. Thus the testing is necessary.

### **Registration, the National Register, and separation of lots**

After approval, the basic material enters the National Register, with each unit of approved basic material having a unique registration reference (Art. 4,2b). Full details of each unit of approval shall be recorded, together with its unique registration reference, in the National Register (Art.10,1). The following information shall be provided as applicable: Botanical name, Category, Purpose (to be stated if use for forestry functions other than timber production is foreseen), Type of basic material, Register reference, Location (for the category Tested: a short title and the exact geographical position where the basic material is maintained), Altitude, Area (size), Origin and an indication “in case of material of category Tested, whether it is genetically modified”.

The registration reference will accompany the material during all stages of production and processing of the reproductive material derived from that basic material, up to the final step of certification. The rules state clearly that lots containing genetically modified reproductive material have to be kept separate at all steps (Art. 13,1k); mixing is not permitted. Mixing of lots of other than genetically modified reproductive material may be allowed under certain conditions.

### Certificates and labels

In the case of forest reproductive material derived from basic material consisting of a genetically modified organism, any label or document, official or otherwise, for the lot shall clearly indicate that fact (Art. 14,7). An official document is the certificate. There are three models for certificates, two of which cover reproductive material that may contain genetically modified material: Certificate of Identity for reproductive material derived from seed orchards or parents of family(ies) and Certificate of Identity for reproductive material derived from clones and clonal mixtures. Among the 21 or 17 items, respectively, to be filled in on the certificates, one is related to genetically modified material and must be answered: “Has genetic modification been used in the production of the basic material: Yes or No?” The same applies for the labels.

Note that ‘clonal mixture’ does not mean a random mixture of anything vegetatively propagated, but is a well defined term. A clonal mixture is a “mixture of identified clones in defined proportions”. And a clone is defined as a “group of individuals (ramets) derived originally from a single individual (ortet) by vegetative propagation, for example by cuttings, micropropagation, grafts, layers or divisions” (Art. 2c). Therefore the clones marketed singly or the clones in a mixture marketed as clonal mixture are identified and remain identifiable during all stages of the production of that material. Also material must be declared as genetically modified that consists only partly of genetically modified organisms, for instance only a few clones in a clonal mixture.

More extended definitions of a clone are given by Ahuja and Libby (1993). Rules for clonal propagules either derived by different methods of *in vitro* propagation or micropropagation including by genetic engineering were presented and compared as long as 15 years ago (Muhs, 1993). An extra category for this material was under discussion at that time, but the development took a slightly different route, which can be seen from the rules above. The genetically modified material is fully integrated in the regulation for marketing of forest productive material.

### ACCEPTANCE

Transparency is an essential part of acceptance. This has been considered in the regulation. To promote transparency the requirement has been adopted that the methodology used for the test and the detailed results obtained shall be made freely available (Annex V, 1e). This requirement is very important for the user and consumer, because they can make up their mind about the suitability of the material for reforestation purposes on the sites in question. If genetically modified material is involved, full information about the pedigree (see above) must be given also. The public can also deal with the matter and raise awareness, which may help to increase the acceptance of the issue of genetic modification of forest trees. But this seems to be a long way off, and to be dependent on many factors, such as the objectives, the methods used, the effects on the environment, and the policy of the breeder or their agency.

The public will gain even more importance as it has the power to influence official policy (in European countries much more than in many other countries). For instance, a bad policy on the part of the forest owner or company interested in growing genetically modified forest trees, by publishing wrong information or concealing information, can lead to strong public reactions against the project, although all necessary permissions according to the Directives have been granted. So far, no genetically modified forest tree has been planted in the EU member states on a commercial scale. This may change in future and those interested in growing genetically modified forest trees should involve the public at an early stage to avoid unacceptable behaviour. The reaction of the public regarding the cultivation of genetically modified crops in the past provides an example, because information from the company was scarce or had been concealed. The result was a reaction rejecting everything connected with genetic modification. It is hoped – and there are promising signs – that transparency and clear declaration and information will reap their rewards.

## **OUTLOOK**

It was far-sighted to establish rules for genetically modified forest trees before breeders start producing such material for commercialization, because they have guidelines on how to proceed. They know that it could take some years to go through all the tests laid down in the rules. That is one of the reasons for their hesitation. In future, methods for the transformation of gene constructs into a genome will improve and methods to address proper positions in advance of where to insert it could be developed. Also, the search for suitable genes, which have a more specific effect, may be successful in future. Thus, after substantial improvement in methods and gene availability, genetic modification may also in future have a chance with forest trees.

Before then, some missing elements of the rules should be developed, in particular the environmental risk assessment with special reference to forest trees. As forest trees are long-lived organisms, experiments with genetically modified trees should be examined over a long period and monitored thereafter up to the end of the rotation. Criteria to be examined and monitored should be developed specifically for forest trees in addition to the general ones set out in Directive 90/220/EEC.

Tests should be extended by regular checks at given intervals for the stability of the gene constructs incorporated in a host genome and their expression. It has been found in many cases that transgene silencing, as well as transgene repeat formation and transgene integration, are sources for unstable expression (Kumar and Matthuis, 2004). Thus, as these factors also show great influence on the expression, additionally the expressivity, which may be defined as the function of the degree of expression in relation to the growth development and seasonal conditions over years, may be analysed. It is not helpful, for instance, if the stability of a sterility gene is lost or the gene will not longer be expressed, before the trees reach the age when they start flowering. It is not even acceptable that expressivity at that age is

reduced to a level that does not fully prevent the formation of fertile flowers. This example can also be applied to many other gene constructs and traits.

Concerns of the public may increase in future regarding the protecting or patenting of cultivars and varieties such as clones or parents of family. The public has experienced some examples in varieties of crops that have been developed by a company and used worldwide, and these varieties have replaced the local, and in many cases well adapted, ones. The company used doubtful methods to urge the farmers to buy its improved seed. As a result, the local farmer and breeder will lose income and the diversity of varieties available will decrease. It is time to think about the future development of the technique of genetic modification and its consequences. The public has great concerns that should be taken seriously.

Another example of unwanted side-effects of poorly framed policy is a case in the United States of America in which a farmer's canola crop was contaminated, without his awareness, by the pollen of a genetically modified variety that his neighbours were growing. The company that had developed the genetically modified variety claimed that the farmer was growing this variety illegally. The unusual court judgement urged the farmer not to grow his own canola variety any longer, but rather to buy the genetically modified canola variety from the company in future. Although the situation is complex, it shows the complications of poor policy. The farmer, Percy Schmeiser, continued to fight for his right to grow his choice of canola, and was recently awarded the Alternative Nobel prize for his efforts to preserve the local and well adapted varieties of various crops (not only canola) bred by farmers around the world.

## **SUMMARY**

The issue of genetically modified forest trees first arose in 1987, when the first transgenic poplar was produced. In 1999, Council Directive 1999/105/EC (EC, 1999) of the EU was enforced as the first regional regulation, and included rules for the marketing of genetically modified forest material moving in international trade. The OECD Scheme as the second regulatory scheme (OECD, 2007) contains no special rules for genetically modified material, although the countries participating in the Scheme have been working actively towards establishing such rules. Their acceptance has been blocked by a lack of unanimous agreement.

The rules for genetically modified material have been discussed in detail above. Requirements to be fulfilled appear in the two EU Directives. After the safety requirements have been satisfied and authorization for release has been granted, the reproductive material has to undergo tests, because it can only be approved in the category "tested". After successful testing the genetically modified basic material will be approved, each unit will be registered individually and listed in the National Register. Certificates and labels will contain a clear indication that the reproductive material has been derived from genetically modified basic material. The regulation supports transparency by obliging the breeder to make freely available details of the methodology used in the test and the detailed results obtained.

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