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Series on Harmonization of Regulatory Oversight in Biotechnology No. 19

REPORT OF THE WORKSHOP ON THE ENVIRONMENTAL CONSIDERATIONS OF
GENETICALLY MODIFIED TREES

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Series on Harmonization of Regulatory Oversight in Biotechnology

No. 19

Report of the Workshop on the Environmental Considerations of Genetically Modified Trees

Environment Directorate

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FOREWORD

  The OECD's Working Group on Harmonization of Regulatory Oversight in Biotechnology started its discussion on tree specific problems in 1997. This discussion was triggered by the growing awareness of the differences between crop plants and trees. The first consensus document on the biology of a tree species was published in 1999, for *Picea abies* (L) Karst (Norway Spruce). Consensus documents on other tree species are also in various stages of completion. Some of these documents present a quite important data set on the organisms in question, but several member states felt some need to address the questions specific to the risk assessment of long lived species like trees. Upon the initiative of Norway, the Working Group agreed to organise a workshop to examine various aspects of environmental consideration for genetically modified trees. The Norwegian Directorate of Nature Management – in close collaboration with the Steering Committee countries (Austria, Canada, Finland, and the Netherlands) – subsequently organised such a meeting.

  This document is a compilation of the presentations and plenary session reports made at the Workshop on the Environmental Considerations of Genetically Modified Trees which was held in Norway, in September 1999. This document was prepared by Norway, the Steering Committee and the OECD Environmental Directorate. The OECD Working Group on the Harmonization of Regulatory Oversight in Biotechnology agreed on the report and the proceedings and subsequently the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology recommended to publish the report.

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OPENING REMARKS: THE BACKGROUND AND OBJECTIVES OF THE WORKSHOP

Bjørn Åge Tømmerås, Workshop Chairman, NINA, Norway

The OECD's Working Group on Harmonization of Regulatory Oversight in Biotechnology started its work to produce consensus documents in 1995. They contain information used for the safety assessment of particular plants and gene products. The "Consensus Document on Biology of Picea abies (L) Karst (Norway Spruce)" was developed by the lead country, Norway and it was published in 1999. Consensus documents on other tree species are also in various stages of completion. The Norway spruce document was discussed at several sessions of the Working Group.

Some of these documents present a quite important data set on the organisms in question, but several member states felt some need to address the questions specific to the risk assessment of long lived species like trees. The discussion on tree specific problems was initiated at the 6th session of the Working Group in 1997. This discussion was triggered by the growing awareness of the differences between crop plants and trees. Upon the initiative of Norway, the 7th session of the Working Group agreed to organise a workshop to examine various aspects of environmental consideration for genetically modified trees. The Norwegian Directorate of Nature Management – in close collaboration with the Steering Committee countries (Austria, Canada, Finland, and the Netherlands) – subsequently organised such a meeting.

The objective of this workshop is to clarify tree specific questions - as long lived species - and identify an adequate way to address these questions. This workshop, held among 47 representative experts from 15 OECD member countries, who have experience in this new field, will have four sessions to treat different aspects of the subject of environmental consideration. The expertise of the participants will certainly facilitate the clarification of tree specific problems and identify a way for the Working Group to consider these questions further. The output of this workshop will be submitted to the OECD Working Group on Harmonization of Regulatory Oversight in Biotechnology.

Why make genetically modified trees and status of the technology

Dave Ellis, Director Biotechnology, BC Research
Vancouver, British Columbia

The genetic improvement of forest crops is still in its infancy and we rely on relatively undomesticated, wild trees for the majority of our wood product needs. While our reliance on forests for wood products will continue, there is a global shift toward tree plantations to meet the growing demand for fiber. A major limitation in maximizing the potential growth and yield in these plantations is the lack of advanced breeding programs in trees. Where huge advances were made in agricultural crops during and since the green revolution with inbred and hybrid lines, such genetic tools for advanced yields in forest crops have not been realized.

The shift toward clonal plantations, a lack of advanced breeding programs and recent advances in the identification of single genes controlling major developmental and morphological traits, provide the necessary tools to capture the huge potential that genetic engineering and genomics can have in a relatively undomesticated crop such as forest trees. Fortunately, the application of genetic engineering into forestry is able to rapidly capitalize on advances already commercially successful and proven in agronomic crops. These same traits, such as insect resistance and herbicide resistance, will likely also be the first generation of genetically engineered trees commercialized.

Of greater significance, however, will be the second generation of genetically engineered trees involving the manipulation of biochemical pathways involved in wood and fiber production. Examples of these include engineering for increased cellulose and wood density as well as modification of lignin to improve pulping yield and reduce costs, both economic and environmental. With this large potential has also come concerns over the use of this technology. Many of the concerns, such as the long-term stability (or instability) of transgene expression, have been addressed in annual crops through the selection of lines with stable expression of the transgene and in trees is being addressed in field trials. Other issues such as the spread of the transgene through pollen are being studied and strategies to lessen such spread are actively being pursued by numerous labs world-wide.

In discussing environmental issues regarding perceived risks of genetically engineered trees, it is crucial that sound scientific analysis of the benefits versus the risks of individual traits are addressed. Genetic engineering is not a panacea that is without risks, controversies, or benefits. However in the history of tree breeding, a tool as potentially valuable as genetic engineering for tree improvement has never before existed. As with plantations of non-engineered trees, the management of the plantations will be critical not only for the proper use of this valuable tool to minimize environmental impacts, but also to maximize returns through increased yields.

Genetically modified plants of Nordmanns fir – for the production of Christmas trees

Jens Find, Tissue Culture Laboratory, Botanical Garden, University of Copenhagen,

The recognition of the potential aims for genetically modified plants is rapidly increasing. Most work has been focused on annual crops, but with the development of methods for mass propagation and more recently, transformation of forest trees, the work in this field has intensified for both broad leafed and coniferous species.

In Denmark, the interest for genetically engineered trees is directed, especially, towards species such as Nordmanns fir (Abies nordmanniana), which is used for the production of Christmas trees and ornamental greenery. This production is characterized as 'agroforestry' and includes intensive application of pesticides and herbicides.

Strong legal restrictions on the use of pesticides and herbicides must be expected in the future and genetic engineering, as an alternative method to reduce the use of pesticides and herbicides, is of great interest.

At present, genetic transformation of conifers is only possible in combination with the methods of somatic embryogenesis. Somatic embryogenesis is the production of plants from somatic cells, i.e. cells that normally do not take part in sexual reproduction. Apart from the earliest steps in embryo formation, somatic embryogenesis is characterized by the same developmental stages as zygotic embryogenesis. The use of somatic cultures as a basis for genetic modification of plant material is furthermore advantageous because: 1) it is a fast method for mass propagation of the transformed genotypes, and 2) the transformed cell lines can be cryopreserved (in liquid nitrogen at -196°C) until superior genotypes have been identified by field trials.

At the Tissue Culture Laboratory in the Botanic Garden of Copenhagen, somatic embryogenesis in conifers has been studied since 1987. Lately, the work has focused on Nordmanns fir, and in the last year there has been a break through in the understanding of processes that control embryo development and plant regeneration. Therefore, it has been possible to develop the methods for somatic embryogenesis in Nordmanns fir. Thus, it is now possible to produce large numbers of plantlets from the somatic cultures (Find et al. 2000A, in prep.).

The collaboration with Forest Research, New Zealand has resulted in the production of several transgenic cell lines (Find et al. 2000B, in prep.) in which the reporter gene, uidA (GUS) and the selection gene, NPT II has been incorporated (see Walter et al., 1998, for further information). These cell lines have shown high embryogenic capacity and more than 1000 plants from transformed cell lines are presently being germinated in growth chambers.

Thus, the present results show that it is now possible to introduce new genes into embryogenic cultures of Nordmanns fir, and that large numbers of plants can be produced from these cell lines.

The next step in this work will be to incorporate specific genes that will have practical importance for growers and thereby improve the value of the plants produced.

In plantations of Nordmanns fir, attacks by the aphid Dreyfusia nordmanniana are a major problem. Insecticides are widely used to protect the plants from this aphid (Kirkeby-Thomsen 1998). Generating aphid resistant varieties using conventional breeding methods is a very slow process for forest trees because of their long generation time. Alternative routes such as transformation and cloning are therefore very attractive for generating pest resistant plants.

The lectin (Galanthus nivalis agglutinin) from the snowdrop plant has been shown to have antimetabolic effects on aphid pests (Hilder et al. 1995), and the gene encoding for this lectin has succesfully been incorporated into tissue cultures of potato (S. tuberosum.) (US patent 4870015), tobacco (N. tabacum) and Indian mustard (B. juncea) (Hilder et al. 1995, Goswami et al. 1998).

Transgenic plants with the lectin gene could possibly reduce or eliminate the need for pesticides in cultures of Nordmanns fir and incorporation of this gene into tissue cultures of Nordmanns fir will have a high priority in future projects for the Tissue Culture Laboratory in Copenhagen.

Conifers are not native to the Danish flora, and in the production of Christmas trees, the trees are normally harvested after approximately 10 years of growth – long before they pollinate. Therefore, Nordmanns fir will be very suitable as a case species in order to study population genetics in relation to risk assessment of transgenic trees.

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Genetically modified Nordic conifers Norway spruce and Scots pine

Hely M. Häggman and Tuija S. Aronen
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1. Introduction

The Eurasian boreal forest zone extends from the Atlantic coast of Norway to the Russian pacific coast, a distance of about 9 000 km. Norway spruce (Picea abies (L.) Karst) and Scots pine (Pinus sylvestris L.) are keystone species of forest ecosystems on large areas in the European part of this forest zone as well as in West Siberia. The spruce and pine roundwood is rawmaterial mainly for pulp production but it is also used for sawmilling and for the plywood and veneer industries. Besides their economical impact, boreal forests are important facilities for recreation, collecting of wild berries, edible mushrooms and lichen, hunting, and reindeer husbandry (Sevola, 1998).

In Nordic countries, Norway spruce and Scots pine have been involved in forest tree breeding schemes as major tree species since the beginning of breeding activities in the 1940's. The main target characteristics have been volume growth or wood production and good climatic adaptation. In the Tree Breeding Programmes for Finland and Sweden (Anon., 1989; Danell, 1993), long-term breeding focuses on assuring the genetic variability and good environmental adaptability among the breeding materials. The short-term activities are intended as a response to new needs and challenges in a changing forestry environment including an effective selection of the best individuals, as well as, transfer of genetic gain into practical silviculture.

The relatively short history of trees as breeding targets is also reflected in the achievements of biotechnological research. In most of the existing transformation techniques, the regeneration of transgenic plantlets is based on tissue culture, which in Scots pine is still the stumbling block. Norway spruce, on the other hand, is the model species of somatic embryogenesis. The main transformation techniques applied to Nordic conifers are Agrobacterium-mediated transformation and particle bombardment. Gene transfer methods together with the breeding goals fix the limits of traits, which could be genetically engineered in trees. The most interesting applications of genetic transformation in the breeding of Nordic conifers could be achieved, if the introduction of single gene effects contributing to important traits were possible. Integration of genetic engineering into practice raises several questions. From the breeding point of view, the best targets for gene transfers would be well known mature trees, but existing transformation methods are based on tissue culture systems that are not applicable for mature conifers. Also, an effective utilisation of transgenic trees in silviculture would require their vegetative propagation, as well as public and official acceptance. Other aspects to be considered are maintenance of genetic variation, proper function of transgenes during long rotation times, escape of transgenes into natural populations and their effects in forest ecosystems.

2. Genetic transformation of nordic conifers - the present state-of-art

Transformation techniques applied for Nordic conifers are Agrobacterium-mediated transformation and particle bombardment. Generally, conifers, which are not natural hosts of Agrobacterium, have been more unaccepting of Agrobacterium-mediated transformation even if in

experimental conditions most of the coniferous species are shown to be prone to bacterial infection (Häggman & Aronen, 1996). Thus, in many conifers including Scots pine (Häggman & Aronen, 1998) and Norway spruce (Newton et al., 1992; Robertson et al., 1992; Clapham et al., 1995), particle acceleration has been preferred as a gene delivery method or for use in gene expression studies.

Thus far, the gene constructs generally used in genetic transformation experiments of Nordic conifers have been selectable marker genes and reporter genes, owing to the fact that the transformation protocols are still under development or developed quite recently. The constitutive CaMV 35S promoter has been appropriate in most applications, but higher transient expression data has been achieved in Scots pine (Häggman et al., 1997; Häggman & Aronen, 1998) and in Norway spruce (Newton et al., 1992; Clapham et al., 1995; Häggman et al., 1997) using ABA-inducible promoters or polyubiquitin promoters. The most frequently used reporter gene is uidA encoding for β-glucuronidase, GUS, (Jefferson et al., 1986) and the corresponding selectable marker gene is neomycin phosphotransferase II (npt II), which inactivates kanamycin by phosphorylation.

One important prerequisite for successful gene transfer is an effective and functional tissue culture method. The most appropriate explants for tissue culture and genetic transformation of conifers have turned out to be embryogenic cultures. From the tree breeding point of view, however, the explants from mature trees would be preferred. The list of stably transformed conifers partly reflects the success of in vitro propagation of the species. Somatic embryogenesis has succeeded in several Picea species, Norway spruce being the first conifer regenerated through somatic embryogenesis (Hakman & von Arnold, 1985; Gupta & Durzan, 1986). Of the Pinus species, Pinus radiata represents the species on which several propagation methods are working i.e. grafting, cutting technology, micropropagation using explants from young as well as adult trees and somatic embryogenesis (Smith et al., 1994). All the other pine species, including Scots pine, seem to be more recalcitrant. For Scots pine, there exist tissue culture methods based on organogenesis (Supriyanto & Rohr, 1994; Häggman et al., 1996) and somatic embryogenesis. The factors affecting inoculation, proliferation, maturation and conversion of embryogenic cultures have been studied (Keinonen-Mettälä et al., 1996; Sarjala et al., 1997; Niemi et al., 1998; Häggman et al., 1998; Häggman et al., 1999) and the plantlets growing in the greenhouse have been produced. However, maturation and conversion phases still need improvement.

2.1 Agrobacterium-mediated transformation

The common feature in Agrobacterium-mediated transformation of Scots pine and Norway spruce is that efficiency is relatively low and both host tree genotype, as well as, bacterial strain affect this factor (Smith, 1939; Clapham & Ekberg, 1986; Ellis et al., 1989; Stomp et al., 1990; Bergmann & Stomp, 1992; Aronen & Häggman, 1995). The supervirulent strain A281 has proved to be able to infect several coniferous species including Scots pine (Aronen & Häggman, 1995) and Norway spruce, in which the strain has been particularly effective (Clapham et al., 1990; Hood et al., 1990). In spite of successful gall formation in the experimental conditions, the attempts to produce stably transformed coniferous plantlets has failed in many cases. This might have something to do with the accessibility of cells competent for both integrative transformation and regeneration of plantlets as in cereals (Potrykus, 1991). Our own studies indicate that the inefficiency of Agrobacterium spp. in infecting Scots pine is not the result of insufficient vir-gene induction but rather is the result of interference by chemical defence compounds during the later stages of T-DNA transfer (Aronen, 1997). From the experimental point of view, it is difficult or impossible to affect the later stages of DNA transfer and therefore the explants with or without negligible amounts of secondary compounds would be favoured in Agrobacterium-mediated transformation. This kind of material might be explants derived from juvenile host tissue e.g. mature or immature embryos, such as cotyledons or embryogenic cultures. Quite recently Wenck et al. (1999)

reported the production of genetically transformed Norway spruces using Agrobacterium-mediated transformation of embryogenic cultures.

Wild type strains of Agrobacterium rhizogenes have successfully been applied to larch transformation (Huang et al., 1991). Generally, however, the goal of oncogenic A. rhizogenes-mediated transformation has been root formation in difficult-to-root conifers (i.e. production of chimeric plants with transgenic roots and non-transgenic shoots) rather than introduction of transgenic plantlets (as reviewed by Häggman & Aronen, 1999). In the experiments with Norway spruce and Scots pine cuttings, hairy or highly branched roots formed on 2-3 % of seedlings of both species after inoculation with oncogenic A. rhizogenes and these hairy roots showed weak production of opines, agropine and mannopine (Magnussen et al., 1994). On the other hand, when in Scots pine over 1000 fascicular shoots were inoculated in vivo (Aronen et al., 1996) root formation increased significantly without any evidence of genetic transformation. The same has also been found in Pinus monticola, Pinus banksiana, and Larix laricina (McAfee et al., 1993). The potential significance of A. rhizogenes-mediated transformation in forest regeneration will depend on the efficiency of this system. Other, important, concerns include, the consequences of releasing opine-producing, genetically engineered trees into the environment. Opine-producing dicotyledonary plants can be found in nature but are not usually large cultivated areas as would be the case of forest trees. Oger and co-workers (1997) reported that opine-producing plants are able to alter their biological environment, more precisely the root-associated bacterial populations. Thus, in long-living trees, risk assessments need to be evaluated carefully and performed case by case. Especially, when considering practical applications, interesting possibilities are opened up by the cases in which A. rhizogenes has acted as a stimulative agent instead of transformation agent in root production (McAfee et al., 1993, Aronen et al., 1996).

2.2 Biolistic transformation

Due to host specificity as well as other difficulties with Agrobacterium-mediated transformation in conifers, the attempts have been focused on a biolistic approach. Biolistic approach means acceleration of microprojectiles, most often gold particles coated with DNA, into the plant cells. There exist a couple of devices, homemade as well as commercial ones, used for particle acceleration. The major advantage of biolistic transformation is the large scale of explants that can be targeted by microprojectiles. Biolistic transformation also allows integration of multiple genes by cobombardment i.e. genes carried on separate plasmids are mixed prior to transfer by particle bombardment (Chen et al., 1998). This opportunity will be of great importance in tree transformation in the future because repetitive insertions of single genes are not possible due to time and effort required for recovery of transgenic trees. Also, the demand of using different selectable marker for each new transformation event is avoided in co-bombardment. In the long run this might also have an environmental impact by decreasing the amount of transgenic plants carrying multiple selectable markers. To date, the biolistic approach has been used to study promoter function and transient expression and to develop methods for production of stably transformed cultures and trees.

In conifers including Norway spruce and Scots pine, embryogenic cultures have proved to be the most appropriate targets for particle bombardment. A lot of information dealing with physical, biological and environmental factors affecting transformation success in Norway spruce (Newton et al., 1992; Robertson et al., 1992; Yibrah et al., 1994; Clapham et al., 1995) and Scots pine (Aronen et al., 1994; Aronen et al., 1995; Häggman and Aronen, 1998) has been gained over the years. Genetically transformed somatic embryo plants of Norway spruce have been reported quite recently by Walter et al. (1997) and Clapham & von Arnold (1998) using particle bombardment. Genetically transformed Scots pine plants have not yet been reported but genetic engineering has proved to be already now a powerful research tool. An alternative method for production of transgenic Norway spruce and Scots pine (Martinussen et al., 1994, 1995; Häggman et al., 1997; Aronen et al., 1998) is the use of bombarded pollen in controlled

pollinations. By this means in vitro propagation could be avoided. The research is still going on and, at present, 8900 Scots pine seeds and 20 900 Norway spruce seeds produced by this method have been sown in our greenhouse and the seedlings will be tested in near future.

At a time, when the genetic transformation of Norway spruce has succeeded only quite recently and when Scots pine transgenic plantlets have not yet been produced, the major advantage of the technology lies in basic studies, such as the role and function of genes and their regulative sequences. With increasing knowledge and availability of important traits, molecular breeding approaches i.e. introduction of transgenic trees with specific traits to breeding programs, will become possible.

3. Target traits

Gene transfer methods together with breeding goals fix the limits of traits, which could be genetically engineered in Norway spruce and Scots pine. The capacity of the current transformation methods allows, at most, the transfer of a few genes at the same time. On the other hand, many traits, such as growth, yield and adaptability, which are important for forest tree improvement, are quantitatively inherited. From the genetic engineering point of view, the genetic background of these characteristics is still poorly understood, even though the relationships between different yield components have been studied extensively (Mikola, 1985; Velling, 1988; Albrektson et al., 1995; Haapanen et al., 1997). Besides the increased volume growth, the characteristics improving timber quality, e.g. fine-branchiness together with perpendicularity, small number, and good natural pruning of branches, are included among the breeding goals (Velling, 1982; Anon., 1989; Karlsson & Rosvall, 1993). The most interesting applications of genetic transformation in the breeding of Nordic conifers could be achieved, if the introduction of single gene effects contributing to these quality components were possible. The reduction of the lignin biosynthesis in the species used for pulping is globally one of the main goals set for tree genetic engineering (Haines, 1994). Lately, the interest in this approach has been raised also in the case of Nordic conifers. From a breeding point of view, genetic modification of the reproductive system of the spruce and pine could also provide some benefits either through induced sterility or early flowering.

3. 1 Crown and stem characteristics

There are natural mutants of both Norway spruce and Scots pine that represent many characteristics, which have been considered ideal in breeding for improved timber quality. For example, a Scots pine genotype E1101, called "Kanerva pine", has a narrow crown, short and thin branches at a perpendicular angle, minimal tapering, abundance of foliage, vigorous stem height growth and a high harvest index. This tree type is assumed to be a result of one single dominant allele. Unfortunately, the ideal characteristics of the E1101 are connected with a tortuosity of the stem, and often also inherited together with it (Mikola, 1985; Pöykkö & Velling, 1993). If the advantageous effects of the "Kanerva allele" could be separated from the crooked stem form, it would be among the most valuable genes to be transferred into breeding material.

Naturally occuring mutants having a specific tree form have also been found in Norway spruce. These trees called pendula phenotypes have exceptionally narrow crowns with thin branches and strong apical dominance (Pulkkinen & Pöykkö, 1990; Pöykkö & Pulkkinen, 1990). The progeny trials have proved that the pendula characteristics are controlled by a single dominant gene (Lepistö, 1985), parallel to the case of the "Kanerva gene" in Scots pine. While no efforts have been made to isolate the "Kanerva gene", the pendula gene of spruce has recently been mapped by using random amplified polymorphic DNA (RAPD) markers (Lehner et al., 1995). When the pendula gene of Norway spruce is available, it will be extremely interesting to introduce it into spruce and pine breeding material in order to see whether it causes

advantageous effects comparable to the "Kanerva allele", and whether these are accompanied by a tortuous stem or not.

3.2 Lignin biosynthesis

Lignin is a natural phenolic heteropolymer that is associated with polysaccharides in plant cell walls. Its main function is to strengthen cell walls, which is important for trees with extreme needs for both structural support and water transport. Lignin is also important in defence reactions. In conifers, lignin is mainly composed of guaiacyl (G-) units derived from coniferyl alcohol, while in deciduous tree species both G-units and syringyl (S-) units derived from sinapyl alcohol are included. Most of the genes involved in the lignin biosynthetic pathway are known, as well as some of the regulative sequences controlling gene expression in developing xylem tissues (Boudet et al., 1995; Whetten and Sederoff, 1995; Baucher et al., 1998).

Approximately 50% of Scots pine and 40% of Norway spruce roundwood produced in Finland, 11.6 and 10.1 million m^3 in 1997, is used in the pulp industry (Sevola, 1998). The main purpose of wood pulping is to liberate the fibers by removing lignin. In Scots pine and Norway spruce the lignin content of the roundwood is around 27 percent. Following the chemical pulping, the lignin content left in the pulp is about 2-3 percent (Sjöström, 1993). In general, the efficiency of wood pulping is directly proportional to the amount of S units in lignin. The G units of gymnosperms have a C5-aromatic position available for very strong carbon-carbon bonds, which make them fairly resistant to the pulping depolymerization (Boudet & Grima-Pettenati, 1996).

Genetic engineering of lignins should provide both perspectives for the understanding of lignin synthesis, structure and properties, and also economic benefits for utilisation of wood in the pulp industry. Modification of lignin could be achieved either by decreasing the amount of lignin or by changing the chemical composition of lignin. If genetic engineering resulted in a 5-7 percent decrease in the lignin content of wood, it would increase pulp yield approximately from 48 % to 50 %, having a remarkable economic impact. Positive environmental effects due to less need of energy and chemicals are benefits for the whole community. Benefits to be achieved in the industrial scale require, however, the mass multiplication and afforestation of trees with modified lignin. It has been estimated that reductions of 10-15 % in total lignin content should be attainable without a loss of biomass yield (Dean & Eriksson, 1992), but the modifications should be expressed only in xylem in order to avoid problems in the resistance against pests, for example (Whetten & Sederoff, 1991). Otherwise, the lignin content is considered to have minor importance for their adaptability (Ståhl & Ericson, 1991).

3.3 Reproductive system

Strauss and co-workers (1995) have reviewed possibilities of introducing either male or complete sterility into forest trees. The motivation for genetic engineering of sterility raises from concern that introduced transgenes will escape into wild populations. This is particularly true in the case of wind-pollinated Nordic conifers with enormous natural populations. The stimulation of faster wood production has also been mentioned as a potential positive consequence arising from the introduced sterility in trees. The two primary options include the ablation of floral tissues via floral promoter-cytotoxin fusions and the disruption of floral gene expression by various methods of gene suppression. The recent studies of Norway spruce have indicated that at least some genes controlling floral development are homologues of the corresponding angiosperm genes, despite the great differences in the structure of reproductive organs (Tandre et al., 1995, 1998). When the introduction of sterility is examined from the practical point of view,

a prerequisite for it, i.e. an effective vegetative propagation method does exist for Norway spruce, while in Scots pine it is still under development.

Another interesting application of the genetic engineering of tree reproductive systems is introduction of early flowering characteristics into breeding material. Norway spruce and Scots pine start flowering and seed production at the age of 20-30 years (Chalupka & Cecich, 1997) or 8-20 years (Sarvas, 1964), respectively. Grafted individuals of Scots pine in seed orchards and clone collections form megasporangiate strobili from the age of 4-10 years with pollen production starting a few years later (Bhumibhamon, 1978). In Norway spruce, it may take even longer, up to 20 years, before grafts produce considerable amounts of flowers (Ruotsalainen & Nikkanen, 1989). Although, grafting itself can induce flowering in a few following years (Nikkanen, pers. comm.). Breeding of Nordic conifers is also hindered by variation of their flowering abundance between the years, especially in the case of Norway spruce (Sarvas, 1968; Pukkala, 1987). An Arabidopsis-derived LEAFY gene in hybrid aspen has stimulated flowering within several weeks (Weigel & Nilsson, 1995). Homologue genes for LEAFY, as well as several MADS-box genes, and a number of male cone-specific genes that might be useful in the early induction of flowers in conifers have been isolated from radiata pine (Walter et al., 1998). If these transgenes also showed Mendelian segregation in R1 progeny, i.e. half of the progeny of the early flowering-transformants being normal, as observed with marker genes in apples (James et al., 1995), it would enable acceleration of the breeding work and selection without necessity of using transgenic trees in practical silviculture.

3.4 Resistance against pest and pathogens

The introduction of genes conferring resistance against biotic damage agents into the Nordic conifers does not seem relevant at the moment. Even though Norway spruce and Scots pine are hosts for many fungal pathogens and insect species (Rummukainen, 1960; Kurkela, 1990; Stephan, 1991), the damage caused by these agents or mammal herbivores have only a local role in forestry. For example, in Finland the most problematic single damage agents are the fungi Gremmeniella abietina (Ascocalyx abietina) and Cronartium flaccidum (Peridermium pini), but only 1.1 and 0.9 % of the forest land area, respectively, is attacked so seriously that the stand quality is reduced. Altogether, noticeable fungal damage is found on 6.9% of forest area, and damages caused by voles, moose, other vertebrates, and insects occurs on only 1.6% of the forest area. For comparison, wind, snow and other abiotic factors such as frost, drought, moisture, nutrient imbalance, fire, or harvesting, cause damage on 6.1 % of forest area (Sevola, 1998). Moreover, in the Tree Breeding Programmes (Anon., 1989; Karlsson & Rosvall, 1993), susceptible trees are generally discarded when choosing material for breeding or seed production populations. Global warming or climatic change may, of course, facilitate the invasion of both existing or new pests and pathogens, and under such circumstances, re-evaluation of the target traits for genetic engineering might also be necessary.

4. Practical applications

Integration of genetic engineering into forest tree breeding programmes can be performed in different ways. In conifers, juvenile material from selected families could be transformed, and clonal testing could be conducted at the same time as testing for novel trait expression. This approach takes into account the general recalcitrance of older trees for regeneration. This is, at present, applicable only for Norway spruce on which clonal forestry can be carried out on a practical scale.

Other possibilities to integrate transgenic trees into breeding programmes involve their use as parents in controlled crossings, in seed orchards, and in the transformation of pollen. If transgenic trees are

used as parents, they cannot be sterile, and the transgenes will escape to natural populations. Transgenic pollen grains, on the other hand, could be used for producing either fertile or sterile progenies (Haines, 1994). Both in Norway spruce and Scots pine pollen, transient transformation frequencies are high (Häggman et al., 1997), and a method for using bombarded pollen in controlled crossings has been developed (Aronen et al., 1998). So far, however, no transgenic progenies have been obtained. At the moment it seems improbable that this technique could be used directly for producing seedlings for reforestation. The pollen transformation method is, however, also compatible with the vegetative propagation techniques, if the embryos or young seedlings born from the controlled pollinations with transgenic pollen are used as explants.

The specific characteristics of the Nordic conifers should be taken into consideration when planning integration of genetic engineering into breeding programmes. Maintaining large variation and good adaptability would require introduction of transgenes into numerous genotypes. In theory, the easiest way to achieve this goal would be the use of pollen as a target for genetic engineering - not only can the pollen donors be selected among the best elite trees, but also the female parents for the controlled pollinations. Even though all the pollen lots are not equally compatible for transformation (Häggman et al., 1997), the collection, handling and testing of pollen samples from different individuals is a relatively fast and easy task. In other words, there are good possibilities to maintain genetic diversity while introducing novel traits.

The long life span of Norway spruce and Scots pine also adds some specific demands for the genetic engineering approach. In order to be economically justified in practical silviculture, actual benefits of genetically engineered forest trees should exceed the costs of their production. However, the most important question is how the proper function of the introduced transgenes can be guaranteed when trees mature and are exposed to varying environmental conditions during their long rotation? Moreover, this question can not really be answered without first testing transgenic trees for decades. Foreign genes transferred into forest trees would not necessarily have to be expressed throughout the life-span of the tree, but rather during a specific developmental stage or a given time period. Good examples of this sort of transgene are those that confer resistance against herbicides used in tree nurseries. Anyhow, it is probable that unique gene constructs with specific promoters, preferably of tree origin, will be needed for the long-term expression of foreign genes in forest trees (Tzfira et al., 1998; Walter et al., 1998). On the other hand, in forest tree breeding, the back-crossing programmes which are performed in order to minimise undesirable pleitropic effects of the transgenes (Jones & Cassells, 1995; Oud et al., 1995), are not possible owing to the long generation intervals and the inbreeding depression (Williams & Savolainen, 1996).

4.1. Environmental aspects

Whichever transgenes are introduced into Norway spruce or Scots pine, careful consideration as to whether these genes can disperse to the surrounding natural populations has to be appended to the genetic engineering programme. Pollen dispersal is effective (Koski, 1970), probably causing a high rate of transgene flow to natural populations. On the other hand, the potential ecological effects of the transgenes in natural populations depend on the characteristics of these genes. For example, the dispersal of the spruce pendula gene or the pine "Kanerva allele" to the wild populations may not necessarily have any harmful effects. Narrow crowned individuals already exist naturally, and moreover, their flowering abundance and fitness is lower than that of trees with a wide crown and thick branches (Nikkanen & Velling, 1987; Hertel & Kohlstock, 1994). The genes conferring herbicide resistance present an opposite example – under certain conditions they can give a selective advantage over the wild-type plants. In the case of the Nordic conifers, the most potential target traits for genetic engineering, i.e. stem and crown characteristics and lignin biosynthesis, hardly present a big risk in increasing the weediness or invasiveness of the species. If the dispersal of the introduced foreign genes to natural populations is after all considered undesirable,

transgenic trees could also be engineered to carry reproductive sterility. Given the difficulty to assure stability of transgene expression over long rotations, it is, however, unlikely that "risky" transgenic trees would be approved solely on the presumption of permanent sterility (Mullin & Bertrand, 1998).

Besides the escape of introduced genes from transgenic plantations into the surrounding natural populations of the given species, there is also a concern that transgenes could be transferred to related species sexually or to other organisms by non-sexual means. In the case of the Nordic conifers, these risks can be assessed as low because there are only a few natural or artificial hybrids of Scots pine or Norway spruce. The risk that introduced genetic material could be transferred from transgenic trees to another organism by non-sexual means has also been considered irrelevant (Mullin & Bertrand, 1998).

Taking into account the large species-level biodiversity of forest ecosystems, it can be concluded that many non-target species will be exposed to a transgene and its products. Of particular concern are trees with introduced genes conferring resistance against pests and pathogens. The expression of broad-spectrum antimicrobial components may not only suppress pathogens, but it may also affect tree symbionts and microorganisms involved in decomposition and nutrient cycling (Mullin & Bertrand, 1998). A related risk is presented by selectable marker genes. Adding novel traits into forest trees by genetic engineering may modify interactions within the forest ecosystem to some extent. Genetic change may expand the range over which the species is adapted, and may lead to changes in forest management and altered use of trees (Tommerås et al., 1996). Increased fitness of one species can affect ecosystem processes through changes in competitive relationships or species displacement. According to Angle (1994), however, most forest ecosystems are diverse and relatively stable compared with agricultural lands. Therefore, they are less vulnerable to rapid changes in soil life due to the addition of transgenic products.

Long rotation times of forest trees and their role as keystone species do have a special impact on ecosystem interactions within stands containing transgenic trees. In the case of trees with transferred insect resistance, the environmental risk associated with the use of transgenic trees is the counter-evolution of the insects to overcome the introduced resistance. Unlike in annual crops, in forest trees new genotypes may not be substituted as problems develop. The long life span of forest trees should already be taken into account when producing transgenic trees by using multiple genes for resistance, and by inserting transgenes into a wide variety of genotypes to maintain the genetic diversity in the forest (Strauss et al., 1991). The accurate regulation of transgene expression, for example in a wound-inducible manner (Bradshaw et al., 1991), would be needed during the decades of the tree's life cycle. Generally, transgenic defence strategies must consider the many evolutionary responses of both target and non-target organisms, and incorporate management of non-transgenic refugia to reduce the possibility for co-evolution with adverse consequences (Mullin & Bertrand, 1998).

Plantation forestry is often accused of reducing biodiversity at all levels, i.e. at genetic, species, and landscape levels. Reforestation with cloned material is currently not widely used for the Nordic conifers, and it is regulated by law in order to guarantee sustainable silviculture. The principals of the regulations aim at maintaining a safe level of biodiversity by limiting the total amount of plantlets being produced per one genotype and by controlling the number of different genotypes included in commercially available mixtures of clones. According to Mullin and Bertrand (1998), transgenic trees can affect species integrity and biodiversity in two quite opposite ways: They can obviously increase biodiversity by adding new genes into the gene pool of the target species. On the other hand, the increased value of transgenic forest products may encourage expansion of planted forests with all their associated risks and effects on biodiversity.

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Genetically Modified Fruit Trees

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Breeding of perennial fruit trees by traditional means is a longterm undertaking. Conventional breeding by crossing is limited by reduced availability of suitable resistance genes in the genepool. Breeding of woody plants requires long time periods due to the long generation time, the degree of heterozygozity is high and multiple backcrosses are required, to eliminate undesired traits. In many instances, resistance genes against biotic and abiotic factors are not even available in closely related species of cultivated fruit trees, but occur in wild species or non-commercially produced cultivars, which have only a poor fruit quality.

To our mind the greatest challenge for genetic engineering lays in the field of plant protection, i.e. in the insurance of yield by a more direct control of different pathogens.

Molecular biology allows the study of host pathogen interactions, as they happen on a molecular level. This gives scientists, for the first time, the opportunity to understand and use the plants own defense mechanisms. The fact that we slowly learn to understand the biological strategies for plant defense will allow us in the future to chose and transfer the responsible genes and to counteract diseases more efficiently and in an ecologically more sustainable manner than spraying pesticides.

The application of molecular techniques – besides providing a more reliable method for cultivar identification - makes new resistance genes available. It is imaginable, that breeding steps will take less time, if desired traits can be directly introduced into high yielding cultivars, without transferring the entire genetic background, which can contain undesirable traits.

Temperate fruit trees and actual problems with pathogens in fruit production

Many of the important temperate fruit crops including grapes, apples, plums and cherries, in contrast to forest tree species have been vegetatively propagated as cultivar clones for centuries, a factor which has also contributed to the spread of latent pathogens such as viruses. The world`s collection of grapevine varieties, the most important perennial crop worldwide, is estimated to contain about 5-15,000 cultivars (Lopes et al., 1999). However, the number of apple cultivars has drastically decreased from over 7000 described in the last century (Ragan, 1926) to actual supermarket supply of red, green and yellow apples.

Among the leading deciduous fruit crops, apples with an annual production of about 40 million tons rank second to grapes that have an annual production of about 68 million tons. Plums and peaches are ranked 10th with approximately 7 million tons, while cherry production reaches approximately 3 million tons. Strawberry production amounts to 2 million tons and apricot production is 1.5 million tons annually (FAO Yearbook, 1991). Considering their nutritional and dietetic value, fruit crops contribute significantly to an improvement of human nutrition and world food production. Records are not kept on the amount of

apples produced in private home orchards, but this is considerable in many areas of the world, e.g. in the UK about one third of all nursery apple trees are sold to home gardeners.

Current fruit production faces problems with biotic and abiotic stresses during production, harvest and storage. Generally there exists little consciousness of how strongly plant pathogens may interfere with crop production and therefore with human life, e.g. tea production in Ceylon replaced coffee plantations affected by fungal pathogens, the famine in Ireland was caused by Phytophtora rot of potatoes. Even today the production of grapes worldwide suffers from yield losses caused by grapevine viruses spread either by soil- or by airborne vectors and numerous treatments with pesticides are applied against fungi and insects in fruit production.

A particular case is the threat of Plum Pox Virus to apricots, which, being the most susceptible species within the genus Prunus towards this pathogen, are systematically being replaced by more tolerant species and therefore are silently disappearing. To our understanding of sustainability this would be a perfect example of a crop worthwhile to maintain with all efforts, in order to guarantee to future generations the delicious taste of its fruits.

General overview of transformed fruit trees

Traits modifiable in fruit trees by a transgenic approach from an agricultural practice point of view do not differ much from those addressed also in other tree species. It is important however to keep in mind that fruits as final products end in the human consumption chain either as raw or as processed products, and therefore a higher degree of caution is requested, when compared to forest trees transformed with the aim of improved wood and fiber production.

Different traits have been modified in transgenic fruit trees (table 1), which comprise altered processing and storage qualities, and resistance to abiotic and biotic stresses.

It is obvious that the main obstacle for transformation of fruit tree species is the regeneration of transformed plantlets. Although Haberlandt (1902) postulated this totipotency of all plant cells, we definitively ignore in some instances the right triggers to bring it about. Attempts to improve crop plants by genetic engineering techniques will therefore depend very strongly on the availability of reliable protocols for transformation, selection and regeneration. To this purpose different explants with their different developmental patterns are suited in a different manner, the choice of mature vs. juvenile material influencing strongly the conservation of the genotype. We compared the use of leaf discs from some species (apple, plum, grapevine) and somatic embryogenesis (cherry, grapevine); the latter definitively offers the advantage of single cell regeneration and therefore currently appears to be the most promising approach to introduce new genes in woody crop species (da Câmara Machado et al., 1995a).

Table 1: Survey of transgenic fruit trees:

Family / Plant	Transformation	Genes	Explant	Reference
Actinidiaceae *Actinidia deliciosa*	*Agrobacterium* LBA 4404	*rol* A,B,C for improved rooting	leaf discs	Ruggini et al. 1993
Caricaceae *Carica papaya*	Particle bombardment	PRSV cp	Immature zygotic embryos	Fitch et al. 1992
Caricaceae Carica papaya	*Agrobacterium* C58	PRSV cp for virus resistance	Hypocotyl, embryogenic callus	Fitch et al. 1993
Ebenaceae *Diospyros kaki*	*Agrobacterium rhizogenes* A4	*Rol* B and C for improved rooting	Stems of *in vitro* shoots	Tao et al. 1994
Juglandaceae *Juglans regia*	*Agrobacterium* Wag11	*Cry*A for insect resistance	Embryogenic callus from immature cotyledons	Dandekar et al. 1994
Rosaceae *Malus domestica* M 26	*Agrobacterium rhizogenes* A4	*Rol* genes for improved rooting	Stems of *in vitro* shoots	Lambert and Tepfer 1992
Malus domestica M 26	*Agrobacterium* LBA 4404	Attacin E for Fire Blight Resistance	leaves of *in vitro* shoots	Norelli et al. 1994
Malus domestica Royal Gala	*Agrobacterium* LBA 4404	Acetolactase synthase for Herbicide Resistance	leaves of *in vitro* shoots	Yao et al. 1995
Prunus armeniaca Prunus domestica	*Agrobacterium* LBA 4404	PPV cp for virus resistance	Cotyledons of immature embryos	Laimer da Câmara Machado et al. 1992
Prunus domestica	*Agrobacterium* LBA 4404	PPV cp	Hypocotyl slices	Scorza et al. 1994
Prunus domestica Stanley	*Agrobacterium* C58	PRSV cp	Hypocotyl slices	Scorza et al. 1995
Solanaceae *Cyphomandra betacea*	*Agrobacterium* LBA 4404	*als* for herbicide (clorsulfuron) resistance	leaves of *in vitro* shoots	Atkinson and Gardner 1993
Vitaceae Vitis vinifera *Chardonnay*	*Agrobacterium* LBA 4404	GFLV cp for virus resistance	Anther derived embryogenic cultures	Mauro et al. 1995
Vitis berlandieri x V. rupestris *110 Richter*	*Agrobacterium* LBA 4404	GCMV cp for virus resistance	Anther derived embryogenic cultures	Le Gall et al. 1994
Vitis berlandieri x V. rupestris *110 Richter*	*Agrobacterium* LBA 4404	GFLV cp for virus resistance	Hypocotyl and anther derived embryogenic cultures	Krastanova et al. 1995
Vitis vinifera	*Agrobacterium*	Chitinase, Glucanase and RIP for fungal resistance	Embryogenic cultures	Töpfer and Harst 1999
Vitis vinifera *and* Vitis berlandieri x V. rupestris *110 Richter*	Vitis berlandieri x V. rupestris *110 Richter*	Cps of GFLV, ArMV, GVA and GVB for virus resistance	Immature embryos and anther derived embryogenic cultures	Gölles et al. 1998

Pathogen-mediated virus resistance

We have focused our efforts on virus resistance breeding by biotechnology, since there does not exist any chance to control these pathogens by chemical means, and the chemical control of their vector organisms, e.g. aphids, nematodes, etc. appears, ecologically, highly questionable.

In recent years, a viral pathogen became the major threat to stone fruit cultivations in large areas of Central and Southern Europe and other mediterranean countries, being responsible for considerable economic losses and reduction of production areas. Plum Pox Virus (PPV), the causal agent of Sharka disease and a member of the potyvirus family, was classified by US and EC plant quarantine agencies as the most important pathogen in apricots, plums and peaches (CABI/EPPO, 1992) (the only plant pathogen for which an APHIS plan exists (Scorza, 1991). Sharka infection data from Spain, Greece, France and Italy clearly demonstrate the economic consequences of this threat (COST, 1993), as apricot cultures are increasingly being replaced. Apricot appears to be the most sensitive stone fruit towards infection with PPV. Apricot production was practically erased in the late 70s in some valleys of Northern Italy, e.g. Vintschgau, and is seriously threatened in some Austrian valleys, e.g. Wachau (Pieber pers. comm.).

Considering the severity of the disease, the difficulty to control its spread, and the lack of resistant cultivars, the necessity of resistant cultivars is evident and a strait-forward strategy is required. Biotechnology offers completely new approaches through the isolation and transfer of "resistance" genes.

"Cross protection" was originally described as the phenomenon of protection of a plant against the invasion of a severe disease-causing virus due to prior inoculation of the plant with an attenuated virus strain (McKinney, 1929). Hamilton postulated in 1980 that the expression of sequences from the viral genome, if expressed in transgenic plants, could possibly cause a protection against viruses. In fact by the expression of the viral coat protein gene in transgenic plants similar effects could be obtained, and it was therefore distinguished as coat protein mediated protection (Beachy et al., 1990).

Following the pathogen-mediated protection approach, we have isolated the coat protein gene of the stone fruit pathogens PPV (Laimer da Câmara Machado et al.,1992), PNRSV (unpublished) and of 4 grapevine viruses (Gölles et al., 1998; Minafra et al., 1998) and transformed different explants of different woody species.

For the in vivo challenge infection experiments, we chose the green cutting-grafting technique, because a) it is also a natural way of spreading of the virus b) high virus titers were to be applied, and c) further data on virus distribution were to be expected.

Transgenic apricot plants regenerated from cotyledons of immature embryos containing the coat protein gene of PPV (Laimer da Câmara Machado et al., 1992) were acclimatized to greenhouse conditions and used for challenge infection studies.

Different transgenic lines were grafted as green cuttings on heavily infected rootstocks. Healthy, non-transgenic apricot and plum were used as negative control. Visual observation of the outgrowing buds was accompanied by monthly ELISA checks of the newly developed leaves. Tests were repeated during 2 following growing seasons with an intercalated winter rest and can therefore be considered representative for 2 years (da Câmara Machado et al., 1995b).

Repeated ELISA readings were never able to detect the virus in the transgenic scions, as well as visual inspection never detected virus specific symptoms, whereas the infected rootstock was visibly positive for PPV.

For the challenge infection experiments, in vitro, we chose to graft transgenic apricot shoots containing the coat protein gene of PPV (Laimer da Câmara Machado et al., 1992) onto heavily infected shoots of apricot. Healthy, non-transgenic apricot and plum were used as negative control. Visual observation of the outgrowing buds was accompanied by weekly scoring of the newly developed shoots by immuno-tissue-printing by standard methods developed in our lab (Knapp et al., 1995).

The integration of the various viral coat-protein genes as well as of some marker genes into Prunus and Vitis has been demonstrated by PCR and by Southern Blot analyses (Gölles et al., 1998; da Câmara Machado et al., unpublished). The majority of transgenic plants analyzed so far contained only one or a few copies of the transgene, which in terms of further evaluation and segregation seems advantageous.

Future developments

For an appropriate evaluation of the risks and benefits of a genetically modified tree, we consider a trait/construct dependent approach as the most meaningful way. We see a lot of potential in the optimisation of constructs, in a sense that expression of the transgene will be limited in time and space, e g. in a certain tissue during a certain period of development of the plant.

Furthermore to our understanding there exists no contradiction between biological production and the use of certain, obviously not all, GMOs. Hopefully, the restraint of excluding initially available GMOs from biological production will be lifted in a near future, when a certain differentiation will be made. This will avoid excluding unjustifiedly certain crops, that may make an important contribution towards a healthier production of food (Ammann, 1998).

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Agrobacterium -mediated transformation of the commercial apples

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Introduction

Apples are one of the most popular fruit trees and genetic transformation studies (Bondt et al., 1996; Puite and Schaart, 1996) have been often atempted by many resarchers in the view of molecular breeding. However, most major commercial cultivars, including 'Fuji',are recalcitrant to genetic transformation. In order to introduce the useful traits such as disease-resistance into apple plants, we have been studying the establishment of an efficient transformation system. In addition, at present, the biosafety evaluation of transgenic apples is being carried out according to the Science and Technology Agency's of Japan "Guidelines for DNA Gene Reconbination Experiments"(in a closed-greenhouse).

Materials and methods

Plant materials:Malus x domestica Borkh.cv.Fuji, Jonagold
Target tissues:Leaf explants excised from in vitro grown shoots
(ca.one month after multiplication)
Gene delivery method:Agrobacterium -mediated transformation
(A.tumefaciens strains EHA101,LBA4404)
Genes: hpt(hygromycin phosphotransferase)
bar(phosphinothrcin acetyl transferase)
uidA(•-glucuronidase) chi1[chitinase of Rhizopus oligosporus (Yanai et al., 1992)]
Binary vecter:pIG121Hm, pBB-Chi
GUS asay:Histochmical GUS assay
DNA analysis: Southern blot analysis
Volatile-compunds:GC-analysis[SHIMAZU:GC-17A column•J& W Scientific DBWAX(0.25mmX30m)]
Culture media used for apple transformation
Shoot prolification medium
[Murashige &Skoog basal medium, 3%sucrose,1ppm6-benzyladenine(BA), 0.8%Bactoagar]
Regeneration medium
[Nitsch & Nitsch basal medium, 3% sucrose, 0.5ppm indole-3-butyric acid (IBA), 5ppm thidiazuron
(TDZ), 0.8% agar] supplemented* with 500ppm cefotazime and 5 ppm hygromycin (3ppm bialaphos).
*No cefotaxime and hygromycin(bialaphos) was added during coculture with A. tumefaciens.

Biosafety evaluation

The evaluation of transgenic apples is being carried out according to the Science and Technology Agency's of Japan "Guidelines for DNA Gene Reconbination Experiments"(in a closed-greenhouse) (Tabei et al I, 1994; Tabei et al. II, 1994).

Results and discussion

The commercial apples were successfully transformed by Agrobacterium-meditated transformation. Transformation of the apples (Malus x domestoca Borkh. cv. Fuji and Jonagold) was attempted using the bar (phosphinothricin acetyl transferase) and hpt (hygromycin phoshotransferase) genes as the selectable markers. Binary vectors pIG122Hm [encoding an intron-containing•-glu curonidase (uid A) and hygromyc in phosphotransferase (hpt)] and pBB-Chi[encoding bar and chi (chitinase from Rhizopus oligosporus)] were introduced into the A. tumefaciens strains EHA101 and LBA4404, respectively. Leaf sections excised from the in vitro cultures of the apples were cocultivated with the bacteria for 3 to 5 days in the dark, after which they were transferred to the selection medium containing 5 ppm hygromycin(or 3 ppm bialaphos) with 500ppm cefotaxime.

The shoots were excised from the drug-resistant calli ca. 2 months after cocultivation and then proliferated under a light/dark cycle of 16/8 h. Histochemical GUS assay of the shoots surviving on the selection medium showed various intensities of GUS expression, depending on the clones. Southern blot analysis of the drug-resistant shoots confirmed the integration of the transgenes in the plant genome. The shoots transformed with chi1 were grafted on the rootstocks for assessment of disease-resistance. The disease-resistance of the transformants are being experimented and evaluated in a closed greenhouse. There were no differences between transgenic and non-transgenic apple on the evaluation of harmful volatile-compunds using Gas Chromatography(GC)analysis.

In our preliminary experiments, no transgenic apples were produced on the kanamycin selection using the nptII gene as the selectable marker that had been used in apples transformation (Bondt et al., 1996; James et al., 1989; James et al., 1996; Puite and Schaart, 1996; Yao et al., 1995). Since kanamaycine was also reported to inhibit shoot regeneretion from leaf explants of apples (Yepes and Aldwinckle, 1994), we used bialaphos and hygromyscin as the selectable reagents in this study. Thus, using the bar or hpt gene, we succeeded in producing transgenic apples (cv. Fuji and Jonagold). In particular, 'Fuji' is the most important cultivars that has a 50% of the commercial market in Japan, but no transgenics had been obtained because of its difficulty of culture. The transformation procedure shown in this study that uses other than the nptII gene as the selectable marker will also be applied to several other commercially significant apple cultivars.

We are now studying the expression of introduced chtinase gene (chi1), which was reported to give resistance to some filamentous fungi in transgenic tobacco plants (Terakawa et al., 1997). Transgenic apples are now being proliferated and grafted on the rootstocks to evaluate resistance to the filamentous fungi. At present, the biosafety evaluation of the transgenic apples are being carried out according to the Science and Technology Agency's of Japan "Guidelines for DNA Gene Recombination Experiments"(in a closed greenhouse) for assessment of influence to an enviroment.

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Summary

1) Apples of commercial cultivars 'Fuji' and 'Jonagold' were successfuly transformed by Agrobacterium-mediated transformation.

2) Both the bar and the hpt genes can be used as the selectable marker genes in apple transformation.

3) The foreign gene (uidA) was observed to express in the shoots of transgenics that had been transformed with pIG121Hm.

4) Integration of the foreign DNA into the plant genome was confirmed by Southern blot analysis in the primary transformants.

5) The Fungi-derived chitinase gene (chi1) was successfuly transformed into two commercial apple cultivars.

6) There were no differences between transgenic and non-transgenic apples on the evaluation of harmful volatile-compunds using Gas Chromatography (GC) analysis.

7) The biosafety evaluation of the transgenic apples are being carried out according to the Science and Technology Agency's of Japan "Guidelines for DNA Gene Reconbination Experiments" (in a closed-greenhouse) for assessment of influence to an enviroment.

Genetically Modified Poplar

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Lessons from the IUFRO position statement on transgenic forest plantations

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This year the International Union of Forest Research Organizations (IUFRO) Working Party on Molecular Biology of Forest Trees issued a position statement about the benefits and risks of transgenic plantations. The motivation for this statement was the perception that the public debate about transgenic crops has been dominated by misrepresentation of scientific facts and perspectives, and therefore that an early contribution from molecular forest biologists would help to inform the debate as it unfolds within forestry.

The text of the position statement, including the results of the vote, are presented below and can also be found at: http://www.fsl.orst.edu/tgerc/iufro_pos-statm.htm.

Below I highlight several points from the position statement and related issues raised at the OECD meeting.

Transgenics will be used in intensive plantations, not wild or extensively managed forests. Because of their multiple uses, extensive management, and long rotations, most of the world's forests and plantations are unlikely to be suited for genetic engineering for the foreseeable future. The use of transgenic plantations is under consideration only for intensively grown plantations for which wood products are the dominant motivation for establishment and management. Many of these plantations are effectively "tree farms." Regulations need to be formulated with these intensive systems in mind.

Tree plantations are a valuable and growing source for renewable, environmentally sound products but require increased production per unit area to meet growing world demand. From environmental and economic viewpoints, forest plantations are a preferred source for energy, fiber, chemical, and solid wood products compared to wild or extensively managed forests. They are also more energy efficient and less polluting compared to alternative products such as metals for building materials and fossil fuels. Due to the increasing need for land for wildlife preservation, food production, and human habitation, it will be essential to produce more wood per unit area in the future. Biotechnology has already demonstrated the ability to generate new crop varieties that produce more and higher quality product per acre with less input of undesirable pesticides, and can clearly make similar contributions to plantation forestry.

There is a high potential for genetic innovation via genomics and biotechnology. The new tools of genomics will provide a continuous stream of genetic advances about important traits such as pest resistance, yield, wood quality, adaptability, and flowering. The most efficient means for using this

information to increase productivity in an environmentally sound manner is via transgenics, which allows specific traits to be manipulated in a rapid manner. This will allow the effective domestication of trees to increase plantation productivity, a process that would require hundreds to thousands of years via traditional breeding. If burdensome national and international regulations make the use of transgenic plantations costly and politically contentious, it could effectively foreclose this great source of scientific progress for plantation management.

Transgenic traits can be highly stable. Many research scientists have confused the instability of gene expression and the incidence of genetic mutations due to transformation (somaclonal variation) that are routinely observed in basic research with concerns for instability in commercial products. During breeding approximately 99% of transgenics are removed as part of normal screening during years of testing. Experience to date with our several years of field trials of transgenic poplars has shown that virtually all transgenic trees are highly stable in expression of their transgenic traits. Indeed, the growth of transgenic crops on millions of acres, and their extremely rapid adoption by farmers in the US, Canada, and Argentina over the last several years, demonstrates the reliability of transgenic traits on large spatial and temporal scales. Among the traits adopted commercially is floral sterility, demonstrating that it is an achievable option for transgene containment in forestry.

Testing requirements should be based on the traits imparted, not the method of introduction. Both conventional tree breeding and genetic engineering pose risks and uncertainties. Conventional breeding for yield and wood quality can result in substantial changes to important adaptive traits such as shoot phenology and stem strength, whose long-term ecological effects are difficult to predict--particularly with the expectation of significant climate change. In contrast, many transgenes in use today seek to add novel traits while maintaining basic adaptive and growth characteristics intact. For this reason, imposing longer requirements for testing of all transgenic trees than for products of conventional breeding is not scientifically defensible; in fact, shorter testing periods are likely to be warranted for simple traits like herbicide and insect resistance. However, for transgenic traits that may significantly affect adaptation (e.g., engineered wood quality), or traits that are important to biosafety (e.g., floral sterility), a longer testing period, or monitoring during early stages of commercial use, would be desirable compared to gain-of-function transgenics. However, in many cases their risks will still be comparable or less than those posed by traditional breeding or use of exotic tree species, depending on the populations, species, breeding method, and environments under consideration. A flexible, case-specific testing system that does not discriminate against transgenic trees is scientifically warranted.

The potential for significantly enhancing tree fitness via most transgenes is low. By far the largest concern over use of pest-resistant transgenic crops under development is not that they will invade wild systems, but that the genes are themselves insufficiently strong to have sustained value in the face of continuing pest evolution. Many of these traits may not even be of benefit outside of agricultural and plantation systems because of their expected pleoiotropic effects (e.g., due to overexpression of pest resistance proteins). The weak concern over serious ecological effects of pest resistance transgenes for wild systems was strongly agreed upon at a recent multidisciplinary workshop that covered several crops, including poplars (Traynor and Westwood 1999: http://www.isb.vt.edu/). The transgenes in use today, with their limited number of physiological mechanisms and simple regulation compared to native pest resistance transgenes, are unlikely to cause serious ecological impacts outside of managed systems. Traits such as modified wood, sterility, and herbicide resistance are likely to either reduce fitness or be of concern only within managed systems—where multiple mitigation/management options exist to deal with unwanted effects and slow pest evolution. Although it will be prudent to monitor ecological effects from transgenic plantations to verify these expectations, for the foreseeable future they do not deserve to be treated as sources of significant ecological hazard.

Ecological impacts of transgenes within plantations are expected to be small compared to the ecological effects of the diverse forms of forest management commonly practiced. Within managed systems, the ecological effects of genetic engineering are likely to be dwarfed by variations in routine forest management. For example, changes to nutrient cycling and herbivore populations caused by genetically modified wood quality are likely to be extremely small compared to variations in site preparation, plantation density, harvesting frequency, and choice of plantation species.

Concluding thoughts

Transgenics raise not just scientific questions but a number of cultural and social issues. Rights to use the technology are dominated by multinational corporations, whose citizenship and regard for the environment are viewed with suspicion by many citizens. Environmental groups have seized upon biotechnology as a tool for mobilizing the public not just against genes, but against the methods and agrochemicals that characterize intensive agriculture and forestry. "Genetic engineering" or "GMOs" simply does not sound pretty; it is therefore easily associated with the ugly side of technology by its opponents at a time when the world is demanding greener industries and cleaner forestry practices. And although very few in the academic biotechnology community have serious scientific concerns, few are also speaking out in favor of it as part of the social debate. I suspect that this is not only because the issues are complex and hard to communicate, but because they also feel victimized by the strict corporate dominance of the use of research results.

I have tried to argue that the benefits from biotechnology can be great, and that scientific risks from transgenics are largely comparable in magnitude to what we take for granted in forest management. Thus, scientific concerns alone do not dictate a very strict, cautious approach to the technology. However, until the socioeconomic factors that fuel the world debate resolve themselves, the prospects for wide use of this technology in forest plantations are likely to remain dim.

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Genetic variation in Norway spruce populations

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Norway spruce (Picea abies (L.) Karst.) has a wide natural distribution covering 31 degrees of latitude, 150 degrees of longitude and from sea level up to 2300 m in altitude. During the last ice age the species survived in Europe in two refugial areas from where it migrated to its present distribution. The origins and migration history have important implications for the patterns of genetic variability observed among and within populations across Europe. Other factors influencing the genetic variability in spruce are natural selection, gene flow and phenotypic plasticity.

Estimates of genetic distance from isozyme studies indicate that small differences exist among populations, even if some clinal variation has been found among populations at the single locus level. For metric traits: growth, growth rhythm, survival and quality characters, a much larger differentiation among populations has been found than for the isozyme marker genes. The variability patterns observed in common garden experiments are clinal, following latitudinal or altitudinal clines. These patterns are in particular strongly expressed for traits characterising the annual growth rhythm: the dehardening in spring, the timing of bud flushing and cessation of growth, and the development of frost hardiness in the autumn. Seedlings from northern and high altitude populations generally have an early growth start and growth cessation and develop frost hardiness earlier than those from more southern and low altitude populations. This variation has been assumed to be an adaptive response to selection for viability to avoid frost exposure. The growth potential of populations is strongly related to the duration of the growth period.

Within Norway spruce populations a substantial variability exists for the same traits that vary at the population level, even in populations at the climatic margin of the species. Such variation is also present in resistance to fungi and insects. Results from experiments with controlled cross families have shown that the genetic variation in the metric traits chiefly is additive. The genetic relationships between traits calculated for families within the same population are different from those found at the provenance level. Families can be found which have a late growth cessation and still have an early development of autumn frost hardiness.

Recently, evidence has been accumulating that the climatic adaptive process in Norway spruce may be less clear-cut than earlier assumed. Several independent tests have shown that the climate and weather conditions during sexual reproduction influence the adaptive properties of the progenies. The phenomenon is expressed in spruce seed orchards established by moving parent trees, propagated as grafts, from north to south, from high to low elevation, or from outdoor to indoor greenhouse conditions. The progenies exhibit a changed phenology and a delayed development of frost hardiness in the autumn compared to their siblings reproduced in a colder environment. Observations on seedlings from seeds harvested in northern stands of provenances transferred from south to north show that they had adapted their photoperiod towards the response of the local provenance. The most likely explanation for these phenomena is the existence of a regulatory mechanism, sensing the changes in temperature and/or photoperiod at some stage during the reproductive process, and then modifying the expression of genes controlling these adaptive traits in the progeny.

Molecular genetic studies have recently been initiated in Norway spruce with the aim to identify molecular markers associated with important traits, in particular wood properties and resistance to fungi.

Genetic variation and hybridization in black poplar (Populus nigra L.) populations

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Introduction

European Black poplar (Populus nigra L.) is a typical pioneer species of riverine areas. It forms a characteristic part of floodplain forests, which are considered to be important to the natural regulation of flooding and water quality. Apart from its economic value in providing wood pulp and timber, it also plays an important role in poplar breeding programmes (Frison et al., 1995).

Although once widespread, it is now under threat of extinction in many parts of Europe. This is mainly due to the disappearance of habitat by regularisation of rivers and the conversion of floodplains into agricultural use. Another threat is formed by the widespread planting of clones of P. X euramericana (also known as P. X canadensis), a hybrid of the American Eastern cottonwood (P. deltoides) and Black poplar. In addition to occupation of growth sites of P. nigra, backcrossing of the hybrid clones to wild P. nigra could lead to introgression of P. deltoides germplasm into P. nigra, leading to erosion of the original wild gene pool (Frison et al., 1995).

In recent times, a start was made to partly restore the natural ecosystem along the river Rhine and its branches in the Netherlands. Re-establishment of the Black poplar as a characteristic member of the natural riparian forest is an important element in this restoration. However, few populations have remained that could act as sources for recolonization (Arens et al., 1998). Therefore, research has been initiated on the genetic diversity of these populations in the Netherlands. These results will have to be compared to the situation in other river systems throughout Europe to establish to what extent the Dutch situation may deviate from the natural one.

With regard to the present issue of the potential environmental effects with the introduction of genetically modified trees, it is most relevant to establish the contribution that molecular marker systems can make to the study of hybridization and introgression in wild P. nigra. To evaluate the effects of hybridization, it is also necessary to know the present state of the genetic diversity in wild P. nigra and how it is partitioned across Europe. P. nigra could even serve as a species model to evaluate the risks of introgression of transgenes from cultivated material, since it is already under the threat of introgression by P. deltoides genes through backcrossing to P. X euramericana, as described above. In the following, the present state of our research on P. nigra is discussed in the light of these issues.

Black poplar in the Netherlands

Arens et al. (1998) made a first inventory of the genetic diversity of P. nigra remaining in the Netherlands, using the molecular-genetic marker technology of AFLP (Vos et al., 1995). This method allows an extensive sampling of the whole genome in an efficient way. It involves scoring of the presence

or absence of specific genomic fragments (this is called a dominant marker). They focussed on two populations along the river Rhine that consisted of mature flowering trees and thus could serve as source populations for re-establishment of P. nigra downstream. It was shown that they comprised a very limited number of genotypes, which had produced a large number of ramets through local vegetative propagation. This vegetative propagation appeared to occur only locally, as young trees downstream were apparently the products of generative propagation. The genetic variability among the young trees was shown to be within the range of the mature trees. Generative recolonization is feasible, since P. nigra seeds show a good retention of germinability compared to members of the Salicaceae in general. The seeds are capable of germination in the water and survive under these conditions for about two weeks (Van Splunder et al., 1995).

Black poplar in Europe

For assessing whether the state of the genetic variation in the Netherlands suffices to re-establish a metapopulation with sustainable levels of genetic diversity, it is necessary to compare the situation to other, more natural river systems elsewhere in Europe. A study by Legionnet et al. (1997) using DNA as well as isozyme markers indicates that vegetative propagation is relatively rare in natural stands of P. nigra in France. The Netherlands being at the northern border of the distribution area, these differences in reproduction strategy could be related to environmental circumstances, since seedlings and root suckers show different ecological preferences. However, it may also indicate that the low genetic diversity in the Dutch populations resulted from human interference.

At the moment, more river systems are under study in the EU project EUROPOP. In addition to the AFLP technique, a codominant marker technology, based on microsatellites, was developed for P. nigra. Since microsatellites in principle enable scoring of both alleles in heterozygotes, they extend the possibilities of population genetic analysis, and allow establishing parent-offspring relationships and thus gene flow along the river systems. Microsatellite markers were developed according to the method described in Van de Wiel et al. (1999). Nine primer pairs showed sufficient quality for scoring alleles unequivocally (Van der Schoot et al., submitted), and they were used to assess the genetic diversity present in a set of 23 P. nigra genotypes from the Core Collection of the EUFORGEN network. The Core Collection aims to represent a very diverse set of plants from all over Europe (Turok et al., 1998). In these 23 individuals, the nine microsatellites amplified between 10 and 19 alleles each. Obviously, it was no problem to identify each individual with its own unique pattern. Using these loci, the plants showed a high level of heterozygosity (average 0.71, ranging from 0.50 to 1.00 with only a few exceptions). This indicates that, in general, these plants come from populations, which have suffered no loss of heterozygosity.

Hybrid poplars

The extent of the threat to the diversity of P. nigra's gene pool posed by the possibility of hybridization to the introduced clones of P. X euramericana has been difficult to assess by traditional methods, because poplars in general show a large intraspecific morphological variability. Therefore, hybrids are difficult to distinguish from pure P. nigra plants in the field. Molecular markers could be a method of choice for this distinction. Thus, Faivre-Rampant et al. (1992) showed that variation in the region of the ribosomal RNA genes could be used to distinguish between P. deltoides and P. nigra. Heinze (1997) showed the same for one locus analyzed with a PCR-based sequence-tagged site (STS) approach. Both markers appeared to be useful for tracing introgression. For obtaining a more complete picture of the occurrence of introgression, more loci spread across the genome will have to be studied. Arens et al. (1998) showed that the multilocus AFLP method could be useful in this regard. Out of 319 bands scored

with six AFLP primer combinations, 111 appeared to be specific for P. deltoides and P. X euramericana clones. Analysis of the AFLP data on field populations by PCR showed a number of trees grouping with P. deltoides and P. X euramericana. Some of these trees had initially been identified as non-P. nigra on morphological characteristics, others were found to have aberrant morphology upon second inspection. A few trees were identified as non-P. nigra by AFLP that could not have been recognized as non-P. nigra on morphological characteristics alone.

Conclusions

The two molecular marker systems discussed above have their own possibilities and limitations. The microsatellite markers developed will be useful for determining the level of diversity in the Dutch trees in comparison to other populations in Europe. From this, conclusions can be drawn with regard to genetic management of the species in The Netherlands. The high degree of variation in microsatellites implies that there is a chance that alleles that have arisen independently in different species, nevertheless are identical in size and thus cannot be distinguished. This renders microsatellites less useful as species-specific markers and thus as markers for introgression from one species to another. On the other hand, the large number of loci in the AFLP patterns that are different between P. nigra and P. deltoides imply that AFLP markers can be used as indicators of introgression.

A more extensive study will be performed to corroborate this, including larger numbers of P. X euramericana genotypes. A good basis for this study is the fact that only a limited number of well-known hybrid P. X euramericana clones (genotypes) have been planted at a large scale in Europe. Therefore, there is only a limited amount of genetic material that needs to be surveyed to establish the gene pool of the hybrid before the actual measurement of the degree of introgression from this gene pool into P. nigra can take place. Such a study, which establishes the actual degree of introgression that has occurred in the past, will contribute directly to estimating ecological risks of introducing transgenic poplars.

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Recent Trends in Forest Management - The Plantation Experience

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Abstract

Despite the natural emergence of the service sector in most mature economies timber harvesting remains a significant global contributor, both in economic and environmental terms. The total annual timber harvest is about 3.36 billion m^3 and the value of forest product trade is US$135 billion. Fuel wood constitutes 55% and industrial roundwood the remainder of the total harvest. Demand for timber and forest products is directly related to population and income levels. In the absence of price and product substitution effects, the annual incremental demand for timber is estimated at 57 million m^3. From an environmental perspective, timber production, when managed in an ecologically sensitive manner, is inherently renewable and timber affords an advantageous environmental impact compared to competing construction materials. Life cycle analysis illustrates the relative benefits in terms of energy use, water use and greenhouse gas emissions in wood products.

Clearly, the forest science challenge is to meet the demand for increased forest products with concomitant demands for social and environmental objectives. One constructive option is proving to be the development of timber plantations. These range from minimum-management stands of native species to genetically selected stands of exotics species. Currently about 8% of the industrial roundwood harvest is sourced from rapid-growth plantations (Wilson, et al., 1998; Jaakko Poyry, 1994). The bulk of the remaining industrial harvest is from second or third generation stands.

This paper discusses the contribution of a selection of parameters in the expansion of plantation timber production. The parameters include the increased pressure on land use, social concerns about forestland management, the financial realities of investing in timber production, genetic selection and engineering in timber production, and the substitution effects of manufacturing technology.

Introduction

Despite the dominance of the service sector in the developed economies, the forest sector remains a significant contributor to the global economy. Less apparent, although perhaps more significant, is the contribution of the forest sector to meeting global social and environmental objectives. A key aspect to this contribution is that forests managed in a sustainable manner (allowing for multiple objectives and risk factors) to produce a flow of benefits rather than a stock. The challenge is to manage this flow in response to product demands, the physical characteristics of a forest, the dynamic nature of social expectations, and the inertia or drag seemingly standard to any institutional setting.

For example, forest product demand is basically a function of population and income levels. Given current population and consumption rates the global demand for timber increases about 57 million m^3 per annum. Rather than ascribing to a Malthusian or Limits to Growth outcome, this determination is employed merely to illustrate the magnitude of the pressure on global timber stocks. The actual demand

for timber products will quite predictably include technological gains and both price and substitution effects, all of which serve to bring a balance between supply and demand.

With respect to global timber supply, the main determinants are product prices, the access to and cost of timber in established production regions, Russian production levels (see Figure 1 for a summary of global timber stocks by region), and the rate of plantation investment (Wilson, 1997). This paper will focus on the contribution of fast-growth plantation forests to meeting forest product demand and will discuss a selection of implications related to plantation forestry.
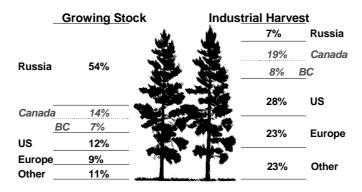

Figure 1: World Softwood Growing Stock and Harvest
Source: Wilson, 1997.

Background

Forestry is a major global industry with annual trade flows valued at about US$135 billion. The FAO estimates that the annual global timber harvest totals 3.36 billion cubic metres (m^3); 55% of this harvest is fuelwood and 45% is industrial roundwood. Forestry is a key component to the economic performance of many economically developed jurisdictions. Figure 2 illustrates the relative contribution of natural resources trade to the economic activity in a selection of jurisdictions. Note that those jurisdictions with the highest dependence on natural resource exports are also major forest product exporters. For the most part these jurisdictions are working with second or third rotation forests although some, such as British Columbia, are working in the natural endowment forest.
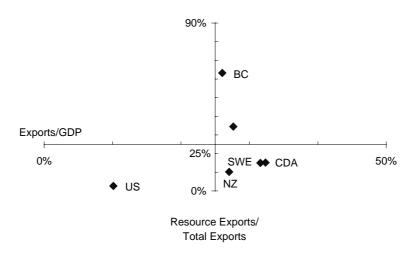

Figure 2: A Measure of the Economic Contribution of Forestry (Source: Wilson et al., 1998)

In addition, to the economic contribution of forests, there is an equally important but a more subtle environmental contribution. Wood is readily utilized, has good structural properties, provides a broad range of products, is renewable, recyclable and wood is relatively environmentally friendly. In comparison with alternative products wood affords a more positive environmental legacy. Specifically, wood is less energy demanding than steel, concrete and brick building materials; wood framing requires much less water than steel framing; and wood framing contributes less greenhouse gas than steel framing (see Figure 3). The objective is to capture these benefits in sustainably managed forests.
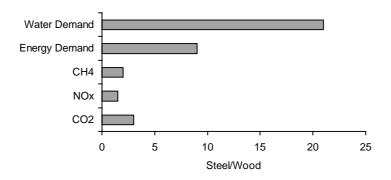
Figure 3: Environmental Legacy of Wood Versus Steel Framing
Note – Methane, nitrous oxides and carbon dioxide are greenhouse gas emissions.
Sources: Canadian Wood Council, 1997, 1995; Koch, 1992; and Timber Trades Journal, 1991.

Issues in New Forestry

There is increased pressure on global forest resources to meet conflicting consumptive and non-consumptive values. This has, in part, led to the current "new forestry" paradigm. The evolution of commercial forestry typically starts with timber maximization in the primary forests, moves to sustained yield, then to integrated resource management, and to the current modus operandi of ecosystem management. Under ecosystem management, the forests are managed for much more than timber production and the extensive margin for operation is reduced. Both of these restrict timber supply and can serve to promote more intensive timber management regimes on commercially operable forestlands. In combination with technological advances and globalization of capital flows, this trend to intensive management has encouraged public sector investment and more recently private sector investment in plantation forestry.

Increased restrictions on the operable area of commercial forestry are a product of, among other things: the increased interest in non-timber values; the high option-value assigned to wilderness by well-educated and affluent voters/consumer; and the emergence of institutional prescriptions. These prescriptions are in the form of regulations, codes and the redefinition of property rights. The redefinition of property rights includes the imposition of public values on fee-simple property, for example, the use of legislated provisions designed for wildlife habitat on private lands.

The issue is one of meeting increased global demands for forest products despite concomitant demands for reduced environmental impact from and reduced operable area for commercial forestry.

The Contribution of Plantation Forests

Plantations are an important part of the current global forestry equation but for the most part these are second and third rotation forests often with minimal management. Fast- growth plantations[1] constitute about 8-10% of the timber harvest. Given the existing plantation inventory and the expected harvest access to non-plantation forests this contribution is expected to double by 2020. Table 1 provides a summary of total area, by hardwood and softwood species, in fast-growth plantation and the planned use of this timber. The geographic distribution of fast-growth plantations reflects the high relative timber yields in the Southern Hemisphere. The growth rates for radiata pine and eucalyptus varieties, which are the dominant species in fast-growth plantations, are in the 20-25 m^3 per hectare per year versus 3-5 m^3 per hectare per year for NorScan production. Figure 4 provides a summary of the mean annual increments reported for a selection of major timber producing jurisdictions.

Table 1: Plantation Area and Planned Use (millions of hectares)

	Softwood	Hardwood	Total
Total Area	7.65	5.95	13.60
Pulplogs	3.05	5.95	9.00
Sawlogs	4.60	--	4.65
Appearance grade	0.90	--	0.90

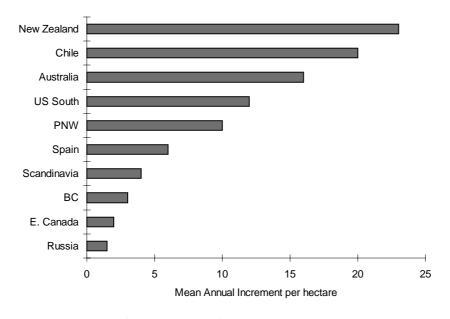Source: National reports, forest industry association reports, and FAO website.

Figure 4: A Comparison of Growth Rates for Selected Regions (Source: Wilson et al., 1998.)

In addition to the social pressure for intensive forestry, there are significant financial drivers. The financial drivers include the capital intensive character of commercial timber production, competing pressure on public funds (limiting funding for public forestry expenditures), corporate divestment, and the interest in plantations from mutual and pension funds. These funds are increasingly challenged to find acceptable returns in a market awash in funds, limited alternatives and high investor expectations. Fast-

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[1] Fast-growth plantations are defined as producing at least 12 m^3 per hectare per year.

growth plantation forestry can provide an attractive alternative for private sector investment. As with all investment the details are what determines the outcome but Figure 5 provides a modest comparison of the net present value of investing in timber production in regions with various timber growth rates.
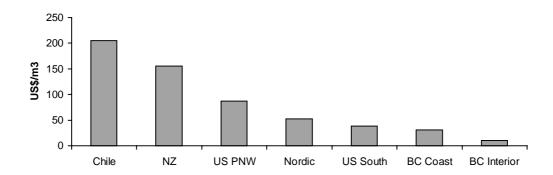

Figure 5: Returns on Timber Invested (Source: Wilson et al., 1998)
Note: Return estimated is the net present value of US$100 invested in intensive management with an 8% discount rate.

Genetics and Plantations

The main objective in fast-growth plantation genetics has been the selection for improved growth rates and this effort, pursued through selection propagation techniques, has successfully produced major gains. The pursuit of growth gains continues but there are ongoing efforts to complement these gains with improved disease resistance and timber structure. These research efforts now include the use of advanced genetic modification techniques (see Hu et al., 1999).

The angst related to plantation genetics is largely from two sources. The first is the fundamental concern surrounding the introduction of exotic timber species and the consequent displacement of indigenous species. Various environmental groups strongly espouse their opposition to any expansion of fast-growth plantation forestry with exotic species.[2]

The second concern about plantation genetics is the potential for genetic drift or horizontal gene flow. This is the possible transfer of modified traits to native stands or to other organisms within the plantation. The uncertainty around such transfers and the possible consequences for ecosystem balance have been raised as a source of concern. Any strategies for the employment of genetically modified trees in plantations must address these issues.

Complementary Conditions for Plantations

It is clear that there are substantial benefits, both economic and environmental, to be captured from improvements in global commercial forestry. It is also clear that established pressures will continue to promote the contribution of fast-growth plantations in realizing these benefits. There are a number of conditions which serve to complement the trend toward an increase in plantation area and improvements in the management of these plantations. There is a need to quantify the net benefits of the contribution and potential of plantations in meeting global needs. In addition, the economic benefits arising from plantations must be more equitably distributed. It will prove problematic if the displacement of indigenous

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[2] Some of these same groups are similarly opposed to commercial forestry in indigenous forests.

peoples is typical of plantation and equally problematic if the gains of genetic modification accrue disproportionately to the holders of the "rights" to this technology (Economist, 1999). A balance in the distribution will encourage stakeholders to be supportive of the genetic techniques and the resulting products.

There are a number of research questions related to plantation genetics which need to be examined. These include information on release risk in terms of the ecological impact of any resulting change in competitive advantage; the long term physical and economic performance of the modified timber; the risk of horizontal transfer or genetic drift; and the risk of collateral impacts on the ecological balance.

The current international institutional setting is not supportive of genetic modification (Globerman and Vertinsky, 1995). Witness the international setting for cloning and genetically modified foods. It is important that the potential contributions (both economic and environmental) of plantations to meeting social needs be considered in context with a renewal of the international institutions which define the options for plantations. Institutional factors such as market access, the mobility of investment funds, the protection of intellectual property rights, and the detail of international research protocols are critical ingredients to the evolution of genetic engineering.

Conclusions

Forestry is a significant source of global economic activity and the relatively benign environmental legacy of products from sustainable forestry make them valuable contributors to meeting expanding global needs. These needs reflect a moving balance among economic, environmental and social expectations. Given the increased restrictions on harvesting, the contribution of plantation forestry to supplying global timber will increase. The research incentives to improve plantation growth rates and timber characteristics will continue to promote investment in genetic modification of the plantation species.

Advancement in genetic modification of timber will advance from an improved distribution of benefits, an informed basis on which to assess public support of such modifications, and a balanced setting among the international institutions which strive to regulate research and commerce. A complementary research agenda will need to include risk assessments on the impact of release, species performance over time, inter-species migration of genetic alterations, and any consequent impact on the ecosystem.

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Indigenous trees used in forestry

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Introduction

Swedish forestry is dominated by two indigenous conifer species: Scots pine (Pinus sylvestris) and Norway spruce (Picea abies). Even though they are only two examples, they serve well as a general illustration of how important indigenous forest tree species are used in forestry. Both species are used for timber and pulp but pine is a more important timber tree and spruce is more important for pulp.

Reforestation and genetic aspects

The reforestation practices differ between pine and spruce. About half of the pine reforestation is done by natural regeneration while spruce is reforestated by planting on almost all areas (Anon., 1998). Improved material is used on most of the pine plantations. For spruce, approximately 25% of the plants are improved due to shortage of such seed. Spruce reforestation material is recommended to be transferred 3-4 latitudes northwards, which means that forestry in southern Sweden imports spruce seed. The best performing spruce provenances come from Belarus, Baltic Countries and Poland. The rather long silvicultural history in Sweden has resulted in many provenance transfers during the 20th century, also for pine, and a considerable genetic variation could be expected in the pine and spruce forests of today.
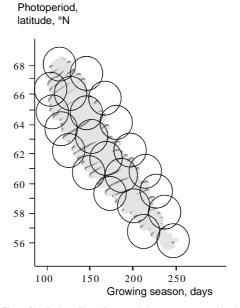

Fig. 1. Distribution of breeding populations on target combinations
of photoperiod climate and length of growing season.

Breeding

Both species are subjected to breeding activities. The Swedish breeding programmes aim at combining high genetic gains with maintained genetic variation (Danell, 1993; Wilhelmsson & Andersson, 1993; Karlsson and Rosvall, 1993). This is accomplished by a breeding strategy where the breeding population is divided into smaller sub-populations (Fig. 1). Each sub-population has a target combination of photoperiod and length of growing season. Within each sub-population, selection in one breeding generation is restricted to provide equal contribution from the parents in the previous generation as shown for pine in Fig. 2. Recent simulation studies have shown that both gains and diversity can be maintained at acceptable levels for 10 generations of breeding with this strategy (Rosvall, 1998).

Scots pine breeding

Crossing
50 parents

2 families/parent

50 full-sib families
5000 progenies

screening in
nursery

500 clones
10 per family

flower stimulation
500 half-sib family x
40 individuals/family
= 20000 seedlings

Progeny testing:
polycross

4 test sites

Selection 50 clones
1 per full-sib family

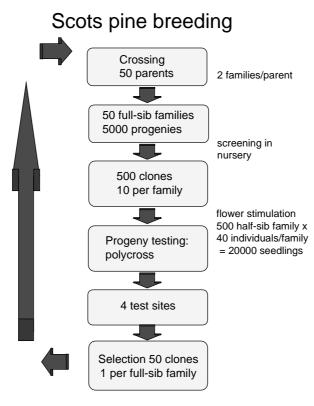Fig. 2. Breeding cycle for Scots pine with application of within-family selection.

Spruce can be propagated vegetatively by cuttings and this is utilised in the breeding programme (Karlsson & Rosvall, 1993). Besides reduction of the time necessary to complete a breeding cycle, this option also makes it possible to use the best genotypes from the breeding programme in a clonal forestry (Fig. 3).

Norway spruce breeding

Crossing
50 parents

2 families/parent

50 full-sib families
5000 progenies

screening in
nursery
vegetative propagation
→ bulk propagation

2000 clones
40 per family
14 ramets/clone

4 test sites

Selection 50 clones
1 per full-sib family
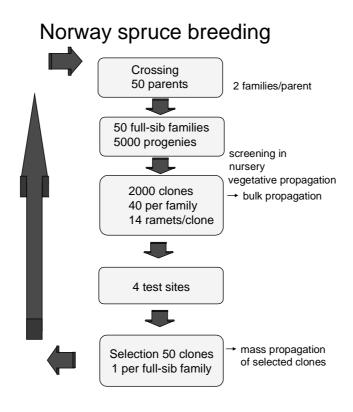
→ mass propagation
of selected clones

Fig. 3. Breeding cycle for Norway spruce with application
of within-family selection and clonally propagated progeny tests.

The most important traits of interest in breeding are survival, vitality, growth rhythm, growth, straightness and branching. Typically, a goal trait in tree breeding should have a long-term validity.

Mass production of improved material

A typical seed orchard for mass production of improved seed contains 30-40 clones, grafted or cutting propagated. The seed orchards are soil-based and open-pollinated, thus allowing a considerable amount of external pollen to contribute. External pollen proportions of 30-70% have been measured in various studies on Scots pine seed orchards (Harju & Muona, 1989; Yazdani & Lindgren, 1991), indicating the tremendous ability of gene flow.

Future production of improved material will probably include indoor seed orchards combined with, if possible, vegetative propagation for scaling up the material, either bulk or clonal propagation. Introduction of somatic embryogenesis into the breeding programme will probably solve the problem with decreased rooting ability by age of donor plants. Cryostorage of embryogenic tissue will provide plants with maintained rooting ability after the field tests and thus give the prerequisites for efficient clonal forestry (Park et al., 1998; Högberg et al., 1998).

Broad-leaf species

Birch (Betula pendula) is the most important broad-leaf species in Sweden but far from the extent of pine and spruce. The characters of interest are essentially the same as for pine and spruce. A rather

intensive breeding programme is pursued also for birch (Werner, 1993), partly because it works as a model species for breeding with short time intervals between generations. The seed is normally produced in indoor seed orchards with high seed yields per area unit.

From a European perspective, several indigenous broad-leaves are important. Although, reforestation methods as well as genetic levels of plant materials vary considerably. For example, poplar (Populus sp.) plantations often consist of few clones and are intensively managed, while beech (Fagus silvatica) to a large extent is naturally regenerated and the use of genetically improved material is marginal. The variation between forest tree species and differences in silviculture and breeding aspects makes a general description impossible. Each species must be described and analysed on a case by case basis.

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Ecological aspects of key species and their use in forestry

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The concept of keystone species

The concept of keystone species goes back to Paine (1966, 1969) and is based on the analysis of marine ecosystems. In the following years the concept was generalized and extended to terrestrial ecosystems. As presently understood, keystone species may be regarded as a functional group with no redundant representation and exerting a disproportionate control within an ecosystem. Its experimental removal (or introduction) causes major ecosystem-level changes in structure, dynamics or nutrient flow (e.g. Bond, 1994, Crawley, 1995, Tømmeras, 1998). However, it is not always obvious which species are keystones, since they may not be abundant. Therefore, a major research challenge is to predict which species in a community have keystone effects or will have such effects under reasonable scenarios of environmental changes (Schulze and Mooney, 1994). According to Bond (1994) keystone species can be classified into several categories: predators, herbivores, pathogens and parasites, competitors, mutualists, earth-movers, system processors, and abiotic agents. Forest trees can play the role of competitors, e.g. in successional replacement, and of mutualists providing plant resources. They may not only have a central position in the food web of a forest, but they may also represent the dominating living environment for hundreds of species and may substantially influence the microclimate and soil conditions as well as the water relations in the ecosystem; thus, providing the basis for biodiversity (Dylla and Krätzner, 1986; Leibundgut, 1990; Ammer and Stimm, 1996; Hattemer and Gregorius, 1996; Stephan, 1996; Schütz, 1996; Tømmeras et al., 1996; Thomasius and Schmidt, 1996).

Forest ecosystems in central Europe

Central Europe is located in the temperate climatic zone with prevailing sub-Atlantic influence. The natural ecosystems from the planar to the sub-montane region are characterized by mixed deciduous forests. In this so called beech zone, Fagus sylvatica is the dominant species, being partially or completely replaced on more acidic soils by Quercus robur, Q. petraea and other deciduous species. In drier, more continental habitats and in the montane region, mixed forests of deciduous and conifer species (mostly Pinus sylvestris, Picea abies, Abies alba) are found. Under native conditions, P. abies forms the climax forest only in the sub-alpine region (Rubner and Reinhold, 1953; Braun, 1982; Leibundgut, 1993; Ellenberg, 1996).

The mosaic of different habitats and the various climatic influences led to the high natural diversity of forest ecosystems in central Europe, where more than one hundred types of forest communities and numerous local races of the forest key species are known (Rubner ,1953; Leibundgut, 1990).

The deforestation of central Europe reached its maximum in the middle ages. After the 15th century there was no further notable decrease of the forest area, which is at present 30% of the total area. However, predominately due to the anthropogenic activities in the last centuries, large areas of natural

forests have been changed in species composition or replaced primarily by pure stands of Picea abies and Pinus sylvestris. In the case of these species, foreign provenances, often of unknown origin and less adapted, were widely used (Hasel, 1985; Schmidt-Voigt, 1986; Tabel, 1996; Thomasius and Schmidt, 1996). As a consequence, ecological stability and biological diversity declined (e.g. Tabel, 1996). Therefore, the long-term strategy for the stabilization of forest ecosystems in Germany is to overcome monocultures through the use of native mixtures of key forest trees. The "Concept for the conservation of forest genetic resources in the Federal Republic of Germany" developed between 1985 and 1987 (see Behm et al., 1997), establishes the research needs and goals for the conservation of genetic resources and for the multiplicity of all native tree and shrub species. As well, similar needs and goals are established for important, tested foreign provenances and for introduced forest tree species. In addition to the German Law on Forest Reproductive Material (Anonymus, 1979) and international regulations for marketing of forest reproductive material, this concept provides the basis for the consequent use of certified plant material for reforestation (see Liepe, 1996; Muhs, 1996) and the framework for the coordination of the activities of all German states in this field.

Abies alba – a keystone species of mixed mountain forests in southern Germany

Silver fir (Abies alba Mill.) was chosen as an example for a more detailed consideration of biology, ecology, use in forestry and conservation of genetic resources of a keystone species. One of the reasons for selecting A. alba was that the genus Abies will probably be one of the first in which transgenic cultivars can be expected on the European market for use not in forestry but in Christmas tree plantations. In this context, the considerable progress made in clonal propagation and transformation of Abies nordmanniana is noteworthy (Find, 1999, in this volume).

Although Silver fir is a productive and valuable forest tree, its economical importance is relatively low due to silvicultural limitations and other problems (Larsen , 1994). In contrast, it is known to have exceptional ecological importance. In 1943 Leiber emphasised that Abies alba had an outstanding status in, for example, forest ecology, soil biology, silviculture, timber yield and landscape protection, and its loss could not be compensated for by any other species. The similarity of his characterization with the present understanding of the importance of keystone species should be noted!

Among the Abies species in Europe and Minor Asia, A .alba has the largest area of distribution and is the only species that occurs in central Europe. After the last glacial period A. alba migrated to central Europe from its refugia in the southern Balkan, central Italy and central or eastern France following different routes. It reached the northern limit of distribution – the Beskides in the East, the Ore mountains (Saxony) and the Thuringian forest in the central part and the Vosges in the West – about 4000 years ago (Konnert and Bergmann 1995; Llamas Gomez and Braun, 1995). Where Silver fir occurs natively, it is a character species of mixed mountain forests. In the lower montane zone it is mixed with Fagus sylvatica. In the montane and upper montane zone beech is gradually or completely replaced by Picea abies, locally resulting in Norway spruce – Silver fir forest. Other imported species in these mixed forests may be Quercus robur, Q. petraea, Pinus sylvestris, Acer pseudoplatanus, and in the Alps, additionally, Larix decidua and Pinus cembra (Rubner and Reinhold, 1953; Leibundgut, 1993; Ellenberg, 1996).

In optimal climatic conditions, Silver fir may reach an age of more than 400 years and a height of 65 m. The stems are cylindrical and straight, and the timber is of high value, similar to Norway spruce (Mayer, 1992; Schütt et al., 1992)). The flowers are wind-pollinated. The seeds are autochoric and distribute after maturation in autumn. Self-fertilization results in a high degree of inbreeding depression (Llamas Gomez and Braun, 1995).

Silver fir grows best on damp calcareous or silicate soils and prefers humid temperate climate with a precipitation • 1000 mm per year .The development of the root system starts with the formation of a tap root, but represents in the mature tree a deep heart-shaped root system. Therefore, it has a considerable positive influence on soil structure and also on humus formation without the strong acidification known from Picea abies (Mayer, 1992).

Young Silver firs have a high shade tolerance. Therefore, natural regeneration develops optimally in closed stands. Under these conditions the young trees show a high competitiveness but grow slowly for decades until openings in the canopy appear. The juvenile period may last 30 to 50 years. Later it shows a fast continuous growth over a long period resulting in a higher productivity compared to Picea abies (Mayer, 1989, 1992).

Silver fir can not be used for reforestation of cleared areas under most environmental conditions. Nor can it compete with light-demanding and fast growing species. Additionally, the species is highly sensitive to strong winter frost, late frost and browsing by herbivoric mammals (Rubner and Reinhold, 1953; Mayer, 1992; Llamas Gomez and Braun, 1995; Eder, 1996; Prien et al, 1996; Waldherr, 1996). In the past, natural regeneration dominated. Therefore, most of the older fir forests are autochthonous (Konnert and Bergmann, 1995).

Besides its general importance as an essential component of mixed mountain forests, there are numerous species directly depending on A. alba as host or nutrition plant, e.g. Hericium coralloides (Scop ex Fr.) S.F. Gray, a Basidiomycete (Haupt, 1988) and Viscum album spp. abietis (Wiesb.) Abrom. (Schmidt, 1989). Crossability can be judged from introgressive hybridisation with A. borisii regis or A. cephalonica, respectively, in the Balkan and controlled hybridisation with species of the genus Abies, e.g. A. cephalonica, A. nordmanniana (Mattfeld, 1925; Vookova et al., 1989; Gajdosova and Vookova, 1990; Aas et al, 1994; Matschke, 1999). Against the background of an assumed future cultivation of transgenic Abies nordmanniana in areas of natural distribution of A. alba (e.g. Bavaria) or of use in forestry (e.g. northern Germany, Denmark. southern Sweden) (Larsen, 1994), the possibility of hybridisation must be taken into consideration.

Decline of Silver fir

Several periods of Silver fir decline have been reported for the last two centuries. A forced decline was observed during the last decades, obviously in connection with the increased anthropogenic influence. Consequently, populations have decreased and currently are scattered throughout the entire range of it original distribution (Wachter, 1978; Mayer, 1992; Larsen, 1994; Konnert and Bergmann, 1995).

A dramatic decline occurred in the northern distribution range. As late as the 18th century A. alba accounted for 15 to 20% of the forest trees in its original habitats. It has declined, for example, in Bavaria to 2% (Waldherr, 1996), in Thuringia to 1% (Hosius, 1996), and in Saxonia to below 0.03% (Llamas Gomez and Braun, 1995). In large areas it has disappeared completely. On the one hand, the reasons for disappearance were anthropogenic: replacement of mixed forests by monocultures, non-adequate forest management (clear felling) and high population density of herbivoric mammals. On the other hand, there is strong evidence for biological reasons for Silver fir decline. Genetic analyses of the recent years underline, that A. alba has, especially along the northern range of distribution, a strongly reduced genetic variation and consequently, a low adaptiveness and adaptability (Larsen, 1981, 1986 a, b, 1989, 1994; Eder, 1996). Indeed, the typical silver fir decline is only observed in the northern range (Schütt, 1978; Kramer, 1982; Mayer, 1992) and not in southern Europe, e.g. Calabrian populations, where the genetic variation is considerably higher (Bergmann and Kownatzki, 1988; Larsen, 1989, 1994; Bergmann et al., 1990 a, b;

Aas et al., 1994; Bergmann, 1994). Another indication for low adaptiveness is, that in the northern range the formation of local races or ecotypes did not occur (Rubner and Reinhold, 1953; Mayer, 1992). The low genetic multiplicity might be an explanation for the high sensitivity against abiotic stress (e.g. drought, extreme frost periods, environmental pollution, nutrient deficiency, Al3+toxicity), pathogens (e.g. secondary infection with bacteria, fungi, viruses and disturbed mycorrhiza) and insects (e.g. aphids, bork beetles, leaf rolling moths) (Schütt, 1978; Mayer, 1979; Larsen,1986 a; Mayer, 1992; Eder, 1996).

Reintroduction of Silver fir into mountain forests

The long-term goal in all German states within the native distribution range of the Silver fir is to enhance its frequency in the forest ecosystems by increasing and stabilizing the percentage of Silver fir trees that still exist in mixed mountain forests and by converting monocultures into mixed forests (Llamas Gomez and Braun, 1995; Eckhardt, 1996; Eder, 1996; Hosius, 1996; Waldherr, 1996). Implicit in this goal is an effort to increase the role of the other keystone tree species (primarily Fagus sylvatica) in mixed mountain forests (Braun, 1995; Wolf and Braun, 1995, 1996).
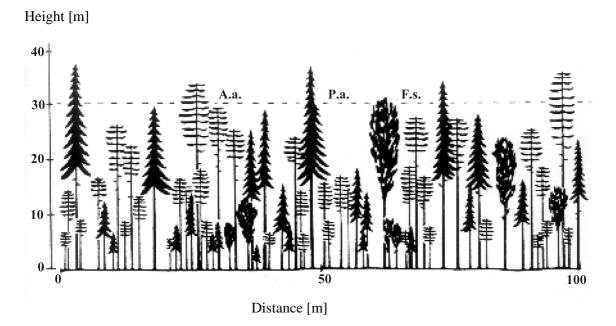

Fig.1: Ideal model of a variable age mixed forest (plenter forest) with Abies alba (A.a.), Picea abies (P.a.) and Fagus sylvatica (F.s.) representing the dominant species. Variable age forests are the most stable forest ecosystems and have the lowest operational risk (according to Mayer 1992)

The example in Saxony underlines the dramatic situation of the Silver fir in its northern distribution range. Prior to human settlement, Saxony was completely forested and broad-leaf trees accounted for 70% of the total tree number. By 1900, the proportion of conifers increased to 98 % mainly through reforestation with Norway spruce and Scots pine (Dittrich, 1993). In this century their proportion was stepwise reduced and is now approximately 80% (Braun, 1999). A. alba made up 30% of the tree population in mountain ecosystems between an elevation of 200 and 800 m as late as the 17th century. By 1917, it was reduced to 0.2 % and, in 1937, to below 0.1 %. By 1955, this percentage had decreased to 0.03% and at present the number of trees older then 60 years has been estimated at 2 000. Of these trees, 94% are damaged or unhealthy (Wolf and Braun, 1995). Isolated single trees or small populations are scattered over a large area, and any efficient natural regeneration is excluded. Another serious problem is

the narrow genetic basis of the remaining autochthonous material (Llamas Gomez and Braun, 1995). Therefore, after genetic characterization, a breeding program was started to increase the genetic variability. This program includes the genetic unification of the isolated populations by controlled pollination and establishment of seed orchards (Wolf and Braun, 1996). Additionally, the development of biotechnological methods for clonal propagation and cryopreservation of selected genotypes is in progress. A further increase of genetic multiplictity can only be achieved by introduction of material from other provenances. The, genetically, most related provenances for use in the western Saxonian Ore mountains and eastern Saxony are found in the Bohemian-Bavarian forest and the Sudetes and Beskides, respectively (Llamas Gomez and Braun, 1995).

The promotion of Silver fir needs an adequate forest management. For conversion of Norway spruce monocultures, A. alba has to be grown 15 to 30 years in advance under shelter, than an opening of the canopy and a stepwise introduction of the other species of the mixed forest can occur. Optimal development of Silver fir occurs in variable age mixed permanent forest (plenter forest, Fig.1). This forest type is characterized by a high biodiversity and ecological stability as well as a continuous and high timber yield. The permanent character of the forest and the natural regeneration of the shade-tolerant climax species, including A. alba, is supported by selective felling methods (plenter and femel felling) (Meyer, 1992; Schütz, 1996).

In Saxony, the long-term goal is to re-establish an area of ca. 120 000 ha of mixed mountain forests with the Silver fir accounting for 10 to 20% of the total tree population (Wolf and Braun, 1996).

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Safety considerations of trees vs crop:
A Canadian perspective

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Considerations: trees vs crops
What makes trees different from crops?

Unique biology of forest tree species
Environment in which transformed trees will be released
Approach to domestication and breeding
How benefits will accrue
Considerations: trees vs crops
Unique biology of forest tree species
large, long-lived organisms
essentially non-domesticated
outcrossing mating system, conifer pollen can travel long distance
Species are well adapted to their natural environment, are ecologically highly competent and maintain high level of genetic diversity

Considerations: trees vs crops
Natural ecosystems
complex and yet poorly understood
many different types
interactions of species
natural populations of the species
Management decisions made over the forest estate. In crops, decisions are made on a field-by-field basis.
Considerations: trees vs crops
Domestication and breeding
long history of breeding in crops:
Gene pool has been used to a large extent. Need to expand to other species and genus.
Forest trees:
still a large pool but generation time makes progress difficult and expensive;
gains primarily from "common" alleles (> 1%);
traits with very low variability.
Questions raised
Gene transfer
to the natural populations of the species (centre of genetic diversity)
to related species and to other species through horizontal gene transfer
Weediness and invasivess
selective advantage
what factors limit increase in range?
Questions raised
Impact on non-target organisms

depends on the genes
Ecosystem interactions
traits which have no coevolutionary history within the ecosystem
Biodiversity

Case-by-case analysis
Species vs natural forests
native species?
Exotic species?   Wheat, potatoes
where does the gene come from?
Are we doing what mother nature does or can do, but much faster?
What does the gene do?
Final remarks
Threat to forest diversity : converting forest land to other uses (deforestation)
Getting into intensive plantations
will allow reduction in pressure on our natural forest
will help increase afforestation (value of the end product worth investment)
Final remarks
Getting into intensive plantations
part of our sustainable development strategy
breeding and biotechnology can make it economically feasible
The big challenge in forest trees compared to crops is that we need wise engineering and deployment strategies as we cannot create new cultivars every 5 years.
Final remarks
Example:
White pine in North America
Introduced pathogen (blister rust)
Natural regeneration impossible in environments favorable to the fungus
No coevolutionary history - no resistance
Biotech a solution to rehabilitate white pine??
Acknowledgments

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Basic Principles of Environmental Assessment of plants with novel traits:

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Introduction

The federal government of Canada has been regulating plants with novel traits[1] (PNTs) since 1988 when it authorized the first confined field trials of transgenic canola and flax. Since then the Canadian approach to the regulation of plant products of biotechnology has evolved into one that is unique amongst those countries that are major developers and/or producers of these plants. The Canadian system is product based; the regulatory "trigger" is the trait expressed in the plant, and not the means by which the trait was introduced. Consequently, transgenic plants are regulated (as they are in the U.S., the E.U., Japan, Australia, Argentina etc.) but so too are plants expressing novel traits that may have been developed using other methods such as chemical mutagenesis, protoplast fusion, or even conventional plant breeding. The decision to adopt a product- versus process- driven regulatory regime was made by the Canadian government after three years of extensive consultation with plant biotechnology stakeholders including other provincial and federal government departments and agencies, industry representatives, agricultural organizations such as trade and farm groups, environmental groups, consumer groups and the general public.

The responsibility for the regulation of PNTs is shared by the Canadian Food Inspection Agency (CFIA) and Health Canada. The Plant Biotechnology Office, CFIA, oversees confined field trials of PNTs and environmental safety assessments of these plants prior to any unconfined release. The Feeds Section, CFIA, is responsible for livestock feed safety assessments while the Plant Health Risk Assessment Unit, CFIA, is involved in the assessment of imported PNTs not destined for deliberate release. Health Canada's Office of Food Biotechnology is responsible for human food safety assessments of PNTs. The Pesticide Management Regulatory Agency, Health Canada, and the Canadian Forest Service, Natural Resources Canada, provide supporting expertise for pesticidal plants and forest trees with novel traits, respectively.

Canada's product based approach to the regulation of PNTs is scientifically defensible because it considers the environmental impact of the whole plant without bias as to how it was produced. However, the product-based system has its own inherent challenges. The more common use of recombinant DNA as a regulatory trigger by other federal governments clearly limits the extent of regulatory oversight to transgenic plants. In Canada, where the scope is considerably broader, scientists and regulators have to determine when a non-transgenic plant is considered novel and therefore subject to regulation. In order to facilitate determinations of what is, and is not a PNT, regulators have developed the Safety Based Model for the Regulation of Plants (Figure 1).

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1 .   A plant variety/genotype possessing characteristics that demonstrate neither familiarity nor substantial equivalence to those present in a distinct, stable population of a cultivated species of seed in Canada and that have been intentionally selected, created or introduced into a population of that species through specific genetic change (AAFC, 1994).
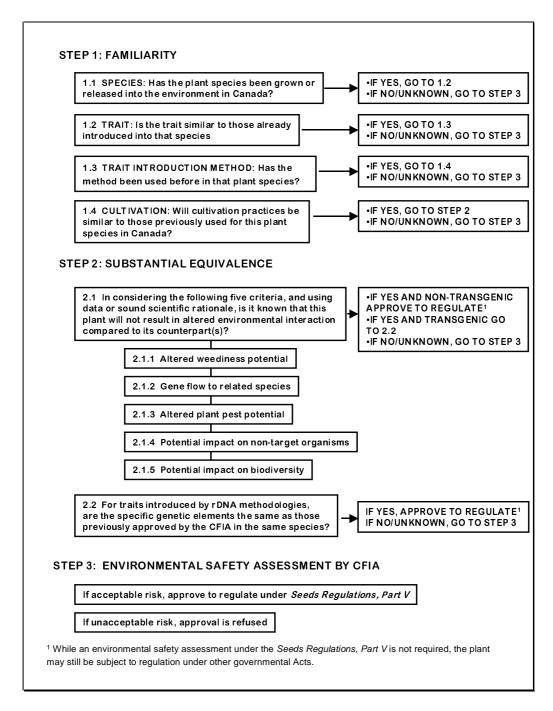
STEP 1: FAMILIARITY

1.1 SPECIES: Has the plant species been grown or released into the environment in Canada?	•IF YES, GO TO 1.2 •IF NO/UNKNOWN, GO TO STEP 3
1.2 TRAIT: Is the trait similar to those already introduced into that species	•IF YES, GO TO 1.3 •IF NO/UNKNOWN, GO TO STEP 3
1.3 TRAIT INTRODUCTION METHOD: Has the method been used before in that plant species?	•IF YES, GO TO 1.4 •IF NO/UNKNOWN, GO TO STEP 3
1.4 CULTIVATION: Will cultivation practices be similar to those previously used for this plant species in Canada?	•IF YES, GO TO STEP 2 •IF NO/UNKNOWN, GO TO STEP 3

STEP 2: SUBSTANTIAL EQUIVALENCE

2.1 In considering the following five criteria, and using data or sound scientific rationale, is it known that this plant will not result in altered environmental interaction compared to its counterpart(s)?	•IF YES AND NON-TRANSGENIC APPROVE TO REGULATE[1] •IF YES AND TRANSGENIC GO TO 2.2 •IF NO/UNKNOWN, GO TO STEP 3

- 2.1.1 Altered weediness potential
- 2.1.2 Gene flow to related species
- 2.1.3 Altered plant pest potential
- 2.1.4 Potential impact on non-target organisms
- 2.1.5 Potential impact on biodiversity

2.2 For traits introduced by rDNA methodologies, are the specific genetic elements the same as those previously approved by the CFIA in the same species?	IF YES, APPROVE TO REGULATE[1] IF NO/UNKNOWN, GO TO STEP 3

STEP 3: ENVIRONMENTAL SAFETY ASSESSMENT BY CFIA

If acceptable risk, approve to regulate under *Seeds Regulations, Part V*

If unacceptable risk, approval is refused

[1] While an environmental safety assessment under the *Seeds Regulations, Part V* is not required, the plant may still be subject to regulation under other governmental Acts.

Figure 1: A schematic representation of the Safety Based Model for the Regulation of Plants (AAFC, 1994).

The Safety Based Model

The concepts of familiarity[2] and substantial equivalence[3] provide the foundation for the Safety Based Model (SBM). In order to assist in determining if a plant should be considered familiar (Step 1,

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2 .   Familiarity is defined as the knowledge of the characteristics of a plant species and experience with the use of that plant species in Canada (AAFC, 1994).

Figure 1) within the context of the SBM, the Plant Biotechnology Office, CFIA, has published seven biology documents for the major crop species grown in Canada. These documents include information on the biology of the plant species, its cultivation and use as a crop plant in Canada; information on the centres of origin of the species; consideration of the species as a volunteer weed; information on close relatives including a summary of their ecology; the potential for inter-species / genus hybridization and introgression of genetic information; and an analysis of potential interactions of the plant species with other life forms during its life cycle. If a plant does not meet the standards for familiarity presented in the SBM, then an environmental safety assessment is required. Conversely, if the plant meets the standard for familiarity it must then be examined in the context of substantial equivalence (Step 2, Figure 1).

To determine if a plant is substantially equivalent to its counterpart, a critical comparison of the new plant with its conventional counterpart is required. The key criteria for such a comparison are presented in the SBM: altered weediness potential, gene flow to related species, altered plant pest potential, potential impact on non-target organisms, and potential impact on biodiversity. Any plant evaluated in accordance with these criteria must undergo an environmental safety assessment if found to have the potential to cause altered environmental interactions upon release. Additionally, for transgenic plants, the specific genetic elements introduced into the plant must be identical to those previously approved by the CFIA in the same plant species. Plants which do not meet the SBM's standards for familiarity and substantial equivalence are regulated by the CFIA in accordance with the Seeds Regulations, Part V. This means that field trials of these plants must be confined and unrestricted release is prohibited until after an environmental safety assessment has been conducted by the competent authority.

Confined Field Trials

Confined field trials of PNTs provide scientists with an opportunity to evaluate experimental PNTs in the field but under conditions which limit the interaction of the plant with the larger environment. Trials of PNTs must be conducted in accordance with government mandated terms and conditions including reproductive isolation, site monitoring, and post-harvest land use restrictions (AAFC, 1995). For each plant species, applicants are limited to five trial sites per Canadian province with a maximum trial size of 1 hectare. Exemptions from the restrictions on trial site number and size may be granted under extenuating circumstances but only for research purposes (CFIA, 1998b).

In 1988, the first year confined field trials were planted in Canada, fourteen trial sites were authorized for two crop species, canola and flax. Since then there has been an explosion in the development and evaluation of PNTs in Canada (Figure 2) and commensurate with this has been an increase in the number of trials (Figure 3), the crop species being altered (Figure 4), and the traits being introduced (Figure 5).

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3.   Substantial equivalence is defined as the equivalence of a novel trait within a particular plant species in terms of its specific use and safety to the environment and human health, to those in that same species, that are in use and generally considered as safe in Canada, based on valid scientific rationale (AAFC, 1994).
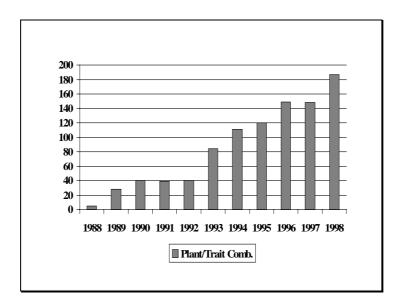
Figure 2: The number of PNTs (plant variety and trait combinations) under trial in Canada has increased from five in 1988 to 187 in 1998.
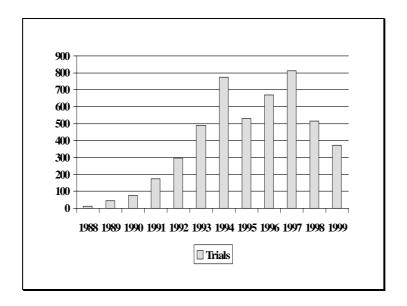

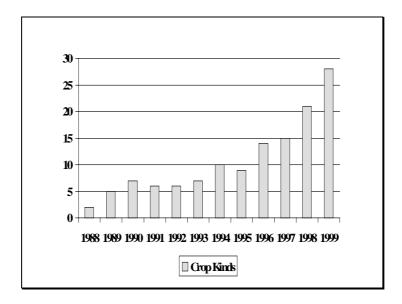Figure 3: Data showing number of confined field trials in Canada from 1988 to 1999.

Figure 4: The number of plant species being modified and grown in confined trials in Canada has increased from two in 1988 to 28 in 1999.
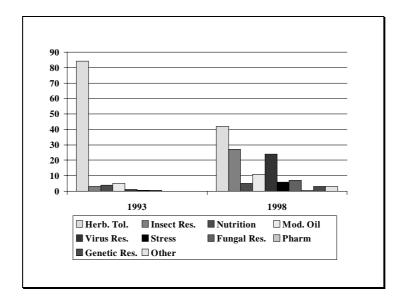

Figure 5: A comparison of novel traits in 1993 and 1998.

In 1993 almost 85% of the PNTs in trial were herbicide tolerant. By 1998 herbicide tolerance was present alone or in combination with other traits in about 42% of the PNTs. One also sees the emergence of "second generation" PNTs with quality or out-put traits such as modified oil profiles and nutritional modifications.

The ability of scientists to conduct confined field trials is integral to the environmental safety assessment process as it is during these trials that data can be accumulated to address the informational requirements for safety evaluations.

Environmental Safety Assessment

All PNTs must be assessed for environmental safety by the CFIA prior to any unconfined release[4]. If risks associated with the release are considered acceptable, the PNT will be granted release, however, the authorization may be conditional on the development and deployment of specific management plans or practices. For example, the unconfined release of Bt corn and potato events in Canada is conditional on the implementation of specific insect resistance management plans designed to mitigate the development of resistance to the Cry proteins expressed in these varieties (AAFC, 1996; CFIA, 1999).

In broad terms, environmental safety assessments require characterization of the PNT and its environmental interactions. The specific information requirements, summarized below, are detailed in Regulatory Directive 94-08 (AAFC, 1994).

Description of the taxonomy and pedigree of the PNT and details on its anticipated use.

Description of the method used to introduce the novel trait(s).

- If the PNT is transgenic, a detailed molecular genetic characterization of the plant is required as detailed in Canada – U.S. Bilateral on Agricultural Biotechnology, Appendix I (CFIA, 1998a).

Data demonstrating the stability of the novel trait(s) over multiple generations.

- A description of the novel traits including: the activity of gene products, breakdown products, by-products and their metabolic pathways; tissue and/or temporal specificity; and toxicity of gene products, breakdown products, and by-products in the environment.

- Information on the reproductive and survival biology of the PNT, including any new adaptations to stress factors. In cases where the gene product is toxic, data is required on the level of exposure and effect on soil micro-flora and -fauna.

- A description of proposed release sites, changes in usual habitat or normal geographic production for that plant species in Canada, changes in cultivation practices or deployment strategies.

- Detailed information on any potential for gene flow from the PNT to related species and any consequences of introgression.

After a PNT has been authorized, post-release monitoring must be carried out by the applicant. Any new information regarding the risk to the environment, including risk to human health, resulting from the release must be reported to the CFIA. At that time the regulatory authorities will re-assess the unconfined release in light of the new information. If the risk is determined to be greater than at the time of the original (or any subsequent) assessment, then new or different conditions on the release may be imposed. Where the new information is such that the risk is reassessed and deemed unacceptable, then the authorization will be cancelled and the applicant must take appropriate action to eliminate or minimize the risk .

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4. An unconfined release consists of the use of the assessed PNT, without requirements for reproductive isolation, site monitoring, and post-harvest land use restrictions (AAFC, 1994).

Forest Trees with Novel Traits

The Canadian model for environmental risk assessment presented in this paper has been applied successfully to agricultural crop species and is currently being adapted by the Canadian Forest Service and the CFIA to meet the challenge of regulating forest trees with novel traits (FTNTs). While the information required for the environmental safety assessment of forest tree species should not differ significantly from that required for crop species, data standards will need to be modified to address the long-lived nature of forest tree species. For example, determining what is considered a distinct, stable population can be challenging in agricultural crop species but is daunting when one turns to forest tree species where there is significantly more intra-specific heterogeneity.

There are currently two confined trials of transgenic poplars in Canada and both will be terminated prior to flowering. It is not anticipated that any FTNTs will be commercialized in Canada in the near term and any future releases will most probably be confined to intensively managed plantations or "fibre farms". It is unlikely that any FTNT will be released for deployment in primary or secondary forests due both to the inherent complexity of the forest ecosystem and the economic reality that cost recovery by the developers of FTNTs will only be realized in the plantation setting.

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Environmental risk assessment of long-lived species

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Introduction

Genetic modification alters the genotype of the target organism in more or less predictable ways. The phenotype of the genetically modified organism (GMO) is even less predictable. As one common approach to risk assessment is to evaluate and regulate GMOs according to their biological properties, it follows that this assessment will be based on very incomplete knowledge. Moreover, when the GMO is released to the environment, uncertainty about environmental properties will add to our ignorance about the possible environmental impacts.

Consensus seems to have been reached for some principles of environmental risk assessment, but not on the procedures for how it should be carried out. For example, transgenic tree species and cultivars raise questions of long-term impacts both on the consequences of gene-flow into natural populations and on changes in occurrence, distribution and performance of the plant in its natural environment and surrounding areas. In particular, the long generation time of many tree species means that risk assessment will be equivalent to making predictions about a very distant future.

In this paper, we take a closer look at some principles of risk assessment for GMOs and discuss how they can be applied to long-lived organisms, such as trees.

Risk assessment

The Ecological Society of America has proposed the following principle for risk evaluations when GMOs are released to the environment (Tiedje et al., 1989): "Genetically modified organisms should be evaluated and regulated according to their biological properties (phenotypes), rather than according to the genetic techniques used to produce them." This acknowledges that the release of GMOs need not differ from the release of other organisms, as regards their possible environmental impacts. Others have pointed out that the technique used in the genetic engineering must also be subject to assessment. There is, for example, little control regarding the final location or copy number of genes inserted in the genome of an organism. This may have consequences for the stability of the genome, for example with respect to regulating gene expression, which at a later stage may be responsible for the occurrence of unexpected phenotypes.

There is broad international agreement that the environmental effects of genetically modified organisms must be evaluated on a case-by-case basis through a stepwise procedure that includes fully enclosed pilot studies (OECD, 1986; Royal Commission, 1989; Tiedje et al., 1989). But even a "case-by-case" and "step-by-step" risk assessment has limitations such as (1) we have to use short-term experience to assess long-term impacts, (2) we can only test the possible problems we are aware of, (3) an inability to document any impact can be caused by problems with the method or that the investigation does not cover

all the relevant effects, and (4) for long-lived organisms, time will always be a limiting factor. Moreover, it has been shown in many cases of deliberate introductions that the persistence of the released population largely depends upon how many individuals were released (Griffith et al., 1989) and that a species' invasiveness may change several generations after it was first introduced (Fremstad & Elven, 1996; Crooks & Soulé, 1999).

There will always be an element of uncertainty involved in the release of genetically modified and other (non-modified) organisms. Our genetic and ecological knowledge is inadequate to allow us to give a priori, precise risk assessment for releases (Simonsen & Levin, 1988; Drake et al., 1989; Ryman et al., 1995). We may provide general guidelines on how to assess environmental effects, but we are unlikely to obtain sufficient knowledge of the ecological interplay within and among species to provide precise predictions. This is because we neither know the genes that are important for adaptations to the environment nor the selective forces acting on those genes. Collectively, these considerations mandate a way of thinking about releases that must incorporate the "precautionary principle" as a guideline for decisions.

"Long-lived" and some correlated ecological features

Many species in seasonal environments, alternating between cold and warm or dry and wet seasons, adjust their life span to a 1-year life cycle. To be long-lived, an organism must differ from this common pattern. We suggest a species to be long-lived if its generation time is (much) longer than 1 year.

A long life often correlates with late sexual maturity, overlapping generations, large body size, K-selected life-history strategy, long dispersal/migration, and the species being a "keystone" or dominant species within its environment and host to a number of other species (Begon & Mortimer, 1986; Stearns, 1992). A species may be termed "keystone" if its experimental removal (or introduction) causes major ecosystem-level changes in structure, dynamics or nutrient flows (Crawley, 1995). For example, in a boreal zone, no species is as important as Norway spruce (Picea abies) for the function, structure, dynamics and total biodiversity of the forest. It is evident that genetic modification of this and other keystone species may have wide-ranging impacts on temporal and spatial scales, and on both the structure and function of whole ecosystems.

Environmental effects of GMOs

The environmental impacts of releases of GMOs can be grouped into three categories (Williamson et al., 1990): (1) effects caused by the genetically modified organism itself, (2) effects resulting from dispersal of genes from the genetically modified organism to other organisms (intra- and interspecific) in the environment, and (3) altered practice in the use of an organism because of the genetic modification (for example, expansion of silviculture to the north following the use of transgenic spruce expressing antifreeze protein genes).

For each of these categories, some combinations of genetic modifications, source populations and release environments will clearly be more critical for assessment than others. Tiedje et al. (1989) have provided a detailed list of questions which can be used as a checklist for identifying releases on a subjective scale from low to high risk. Some critical questions from that list are:

Invasion potential

- is the expression of the genetic modification well documented and understood,
- does the genetically modified trait represent an ecological novelty,
- to what degree is the host organism domesticated,
- is the organism a "keystone species" in the environment,
- is the release environment "contained" by natural barriers for spread of the organism, or can the spread be controlled by human intervention,
- how well can the dynamics of the release environment be simulated prior to, and monitored after, release?

Gene flow

- does the organism have close relatives (i.e., natural or semi-natural populations of the same or closely related species) in or near the release environment,
- how large is the natural gene flow among populations of the species,
- does the release environment put a selective premium on the introduced gene?

Altered use

- does area expansion occur,
- are new control agents favoured?

Invasiveness of trees

Based on Tiedje et al.'s (1989) checklist, transgenic trees must in general be considered "high-risk" organisms, because of (1) their essentially wild (self-propagating) characteristics, (2) the broad geographic range they occupy, (3) the high level of natural gene flow among populations, (4) the important ecological role they play in ecosystems, (5) the difficulty of simulating ecological conditions in experiments, and (6) the typically uncontrolled access for the public to test sites.

On the other hand, long-lived species are often easier to monitor than short-lived ones. Moreover, the long pre-reproductive period makes it possible to call back unwanted effects as long as only immature individuals are used. This suggests that eventual use of long-lived GMOs should be accompanied by good monitoring programmes, and by effective measures to control possible invasions.

Valuable insights for the risk assessment of transgenic trees may be gained by reviewing the release history of non-transgenic varieties of the species. The invasiveness of many tree species is fairly well known. According to Rejmánek (1999), invasiveness of woody species in disturbed landscapes is associated with small mean seed mass, short juvenile period, long seed dispersal period, and short mean interval between large seed crops. Vertebrate dispersal is responsible for the invasiveness of many woody species in disturbed and undisturbed habitats. In making this list, Rejmánek (1999) did not consider "delayed" invaders.

Ecological and genetic knowledge of the target organisms may be combined with the use of mathematical models to look into the future at realistic environmental scales (Williamson, 1989; Hengeveld, 1999). Among other things, the use of mathematical models may help us describe and understand patterns of invasion and introgression, identify critical factors limiting the spread or establish-

ment of the transgenic organism, and perform sensitivity analysis with respect to how variation in parameter values influences the likelihood of an environmental effect.

Norway spruce (Picea abies)

Ecological and genetic characteristics of Norway spruce, particularly as they relate to risk assessment, have recently been reviewed by Tømmerås et al. (1996). Norway spruce plays a key role in boreal ecosystems by making a characteristic microclimate, by changing the soil quality (chemically and functionally), and by creating structure in the ecosystem. Approximately half of the species diversity in Norway is connected to forests, and half of the Red List species as well. Hundreds of species of mycorrhiza fungi are described on Norway spruce. These fungi are in general very important for spruce growth and especially crucial on dry habitats. Norway spruce is important for the forest dynamics in the boreal zone from western Scandinavia and central Europe to Siberia. Levels of gene flow among populations are quite high, and interbreeding between cultured and natural populations is common in the species. Thus, Norway spruce is a relevant model system for evaluating environmental impacts of transgenic varieties of a key species having both cultured and natural populations.

The generation time for Norway spruce is approximately 25 years. This long generation time has a profound impact on the time it takes to estimate important ecological and genetic parameters in the field. For example, calculation of the rates of spread of a Norway spruce transgene having a selective advantage, or disadvantage, respectively, of s = ± 0.1, demonstrates that the two curves look very similar after 10 generations or ~ 250 years, even though the end results are very different (global fixation with selective advantage, stable gene frequency cline with selective disadvantage; cf. Tømmerås et al. 1996). Thus, the time scale for assessment of environmental impacts of releases of transgenic trees may therefore be on the order of hundreds of years before one can expect to obtain precise estimates of critical parameters.

Some possible scenarios for transgenic Norway spruce

Risk assessment of GMOs may be quite trivial when the target organism is fully domesticated, fully dependent on humans or easily controlled by human intervention. On the other hand, a long-lived, widely distributed key species such as Norway spruce shows that risk assessment may be a formidable task.

As a desk study of risk assessment, Tømmerås et al. (1996) considered three different types of transgenic Norway spruce. The genetic modifications were fungal disease resistance, insect resistance and frost tolerance. These modifications differ in perceived risks for the various attributes evaluated according to Tiedje et al.'s (1989) checklist. The ranking of risk associated with the three genetic modifications is uncertain at the current level of knowledge. For example, even for a trait such as frost tolerance, which has been studied by provenance transfer, our knowledge about transgenic, frost-tolerant spruce is far from complete. One aspect of uncertainty relates to the fitness consequences when non-native provenances interbreed with native ones; another uncertainty relates to how far lessons from provenance transfer can be used to study frost tolerance achieved by genetic modification. There is no way around this problem except doing the hard work related to studying risks in controlled (and confined) experiments, paying attention to a long checklist of attributes for each genetic modification to be evaluated.

The types of environmental effects may differ between the various types of genetic modification. For frost tolerance, the main risk is probably invasion to new areas. For fungal disease resistance, effects on the forest where spruce already is the dominating species are probably more important, in particular because of potential changes in the mycorrhiza system. A genetically modified spruce able to resist or

change the decomposition of wood (e.g. insect resistance) is considered as specifically questionable, due to a broad range of implications for ecosystem function, habitats and species diversity.

Lack of scientific knowledge, especially on topics related to the biodiversity and functioning of forest ecosystems, is partly responsible for the uncertainties in predicting impacts by releasing transgenic spruce. Moreover, some short-term and many long-term effects are more or less unpredictable due to stochasticity in climatic and biological conditions and the specific conditions in the area at the time of release (Kowarik, 1996). The uncertainties about the probability of potentially irreversible negative impacts, the wide-ranging consequences, should they occur, and the almost impossible task of alleviating unwanted effects, should lead to severe restrictions on the release of genetically modified Norway spruce today.

The only possibility we can see for transgenic spruce at present, is to base production on only pre-reproductive (immature) stages in physically controlled fields. In that case, the environmental risks associated with reproduction and gene flow would largely disappear. But several aspects of the ecological role played by spruce would still be valid reasons for concern, and the problem of controlling public access to test or growing plantations would not guarantee fully against unwanted reproduction and spread of transgenic individuals or transgenes.

Conclusions

Long-lived, transgenic tree species and cultivars …..

- − cannot be simulated with ecological realism
- − are relatively easy to monitor
- − offer long periods to stop unwanted effects, but are unconfined after reproduction
- − are accessible to the public
- − cannot be assessed regarding selective advantage vs disadvantage
- − often have a well characterized invasiveness, and
- − need efficient control measures.

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Environmental Safety Considerations for Field Test of Genetically Modified Apple

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Abstract

Appropriate regulation is effective in minimizing the potential risks of a new technology to the environment and human health, and to ensure positive public perception.

Some genetically modified apples were already produced and grown to examine agronomic traits in a closed greenhouse. For the field test of transgenic apples, the assessment of the range of gene flow via pollen dispersal and the production of unique allelochemics are important. Apple flowers are entomophilous. The range of pollen dispersal was examined in the greenhouse, screenhouse and field using non-transgenic apple trees. Red leaf color was used as a marker of pollen dispersal and the gene flow through pollination by wind was limited within 1 m. Pollen dispersal by insects was occasionally found at a distance of 150 m from the pollinizer, usually within the radius of 80 m of the test field.

Rootstocks are commonly used in apple cultivation. In the case of genetically modified rootstocks, there is a doubt whether the introduced genes and gene products transfer from the rootstock to the scion. In the genome of scion parts that were grafted on the genetically modified apple rootstocks, no integration of introduced genes from the rootstocks was confirmed by PCR analysis. Moreover, the same experiment was pursued in tobacco. The scions of tobacco were grafted on the genetically modified tobaccos that exhibited resistance to antibiotic, kanamycin. No seeds gathered from the fruits of the scion part could survive on the culture medium containing kanamycin. These results suggest that the introduced genes in the transgenic rootstock do not spread into the environment through the scion part.

Introduction

The progress of genetic engineering of plants has resulted in the production of a range of agronomically important plants. The Ministry of Agriculture, Forestry and Fisheries (MAFF) issued the approval for cultivation of 24 kinds of transgenic crops in ordinary fields to date. However, the risk assessment of transgenic apple trees on environment has not yet been carried out in Japan.

For field testing of genetically modified apple trees, it is important to consider that the unintended gene flow to the environment should not occur. Apple flowers are basically entomophilous, however, we examined the range of pollen dispersal both by wind and insects for the assessment of gene flow using non-transgenic apple trees.

In Japan, most of the commercial apple trees are grafted on rootstocks to improve the productivity. Recently, we have introduced disease resistant genes into the rootstocks also with scion cultivars. Hirata et al. (1990) reported that the introduced gene in the genetically modified rootstock was transmitted and integrated to the scion part across the graft union in tobacco. Considering the use of transgenic apple rootstocks, the movement of the introduced gene and gene products from rootstock to

scion should be confirmed. From this perspective, the experiment for the gene movement from rootstocks in apple and tobacco was undertaken.

Materials and Methods

1. Gene flow via pollen dispersal

1) Plant materials

The apple cultivar 'Fuji' was selected as the pollen recipient in this experiment because this cultivar is the most popular variety in Japan and has many opportunities to pollinate in the orchard. Red leaf crabapple cultivar 'Adams' was used as the pollen donor because it showed good cross-compatibility with 'Fuji'. Characteristics of red leaf color are known to be controlled by a single dominant gene, and 'Adams' possesses this gene heterozygously. Trees for the experiments were potted. The height of trees was 1.5 m on average.

2) Pollen dispersal by wind

The apple cultivar 'Adams' as pollen donor was placed in front of the electric fan whose maximum velocity was about 4 m per second. The wind was blown during blooming period. To detect the pollen dispersal, the petri dishes were set at 1-m intervals from 'Adams'. The pollen dispersal was examined by the presence of pollens trapped on the petri dishes. To investigate the bearing of fruits through pollen dispersal by wind in the greenhouse, the apple cultivar 'Fuji' was placed at 1-m intervals from 'Adams'. The number of seeds in fruits and red leaf seedlings germinated confirmed the fruit development through pollen dispersal by wind.

The experiment regarding pollen dispersal by natural wind was carried out in a screenhouse protecting the trespass of insects. Pollen donor 'Adams' was put at the center of the screenhouse and pollen recipient apple 'Fuji' was set in four directions at distances of 1, 2, 3, 4, 5 and 6 m from 'Adams'. The range of pollen dispersal by natural wind was confirmed in the same way.

3) Pollen dispersal under natural condition

The test field was set up at a distance of 500 m from the apple orchards in May 1994 and 1995. The test field was almost flat, covered with field crops, such as grass and rape. 'Adams' trees, as pollen donors, were placed at the center of the test field, and the apple cultivar 'Fuji' trees, as pollen recipients, were put in four directions (north, south, east and west) at a distance of 0 m to 300 m from the donor. Fruits were harvested at the end of October and the ratio of red and green leaf seedlings was examined.

2. Introduced gene movement from rootstock to scion

1) Plant materials

For the experiment of gene transfer from rootstocks to scions, the neomycin phosphotransferase gene (NPTII) was introduced into the apple cultivar 'Orin' and tobacco 'Xhanti' by Agrobacterium-mediated transformation system (Norelli et al., 1994). Non-transgenic apple and tobacco were grafted on transgenic apple and tobacco rootstocks, respectively. These plants were grown in a separate greenhouse to prevent contamination of pollen from the genetically modified plants.

2) Extraction of nucleic acids

The scions of apple and tobacco were grown for two years and six months respectively, after grafting. Young leaves were collected from every scion for extraction of nucleic acids. The total DNA was prepared from three young leaves according to the protocol of Murray and Thompson (1980).

3) PCR analysis

Polymerase chain reaction (PCR) was performed with AmpliTaq Gold DNA polymerase (Perkin Elmer Japan, Urayasu, Japan) to detect the movement of the NPTII gene from rootstock to scion. Primers for PCR analysis were as follows: 5'-GATGGATTGCACGCAGGTTCTCCG-3' and 5'-ATACCGTAAAGCACGAGGAAGCGGT-3'.

4) Growth test on selection medium

The seeds gathered from tobacco scions were sowed on the selection medium, which is Murashige and Skoog medium (Murashige and Skoog, 1962) containing 300 mg/l of kanamycin, to confirm the integration of NPTII gene into the seeds of scion part. The survival of the seedlings was observed two weeks later.

Results

Pollen dispersal by wind

In the experiment of pollen dispersal by artificial wind, pollen was dispersed 1 m from the pollen donor. Although one fruit was observed on the apple tree, which was placed 6 m from the pollinizer, no seed was found in the fruit.

In the experiment regarding pollen dispersal by natural wind in a screenhouse, three fruits were observed at 1 m from 'Adams' (Table 1), and one out of five seedlings derived from these fruits had a red leaf. The results indicated that the fruit development resulted from the outcross of the pollen of 'Adams'. From these experiments, it is estimated that the gene flow of pollen in apple by wind is limited within 1 m.

Pollen dispersal under the natural condition

Pollen dispersal under the natural condition was examined in the test fields for two years (Fig. 1). The average number of fruits was 8.8 per recipient tree. The number of seeds gathered from 642 fruits was 2291. The existence of red leaf seedlings derived from pollinizer 'Adams' was observed after the germination of these seeds. Red leaf seedlings were found within 80 m in three directions (north, south and east), but 150 m in the west.

Introduced gene movement from rootstock to scion

Young leaves were collected from each apple scion, which was grafted on genetically modified rootstock. The presence of the NPTII gene in the genome of transformed apple trees was confirmed by PCR analysis (Fig. 2). The DNA fragments corresponding to NPTII gene were amplified from the genomic DNA of transgenic apple trees used as rootstocks, but not from the genomic DNA of scion grafted on

transgenic rootstocks. From this result, it seems that the introduced gene does not transfer from the rootstock to scion.

Moreover, the result of the experiment using transgenic tobacco was the same as in the case of apple. No specific DNA fragment was amplified in genomic DNA of scion by PCR analysis (data not shown).

The experiment was conducted to confirm the transfer of the introduced NPTII gene in rootstocks to the next generation through the seeds of scion part in tobacco. Seeds were gathered from scion part and about 60,000 seeds were sowed, but no seedling could survive on selection medium containing kanamycin suggesting that seedlings did not have the kanamycin resistant gene.

Discussion

Many genetically modified organisms were produced and some of them have entered the commercial track after biosafety evaluations. Appropriate regulation is effective to minimize the potential risks of a new technology to the environment and human health, and to ensure positive public perception. Regulation should be based on the product rather than the process and review conducted on a case-by-case basis.

The releases of these genetically modified organisms should be conducted cautiously because both the genetically modified organisms and their genes may spread in the environment and influence an ecosystem. Therefore, the environmental risk assessment is important for the release of genetically modified organisms. In Japan, 24 kinds of transgenic crops were cultivated in the ordinary field under the official approval of the Japanese government to date. The evaluation items, which are commonly used as an environmental risk assessment of annual crop, such as soybean, tomato, melon (Tabei et al., 1994) and others, are as follows: 1) confirmation and expression of the introduced genes, 2) morphological and growth characteristics, 3) reproductive characteristics, 4) evaluation of harmful impact to other plants, 5) influence on soil microflora, and 6) residual Agrobacterium as vector (in case of Agrobacterium mediated transformation system). During the production of transgenic plants, confirmation of existence and expression of the introduced gene is an essential process. In this process, southern blot hybridization analysis is often used to confirm the integration of the introduced gene into the genome. Then, morphological and growth characteristics are usually compared between transgenic and non-transgenic plants. If these characteristics are not different statistically, it is reasonable to presume that unexpected events will not occur by the introduction of foreign genes. This last item is necessary to prevent genetically modified Agrobacterium from releasing into the environment.

When we consider the influence of genetically modified apples on the environment, it is certain that reproductive characteristics, harmful impact to other plants and influence on soil microflora are very important evaluation items.

As regards pollen dispersal, our experiments drew some conclusions. Pollen dispersal by wind was limited within the maximum of 1 m from pollen donor; therefore, there was no concern about the gene flow through pollination by wind. On the other hand, the maximum range of the gene flow through the pollinator was a distance of about 150 m from the pollen donor. Wertheim (1991) reported that the maximum range of pollen dispersal was 40 m from the pollen donor in The Netherlands. The range of pollen dispersal differs approximately four times between Japan and The Netherlands, and it seems that the range of pollen dispersal by insects should be confirmed in every region. The distance of pollen dispersal would be affected by many conditions, such as wind, temperature and the configuration of the ground and

others. We consider that transgenic apple trees should be isolated from the apple orchards by about 200 m in the planned field release in our condition.

On the other hand, little is known about harmful substances to other organisms from apple trees. To compare the characteristics of apple with that of annual crops, the most different point is that an apple is cultivated long term at the same place. If they produce any harmful substances to other plants, like allelochemics, the influence by cultivation of these species may be more serious than the cultivation of annual crops. Repeated cultivation of apple trees is difficult and it is generally known as replant disease. We should evaluate the generation of novel allelochemics by HPLC and bioassay before the cultivation of transgenic apple trees in ordinary fields.

The use of rootstocks is a common style in fruit tree cultivation. Some of the genetically modified rootstocks are expected to indicate the resistance to soil pathogens. In our experiments, it was revealed that the introduced genes in apple and tobacco rootstocks did not transfer to the scion by PCR analysis and survival test of seedlings on selection mediums. Recently, Xoconostlle-Cazares et al. (1999) reported that RNA and peptides similar to viral movement proteins moved together into the sieve element of scion tissue. For the environmental risk assessment of genetically modified rootstocks, it seems that the movement of RNA and peptides derived from the introduced gene between scion and rootstock should be confirmed by northern and western blot hybridization analysis.

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Figure legends

Direction & Distance (m)		Flowers	Fruits	Seeds	Seedlings red	green
North-east	1	36	0	0		
	2	19	0	0		
	3	15	0	0		
	4	42	0	0		
	5	7	0	0		
	6	33	0	0		
North-west	1	7	0	0		
	2	7	0	0		
	3	12	0	0		
	4	23	0	0		
	5	14	0	0		
	6	18	0	0		
South-east	1	12	0	0		
	2	6	0	0		
	3	25	0	0		
	4	11	0	0		
	5	22	0	0		
	6	4	0	0		
South-west	1	22	3	12	2	8
	2	11	0	0		
	3	19	0	0		
	4	29	0	0		
	5	23	0	0		
	6	9	0	0		

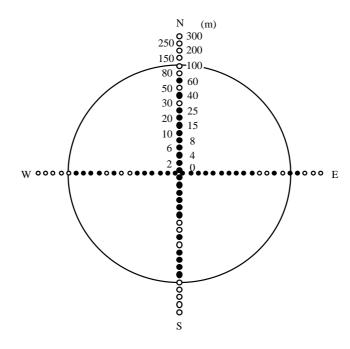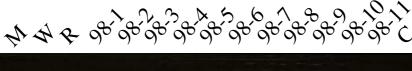Table 1: Fruit development through pollination by wind in the screenhouse

Fig. 1: The range of pollen dispersal under natural condition. The pollen donor 'Adams' was placed in the center. The pollen recipient 'Fuji' was placed in four directions (n; north, e; east, s; south, w; west) from the pollen donor. The numbers denote the distance from the pollen donor. The closed circles denote the trees yielding red seedlings.
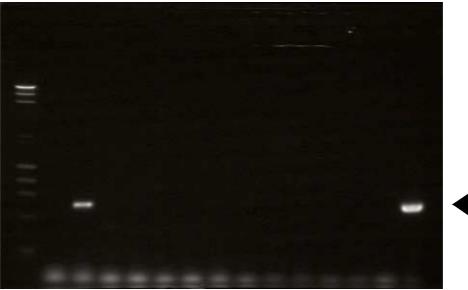
Fig. 2: PCR-amplified NPTII gene from the genome of scions grafted on genetically modified apple rootstocks. The arrow indicates the position of bands corresponding to the size of amplified DNA fragment (725 bp). 'M' denotes the molecular-size markers of HindIII-digested •-phage DNA and HaeIII-digested ⁻X174 DNA. 'W' denotes the original 'Fuji' as negative control. 'R' denotes the genetically modified apple 'Orin' used as rootstock. 'C' denotes the NPTII expression vector as positive control. '98-1 ~ 98-11' denote the scion lines grafted on genetically modified apple rootstocks.

Environmental Risk Assessment of Genetically Modified Trees in Denmark

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Abstract

The work on genetic engineering of plants has previously been directed mostly towards agricultural one-year crops, but now it is also possible to produce genetically modified trees. The aims are often to improve production, wood quality for paper and pulp production, insect and fungi resistance, phytoremediation etc. Transgenic plants may have unintended negative consequences for the environment, human health and production. Therefore, the human and environmental risks of cultivating the transgenic plant are evaluated before field releases and marketing of every new GMP. This presentation will focus on the environmental risk assessment and the data needed to perform the assessment.

Genetically engineered trees require particular ecological concern because trees are long lived organisms. Trees may form their own ecosystems by influencing both abiotic conditions as light, water and soil processes, and the flora and fauna of the system. Most trees are weakly domesticated and act almost like wild relatives and will often be planted near closely related species. Many temperate trees are wind pollinated and therefore efficiently spread their pollen, and some trees become 'weeds' (invasive) and are hard to control after introduction to new continents/regions. These additional aspects need to be included when implementing risk assessment protocols to the evaluation of transgenic trees. We will discuss the data needed with reference to commercially important Danish trees.

Introduction

In most countries risk assessment of every new transgenic plant is required as is the case in the European Union (Council Directive 90/220/EEC). Furthermore, parties contracting the Convention on Biological Diversity are required to take steps to regulate and manage the risks associated with the use and release of living modified organisms resulting from biotechnology. An important principle for risk assessment is that the effects must be evaluated on a case-by-case basis through a stepwise procedure (Council Directive 90/220/EEC; OECD, 1986). But even a risk assessment based on these principles has limitations. First of all we can only test the effects we are aware of, next, there exists a divergence between the available data and the effects evaluated both in time, scale and complexity. This mandates the implementation of the 'precautionary principle' as a guideline for risk management and set-up of monitoring programs and restrictions to the releases as feedback or follow-up mechanisms. An understanding of the biology of the species is needed in order to evaluate the different characteristics of the transgenic line, and to outline the possible responses of ecosystems. Monographs on the species (e.g. OECD, 1997a,b; OECD, 1999a,b) are of great value here. Furthermore, protocols for environmental risk assessment and test guidelines for minimum data requirements (Fig. 1.) have been developed (Strandberg et al., 1998; Strandberg & Kjær, 1998; Kjær et al., 1999). These guidelines, however, need to be improved in relation to the assessment of trees.

The work on the genetic engineering of plants has previously been directed towards agricultural one-year crops. Concerns about transgenic plants, as addressed by Directive 90/220/EEC, centre on seven issues of ecological, agricultural and health relevance:

- transgenic plants may be toxic or allergic to wildlife, humans or domestic animals,
- transgenic plants may become weeds in the field,
- transgenic plants may become invasive of natural habitats,
- engineered genes may be transfered to related weeds or wild relatives (the hybrid offspring may then become invasive),
- transgenes may affect non-target organisms,
- transgenes may affect ecosystems, biodiversity or regionally adapted genetic variation, and
- altered practice in the use because of the genetic modification.

Today, however, genetically modified trees (GMT's) are also produced. Then the question arises if any special concerns need to be taken into account when evaluating risks related to trees and bushes.
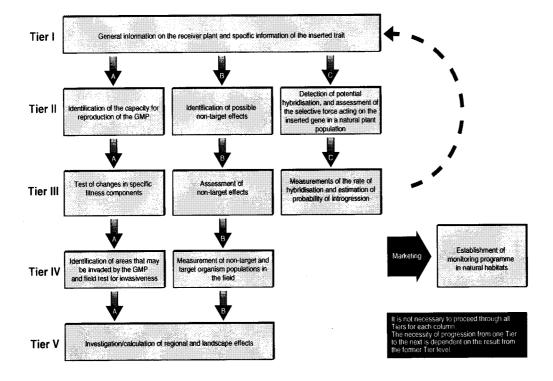

Figure 1. The tiered scheme of data requirements needed to perform risk assessment of annual crops before marketing (Strandberg & Kjær, 1998)

Ecological concern related to risk assessment of GMT's

The production of long-lived transgenic species, for example GMTs, represents a challenge to the risk assessment of environmental effects. Even for well-known species as Norway spruce (Picea abies) there is a lack of information especially on ecosystem related topics (Tømmerås et al., 1996). Full life-cycle experiments will often be impossible. Moreover, stochastic changes in climatic and biological

conditions as well as changes in management practice may be considerable over the full growth period and these parameters may, to a certain degree, affect the uncertainties related to the risk assessment of trees.

In the following, we will address some of the above mentioned key questions related to the ecology of trees.

Domestication

Highly domesticated species are expected to pose little risk to the environment and unwanted effects can be controlled by human intervention. Most trees, however, are essentially non-domesticated and contain, therefore, the whole genetic variation of the species. Temperate trees exhibit large genetic variability due to seasonal environmental stresses, periodic climatic extremes and stress from a range of pest organisms (Mullin & Bertand, 1998). This genetic variability is needed to sustain variation in growth conditions during their long life cycles. However, some selection for e.g. growth and pest resistance has been done in forestry, but essentially the produced plants are 'just' natural varieties with specific traits and rarely cross-bred progeny. Furthermore, these selected varieties as well as future and present GMTs are major ecosystem components adjacent to natural populations of closely related species/provenances. The genetic structure of a planted stand closely resembles wild populations (Mullin & Bertrand, 1998). This raises both the problem of gene flow into the natural populations from the GMT and changes in occurrence, distribution and performance of the tree in its natural area. After reaching the reproductive stage, control of spread becomes extremely difficult as argued for Norway spruce (Tømmerås et al., 1996).

Keystone species

Some species are ecologically more important than others. Such species have been termed keystone species and exert disproportional control within an ecosystem. Its experimental removal or introduction causes major ecosystem-level changes in structure, dynamics or nutrient flow (Crawley, 1995). Trees have the potential of being keystones by: 1) providing structures and resources for a large variety of species including plants, fungi, insects, birds and mammals, 2) influencing local climate, available light, water and nutrients, 3) being competitors with the potential to invade and become dominant in most ecosystems, and 4) being the dominant growth form of climax vegetation in many parts of the world. Therefore, environmental concerns related to the release of GMTs may be large and effects to the environment need to be carefully investigated before release.

Gene flow

One of the main concerns regarding large-scale use of transgenic plants is the risk of gene flow from the transformed crop to other organisms. Gene flow by sexual transfer between the transformed plant and wild relatives is the most likely way of such transfer. For most temperate and boreal forest tree species, gene flow is facilitated by wind-born pollen that may travel long distances. For most tree species, pollen flow is far more important in keeping up genetic diversity than gene flow by seed (Levin & Kerster, 1974). In relation to gene conservation, hybridisation by pollen is presumed to be the largest hazard for plant species (Ellstrand, 1992). As for crops, it would be relevant to state if introgression of insertions between the GMT and other trees in the neighbourhood takes place and to test the long-term stability of the inserted genes.

Long lived organisms

Trees are large, long-lived organisms, and essentially non-domesticated. Life cycles are much longer than for most agricultural crops. During its lifetime, a forest tree must cope with seasonal environmental stresses, periodic climatic extremes and fluctuating pressures from a range of pest organisms.

One of the important differences in risk assessment between agricultural crops and trees is the feasibility of testing. Testing trees over a full generation is not feasible and test procedures for crops should be redesigned in relation to trees.

Species attractive for genetically modification in Denmark

Many native Danish tree species like Fagus silvatica, Quercus ssp, Fraxinus excelsior etc. will hardly be subject to genetic modifications in Denmark. These species are slow growing with a fairly long rotation age. Presently, there are only a few native Danish species, which may be targets for genetic modification. Populus tremula and Betula pubescens are common and are objects for genetic modifications in other countries, but are without commercial interest in Denmark.

About 2/3 of the tree species planted in Denmark originate from non-native species. There are broad interests in using trees from other areas in Europe and North America. This is especially the case with conifers, monumental trees and bushes. Obviously, fruit trees may be relevant for genetic modification. There will be appealing breeding targets on domesticated species of Malus, Pyrus and Prunus. A major environmental concern of using genetically modified fruit trees will be the risk of introducing genetically modified genes to the native stands of Malus ssp. and Prunus avium. Wild cherry (Prunus avium) itself may be interesting for genetic modification and it is easy to clone. Elm (Ulmus glabra) has been important for shelterbelts and other protective plantings in the open landscape and on some locations there is no replacement for it. Elm disease, however, has made it impossible to use elm for plantation. Genetically modified elm, resistant to elm disease, therefore, would be very beneficial for landscape plantations and plants for the city.

Case: Genetic modification of Christmas trees in Denmark

Production of Christmas trees and greenery has become increasingly important in Denmark. The production of Christmas trees occupies about 5% of the traditional forest area, but covers 40-50% of the economic revenue. There will be specific interest for improvement of Christmas trees. Target for breeding could be insect resistance, needle retention, internodial length and resistance to herbicides. Genes for several of these characters have been developed for agricultural crops.

Genetically modified trees of Nordmann fir, Abies nordmanniana, have been produced in Denmark and trees are expected to be ready for field trials within 2-3 years (Find et al., in prep.). A few markers have successfully been inserted within the genome. The next phase probably includes insertion of an aphid resistance gene. The protein lectin (Galanthus nivalis agglutin), that is produced by snowdrops (Galanthus nivalis), are toxic to insects for example aphids (Hilder et al., 1995). The gene, which codes for lectin production has successfully been transferred to crop plants e.g. potatoes (US patent 4870015) tobacco and oil seed rape (Hilder et al., 1995; Goswami et al., 1998). Aphid resistance may significantly reduce the need for insecticide spraying in Nordmann fir cultures.

Biology of Abies nordmanniana

Abies nordmanniana was introduced to Denmark in 1884 and rarely, was it used as a replacement for Abies alba. From 1960 and on, the species became valuable for Christmas tree and greenery production.

The natural distribution of A. nordmanniana is within the eastern part of the Black Sea area on mountain ranges between 600 and 2000 m (at sea level). A number of related species exists within the Abies genera. Most closely related is A. bornmülleriana growing in the western part of the Black Sea area. Other related species are A. equi-trojani and A. alba. Based on isozyme analysis, they are all considered as different species (Konnert et al., 1992; Simsek, 1992).

The European Abies species are easily hybridising (Greguss, 1994; Kormutak, 1994). Hybridisation has often been observed in Danish seed orchards of A. nordmanniana. Pollen from A. alba will freely hybridise with A. nordmanniana and the offspring has no value as Christmas trees because of their intermediate morphology. Trees and stands of A. alba within close distance of a A. nordmanniana seed production area, therefore, are removed if the seed stands should be approved for commercial trade. The specific gene flow pattern (pollen flow, seed dispersal and flowering biology) for A. nordmanniana are poorly known. The pollen of Abies species is equivalent in size and form to pollen of Picea, but larger than Pine pollen.

Normally, Abies trees are fertile when they are 20 years and pollen has the potential to move over large distances. The species are mainly outcrossing, but inbreeding could be expected because of the scattered distribution. Large variation of morphological and physiological traits has been observed between provenances of A. nordmanniana.

A. nordmanniana is used in highly managed plantations in the landscape or within the forests in Denmark. The species are rarely found as mature trees in forest ecosystems for timber and pulp production and used much less than the related A. alba. Flowering normally occurs later in the life cycle and A. nordmanniana is less susceptible to infections by Dreyfusia nordmannianae than A. alba. The growth rate of A. nordmanniana is less than for A. alba and both species are constantly affected by game. A. nordmanniana is not known to invade natural ecosystems and natural regeneration has rarely been observed. Like A. alba, the species can only survive when properly managed including strong game control.

A. nordmanniana is often used for Christmas tree production, and all trees are harvested before they are fertile. Areas carrying older Christmas tree stands are occasionally left for greenery production. The stands are established very intensively with soil management, herbicide treatment and fencing. Because of periodical attacks of aphids and often as a precaution, the Christmas tree stands are intensively treated with insecticides. However, forest areas are regarded as potential water reservoirs, and therefore, the use of insecticides are undesirable and prohibited in the national forest service from 2003, and probably also in the private forests within a short period. Many new research actions have been initiated to reduce the use of pesticides especially in Christmas trees plantations.

Environmental consequences of possible release of insect resistant GM Christmas trees in Danish forestry

A possible release of GMTs will require a careful study of the possible adverse effects to the environment. Here we discuss some potential environmental consequences of the hypothetical case of aphid resistant GM Christmas trees based on the seven issues mentioned above. A risk evaluation for release of a genetically modified Abies nordmanniana resistant to aphids should be based on more analysis

than done here. However, the specific case is quite simple compared to other species and traits, and could be an important hint for future releases of genetically modified trees.

Toxic or allergenic effects

Lectins are known to be toxic to mammals (Els et al., 1998). Browsing by larger animals may be excluded by fencing but the effects to these animals e.g. roe deer and red deer need to be demonstrated. Toxicity to humans, of relevance to the assessment of effects to human health, also needs to be tested.

Effects on non-target organisms

The possible effects on non-target organisms should be demonstrated (Kjær et al., 1999). These effects encompass both toxic effects on other organisms than the aphid and indirect effects on aphid predators e.g. ladybirds and other consumers within the food web. Indirect effects may also occur due to changed management.

Invasion and introgression

Nordmann fir is not invasive to natural ecosystems but it needs to be demonstrated that the transgenic plant does not differ from this. While Abies species easily hybridise, Christmas fir rarely grow to fertility. Introgression of the genetically modified characters on Abies alba can probably not be visualised in risk analysis because of the long rotation time, but magnitude of introgression may be demonstrated through fitness characterisation and estimation of gene flow rates. The latter can be done by parental analysis with molecular markers.

Ecosystem effects

Abies nordmanniana does not exert disproportional control within natural ecosystems and may as such not be considered an ecological keystone species. An inserted gene coding for lectin production, however, may have significant influence on biodiversity within the forest ecosystems. Especially since the insect fauna may be influenced, subsequently, affecting consumers within the food web.

Concluding statements and future activities

The last decade of environmental risk assessment of transgenic plants has been a learning process. Not only members of industry but ecologists as well were slow to provide more than vague guidance on risk assessment. The assessment of adverse environmental effects of GMTs, however, still represents a challenge. Many of the shortcomings related to the environmental risk assessment of annual crops, e.g. the extrapolation of results obtained in small-scale greenhouse or laboratory experiments to large scale and long-term effects under field conditions, become extremely important when assessing risks related to long-lived species like trees. This points to future improvement of existing guidelines and models and the development of new ones that focus on the specific problems related to the risk assessment of trees.

Background information on the biology of tree species are scattered and monographs on the species like the one on Norway spruce (OECD, 1999) will be very useful and expose the gaps in our knowledge of the biology of the species and ecosystem functioning.

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Annex I

Report of the Plenary Sessions

INTRODUCTION

The OECD Workshop on the Environmental Considerations of Genetically Modified Trees was held in Trondheim, Norway from 13 to 15 September 1999. There were 47 participants from 15 countries (Austria, Belgium, Canada, Denmark, Finland, France, Germany, Japan, Korea, The Netherlands, Norway, Sweden, Switzerland, United Kingdom, United States).

The workshop was organised by the OECD's Working Group on Harmonization of Regulatory Oversight in Biotechnology. The need for such a workshop was first identified at the 4th Session of the Working Group in 1997, when it was finalising its first tree consensus document (Norway Spruce). Since then, Norway has played an essential role in organising and hosting this workshop together with other countries in the Steering Committee (Austria, Canada, Denmark, Finland, the Netherlands and Norway) [ENV/MC/CHEM/BIO/M(97)2]. The Working Group discussed the arrangements at several subsequent meetings and decided that the workshop should consider the "environmental effects resulting from genetic modification of long lived species" [ENV/JM/BIO/M(98)1]. The workshop should report back on its findings to the Working Group.

Four plenary sessions were held at the workshop as shown below. There were presentations from 21 experts from 11 countries. The papers presented during these sessions covered the major issues concerning environmental safety aspects of the unconfined release of GM trees. They presented a comprehensive overview of the current technology and progress in risk/safety assessment.

Session title	Chair
Session 1. Opening Session	Dr. Bjørn Åge Tømmerås, Norway
Session 2. Present status and future possibilities in gene technology concerning GM-trees	Dr. Birgit Loos, the Netherlands
Session 3. Contribution from forestry practice and siviculture in risk assessment	Dr. Marja Ruohonen-Lehto, Finland
Sesssion 4. Systems and challenges in assessing environmental considerations	Dr. Pierre Charest, Canada

At the end of the second day, the workshop formed four working groups to discuss the following issues:

- Assessment criteria to cover safety considerations for unconfined release of genetically modified trees

- Information requirements to cover safety considerations

- Safety considerations for unconfined release of genetically modified trees

- Monitoring of genetically modified trees

Following the discussions in the four working groups, a plenary discussion was held on the final day. This identified a number of key points. For example, trees were considered to include both forest trees and fruit trees. The papers indicated the very complex nature of the issues, which must be addressed before long-lived organisms such as trees can be released on a commercial scale. It is their longevity and size, which clearly distinguish trees from other crop plants as regards safety considerations. On the other hand the longevity of trees allows monitoring over a long period of time and the effects of climate and other factors can be evaluated more extensively.

This report presents the proposals from the workshop to the Working Group on Harmonization of Regulatory Oversight in Biotechnology. It also identifies additional points raised during the General Discussion at the end of the workshop.

PROPOSALS TO OECD WORKING GROUP ON HARMONIZATION OF REGULATORY OVERSIGHT IN BIOTECHNOLOGY

Various points, regarding environmental assessment and deliberate release of GM-trees, were identified by the workshop which could be discussed further by the OECD Working Group. In particular, in the workshop, it was agreed that:

- In the foreseeable future, most genetically modified trees will be used in intensively managed plantations for timber production and to a lesser degree for fruit or nut production as well as ornamental tree production.

- As regards assessment criteria, case by case evaluation and stepwise procedures should be the same as for agricultural GM-crops, but with more focus on temporal and spatial scales, since trees normally are long-lived species and will be present often as key species in the environment for a long time.

- The need for long term evaluations and predictions can be in conflict with the possibility of achieving them, e.g., because of (i) scarcity of knowledge and (ii) expanded time means larger variation in stochastic and environmental variables.

In addition, due to the specific attributes of trees including their long rotation time, a number of topics were identified for further consideration, for example.:

- Gene flow – longer distances, bigger scale and larger time frame
- Trees are often key species in ecosystems
- Enduring effects on non-target species
- The long term effects on target species
- Long term stability of genetic modifications
- The multiple uses of forests
- The level of domestication of the species involved

Gene-flow may occur, but what are the biological consequences? Because of the longevity factor, the genes will be in the environment for a long time. Also with changes in land use the outcome of gene-flow will be difficult to predict. The effect of gene-flow, however, will be dependent on the specific GM-tree (species, traits, phenotype) and its interaction with the environment. This situation needs to be assessed on a case-by-case basis.

Long term stability in relation to the expression of the GM-trait(s) itself, to reproduction, to pleiotropic events affecting the phenotype, etc. need to be considered closely for trees because of the fact that they are long-lived species. This increases the chance of instability developing over time either as i) a result of instability in the genome itself or ii) as a result of changing environmental conditions. To ensure long term stability of a GMO is also important to the regulatory authorities because their assumptions and monitoring strategies are based on knowledge of the initial situation.

Introgression/Fitness is not an issue specific to GM-trees as compared to other agricultural crops, but for trees, there is the need to consider the issue more closely because of the longevity factor and relationship to natural populations. The situation can be assessed in the same way as in crops, but there is a need to expand the time-frame and increase the area of observation. It is problematic to obtain data over such a long time, which means the environmental assessments will be more difficult and the situations will be harder to predict. As well, the time of interaction with the environment is much longer than that for crop plants. For instance in comparison to crop plants, which often are rotated – the selection pressure will be extended.

In addition to further consideration of the above points it was proposed that the OECD should:

a) Determine what constitutes a reasonable length of time (part of rotation time) when assessing a genetically modified tree. At present there are no guidelines or rules as to the period over which it should be tested or monitored.

b) Undertake and develop case studies (theoretical) for a number of species in order to identify problems when preparing environmental risk assessment for trees.

c) Gather information on the ecology/biology of a range of tree species, by continuing with the development of consensus documents. This information is required for each species when grown in: (i) natural forests and (ii) plantations.

POINTS AND QUESTIONS FROM THE GENERAL DISCUSSION

A general discussion session was held at the end of the workshop. As a result, the following points were raised and intensively discussed.

First, before entering into the general discussion on the risk/safety assessment of genetically modified trees, the importance of distinguishing between various tree species was emphasised. In other words, the approach of the discussion on the subject should be on "species by species" basis, because many species are not genetically modified yet or are far from being commercialised, while some others are on the verge of being commercialised, e.g. poplar. It is important to be clear on which tree species is under discussion.

Some disagreements were observed on the approaches to potential risks related to the release of GM-trees. In particular, there were different views on the following questions:

- Should the risk assessment of GM-trees be any different from other conventionally bred trees?

- In comparison with GM crop plants, do GM-trees need additional risk assessment because of increased chances of unforeseen effects related to potential instability and pleiotropic effects?

- Are established risk assessment approaches for crop plants – such as the concepts of "familiarity and substantial equivalence" - also good approaches for GM trees?

The general discussion also addressed some specific cases, for example, the environmental safety of a genetically modified tree, whose lignin level is reduced. It was pointed out that lignin is very important for the tree to resist insect herbivory, so that specific environmental safety considerations are needed on this particular type of GM trees. On the other hand, GM-tree plantations with increased fibre content could reduce pressure on natural forests in the future, when economic demand for paper pulp is assumed to be much higher than today. The discussion about the risk of horizontal transfer of antibiotic resistance from GM-trees to microorganisms in the soil is comparable to crops. Consequently, field trials with careful monitoring are important to assess these points.

In addition, it was pointed out that there ought to be reproductive boundaries for the GM-trees (e.g., sterility, harvesting before flowering, not planting when there is a chance of hybridisation with wild species) to minimise the chance of gene dispersal to the natural environment. On the other hand, it was also pointed out that GM trees are poor competitors in natural environments outside of plantations.

Risk assessments and the management of natural ecosystems must take into consideration the fact that forestry is different from the cultivation of crop plants. Trees are normally present for a long time whereas crop plants are rotated in a short period of time.

SUMMARIES OF THE DISCUSSIONS IN THE FOUR WORKING GROUPS

The following four working groups were held at the end of the second day of the workshop.

Discussion Subject of Each Working Group	Group leaders
Working Group 1. **Assessment Criteria** to Cover Safety Considerations for unconfined release of genetically modified trees	Dr. Marja Ruohonen-Lehto, Finland
Working Group 2. **Information requirements** to cover safety considerations	Dr. Jan Højland, Denmark
Working Group 3. **Safety Considerations** for Unconfined Release of Genetically Modified Trees	Dr. Pierre Charest, Canada
Working Group 4. **Monitoring** of genetically modified trees	Dr. Birgit Loos, the Netherlands

WORKING GROUP 1 - ASSESSMENT CRITERIA (TO COVER SAFETY CONSIDERATIONS FOR UNCONFINED RELEASE OF GENETICALLY MODIFIED TREES)

Firstly, to focus the discussion, the following points were clarified. There is a clear difference between fruit trees and forest trees. A difference was also noted between natural forests and intensive plantations (wood farming), and between urban forestry and the growth of ornamental trees. The Working Group decided to concentrate on intensive plantations, urban forestry and ornamental trees.

Secondly, it was noted that the following issues should be carefully considered when assessing genetically modified trees:

- **Stability** of the trait over the life cycle of the species was considered important since trees are long living species and, thus remain in the environment for a long time. Pleiotropic effects may not be noticed in the early part of the life cycle of the GM tree. Also, the integration site in the genome and its possible effects were discussed.

- **Gene flow, transfer into natural populations, introgression and effects on fitness** were also discussed. Tree pollen can travel long distances, and so gene flow is an important consideration. The possibilities and issues affecting introgression were discussed (allele frequencies, selection advantage, genetic drift). Fitness and the difficulty of its assessment were considered.

- **Effects on non-target species** are difficult to assess.

- Trees in natural ecosystems are often **keystone species**. A GM tree, which has such keystone status, should be carefully assessed.

As a summary of the issues to be considered when assessing GM trees, it was noted that: i) Prediction of environmental effects is very difficult when long living species are assessed (mathematical models, stochastic effects etc.); ii) issues mentioned above are not specific to trees (long living species), but must be assessed with a "tree-specific" emphasis.

Thirdly, the following key questions were discussed with regard to the invasive potential of GM trees.

a) Is the expression of genetic modification well documented and understood?
b) Is it an ecological novelty?
c) What is the degree of domestication?
d) Is it a keystone species within the given environment?
e) Do natural barriers exist or is it controllable by human intervention?
f) Can we simulate the dynamics of the release into the environment?

In particular, Issues b, c and d were considered to be of major importance when considering assessment criteria. (See earlier comments and comments of working group 3 as well as the general discussion.)

Fourthly, the final "exercise" was to consider some specific cases of GM Norway spruce, which contains 3 different genes: 1) root rot gene from Norway spruce, 2) root rot gene from Sitka spruce and 3) anti-fungal gene of bacterial origin. The working group considered their long term stability, non-target organisms, gene flow, fitness and effects on target organisms. However, the working group could not reach a consensus on whether the assessment criteria should be different between these different cases. As regards gene flow and fitness, even if the inserted gene originally came from the same species, in that case Norway spruce, allele frequencies could change and the GM Norway spruce's allele may be rare in the natural population and thus be the subject of serious considerations. The working group did not have a consensus on these cases, especially on gene flow and fitness issues.

WORKING GROUP 2 - INFORMATION REQUIREMENTS TO COVER SAFETY CONSIDERATIONS

The following information requirements were proposed as a result of the discussion of Working Group 2.

- Plant biology
  - taxonomy
  - reproductive biology
  - distribution (natural, induced, origin)

- Gene(s)
  - what sequences have been introduced
  - what gene products are expressed
  - constitution of the function of the products
  - the source organism(s) for the sequences

- Interaction with environment
  - concepts of familiarity
  - concepts of substantial equivalence

– management (altered use)
– possible changes in land use
– fitness related parameter(s), e.g. introgression, invasiveness
– susceptibility to biotic and abiotic stress
– non-target organisms (selection of indicator organisms)
– description of ecology (food-web, keystone species)

The discussion in the working group also focused on other points. For example, according to the assessment result, the release of a particular GM tree might need to be restricted to a certain geographical area or during a certain period of time. In that case, the above mentioned points need to be re-examined.

Monitoring

In order to implement the effective monitoring of field trials, the working group recognised the need to discuss further which parameters and indicator organisms should be observed in order for monitoring to provide additional evidence useful in a risk/safety assessment. It was also noted that the interval between re-examination, when needed, may be set on a case-by-case basis.

WORKING GROUP 3 - SAFETY CONSIDERATIONS FOR UNCONFINED RELEASE OF GENETICALLY MODIFIED TREES

Assessment criteria to cover safety considerations for the unconfined release of GM trees should be developed on the basis of the following issues:

- Plant biology
- Gene(s)
- Interaction with environment
- Baseline information

Working Group 3 also developed the following recommendations:

- The OECD should concentrate its effort on GM of trees in plantation systems. Development of GM trees for the use in natural environments should not be encouraged unless they are to be used in an intensively managed context for a predominant product.

- An important exception to the above recommendation and a major benefit of GM technology could be the rescue of long lived species and varieties under threat (elms, chestnut and fruit trees).

- Ecological significance of gene flow has to be evaluated in the context of the species, trait, rotation and management practices.

- Transformation work and resultant commercialisation, if it occurred, should not reduce the genetic base (more of a production issue).

- Horizontal gene transfer is of low risk and does not require additional assessment.

- Monitoring of genes that directly affect environmental safety should be a compulsory part of any regulatory scheme (BT, Sterility). Where the novel trait is primarily a production trait, monitoring should not be a requirement.

- The OECD should consider the development of a number of case studies for GM trees to define and provide information for use in environmental assessments.

- Degree of acceptable risk should be assessed with a similar degree of rigor as that employed in conventional breeding.

WORKING GROUP 4 - MONITORING OF GENETICALLY MODIFIED TREES

The working group recognised that monitoring after commercial release may be needed to confirm assumptions made during the evaluation process. The level and type of monitoring will vary depending on:

- species
- trait
- management practices
- rotation
- environment / ecosystem

Of these issues, management practices, rotation and environment/ecosystem are likely to be of specific importance for trees, when compared with other crop plants.

Level/type/parameters detected for monitoring at the time of commercial release

In particular, Working Group 4 discussed the following issues within the consideration on levels, types and parameters of the monitoring after unconfined release.

- the difference between vegetative and reproductive phases of GM trees

- Possible gene flow to wild relatives
    - Once a GM tree is authorised for commercialisation, it would be deemed that there is an acceptable mechanism in place (e.g., such as natural barrier to cross with wild relatives), which may keep low level of gene flow or its risk.
    - Monitoring should confirm the efficacy of acceptable mechanisms to control gene flow

- Possible gene flow of insect resistance genes
    - non-target effects could change over rotation
    - Diversity should be maintained

- Horizontal gene transfer
    - the issue should be resolved prior to commercial release
    - there was no consensus on its importance/relevance

- Monitoring in a "project-like" manner
    - EU-projects
    - There should be sub-plots and representative plots
        - adequate controls and replicates should be performed
    - The area inside a release
        - the area of monitoring should be defined
        - monitoring of the whole area is difficult
    - Outside release area: monitoring should include
        - Impact on forest/natural area
        - long term impact

ANNEX 2
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