



Genetically modified trees

Status, trends and potential environmental risks

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Abstract

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This report provides a review on the current status and trends observed in the field of genetic modification of trees. Based on a literature review and the consultation of relevant researchers, it further provides an overview of potential environmental risks related to the deliberate release of transgenic trees into the environment. The report focuses on differences between crops and trees and identifies the potential risks of introducing transgenic trees in the Netherlands.

Keywords: genetic modification, transgenic tree, environmental risks

Cover photo: Field trial with genetically modified trees in Beijing District, China.
Sven de Vries, 2001

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Summary

Genetic modification (here defined as 'the alteration of genetic material in an organism in a way that does not occur naturally by mating and/or natural recombination') of trees is expected to achieve important economic and environmental benefits. Currently, *Populus* is by far the most studied tree genus for genetic modification (GM) purposes, and the number of transgenic tree species is increasing in genera like *Populus*, *Pinus* and *Eucalyptus*. The main traits subject to genetic modification in tree breeding include herbicide tolerance, resistance to pathogens and abiotic stress, wood composition and growth rates, and phenology (mainly flowering and fruiting).

Trends

There is a global shift in the forestry sector towards timber production in tree plantations, where the use of GM trees might be considered. Volume increase, faster growth, size and stem characteristics are among the traits subject to GM research in forest trees. A clear trend is the increased interest in breeding new varieties of fast-growing short rotation trees such as pines or *Eucalyptus* for wood and fibre production. Both in forest as well as in fruit trees genetic modification research has a growing focus on traits such as tolerance to biological (plant pathogens or pests) and chemical stresses (use of pesticides) and adaptability to various soils and climate. Controlling pollen and gene flow of long-lived species is important, especially stimulated by concerns of spread and outcrossing of transgenes. Recently the transfer of *Rhizobium* or *Cyanobacterium* N-fixation strategies to trees has become focus of genetic modification in trees.

We expect that especially fruit trees will be increasingly researched for GM deployment in the Netherlands: pesticide tolerance, fungal resistance (like apple scab) and male sterility genes are commercially interesting traits in fruit trees. Among forest trees, fast growing or low-lignin poplar trees seem interesting for the Netherlands. Conifers with increased growth or delayed shedding of needles, could also be interesting for the Netherlands, though this research is still in its infancy.

Empirical data regarding the behaviour of transgenic trees in the environment are largely lacking. General concerns about genetic modification of crops, such as the instability of transgenes, pleiotropic effects, resistance development and effects on non-targeted characteristics seem also to be relevant for trees. However, some of the potential environmental risks may be different for trees compared to annual crops as trees are important drivers of terrestrial biodiversity, due to their long lifespan and the high number of organisms associated with them.

Environmental risk assessment

Aspects that seem to be specific for the environmental risk analysis¹ of GM trees (in comparison to transgenic crops) are:

- Potential environmental impacts may increase or be longer lasting (due to the longevity of trees);
- The impacts on large groups of tree-associated organisms that are potentially exposed to transgenic trees (e.g. insects, birds, litter biota, symbiotic fungi);

¹ Following the EU directive 2001/18/EC on the deliberate release of GM organisms in the environment.

- The potential environmental impact as a result of outcrossing of transgenes among trees (considering the ease with which some species hybridize, and the long distance dispersal of pollen, and the high number of wild relatives);
- The potential impact that GM trees may have on long-term biogeochemical processes like the decomposition of organic material (due to the long rotation time and the use of traits that alter the wood composition).

Samenvatting

Status

Genetische modificatie (hier gedefinieerd als de 'verandering van genetisch materiaal in een organisme op een manier die niet onder natuurlijke omstandigheden geschiedt door voortplanting en/of natuurlijke recombinatie') van bomen zal naar verwachting leiden tot belangrijke economische en milieuvordelen. Momenteel is *Populus* onder de bomen veruit het meest bestudeerde genus voor genetische modificatie (GM)-doeleinden, en het aantal transgene boomsoorten in genera als *Populus*, *Pinus* en *Eucalyptus* blijft verder toenemen. De belangrijkste eigenschappen waarop de genetische modificatie van bomen gericht is zijn onder meer herbicide-tolerantie, resistentie tegen ziekten en abiotische stress, houtsamenstelling en groeisnelheid, en fenologie (vooral bloei en vruchtzetting).

Trends

Er is een wereldwijde verschuiving in de bosbouwsector in de richting van productie uit plantages, waar de toepassing van GG-bomen kan worden overwogen. Volumetoename, versnelde groei en stamvorm behoren tot de eigenschappen voor genetische modificatie in bosbomen. Een duidelijke trend is de toenemende interesse voor het kweken van nieuwe variëteiten van snel groeiende, korte rotatie bomen zoals dennen of *Eucalyptus* voor pulphout of bio-brandstof, in tropische en subtropische regio's. Zowel in bos- als in fruitbomen richt genetische modificatie zich in toenemende mate op het verkrijgen van tolerantie tegen biologische (pathogenen of plagen) en chemische stress (gebruik van pesticiden) en de aanpassing aan veranderde bodems en klimaatomstandigheden. Regulering van pollen- en genverspreiding bij lang levende soorten is van toenemend belang, voornamelijk gestimuleerd door bestaande zorgen over verspreiding en uitkruising van transgenen. Sinds kort vormt ook het overbrengen van N-fixatie strategieën uit *Rhizobium* of *Cyanobacterium* naar bomen een belangrijke focus van het onderzoek naar GG-bomen.

We verwachten dat het GM onderzoek naar met name fruitbomen in Nederland de komende tijd zal toenemen: pesticidetolerantie, schimmelresistentie (zoals appelschurft) en mannelijke steriliteit zijn commercieel interessant bij fruitbomen. Onder de bosbomen lijken populieren met versnelde groei of een verminderde lignine-aanmaak interessant voor Nederland. Coniferen met versnelde groei of vertraagde naaldval zijn mogelijk ook interessant voor Nederland, maar onderzoek hiernaar staat nog in de kinderschoenen.

Empirische data over het gedrag van transgene bomen in het milieu ontbreken veelal. Algemene zorgen over genetische modificatie van gewassen, zoals de instabiliteit van transgenen, pleiotropische effecten, resistentieontwikkeling en effecten op niet beoogde eigenschappen lijken ook te gelden voor bomen. Niettemin zal een aantal mogelijke milieurisico's van bomen verschillen ten opzichte van eenjarige gewassen omdat bomen door hun lange levensduur en het grote aantal organismen dat ermee is geassocieerd, belangrijke dragers zijn van terrestrische biodiversiteit

Milieurisicoanalyse

Aspecten die specifiek gelden voor de milieurisicoanalyse² van GM-bomen (ten opzichte van transgene gewassen) zijn:

- Potentiële milieuvloeden kunnen toenemen of langer duren (door de lange levensduur van bomen);
- De invloed van grote groepen aan bomen geassocieerde organismen die mogelijk worden blootgesteld aan transgene bomen (b.v. insecten, vogels, strooiselfauna, symbiotische schimmels);
- De mogelijke milieuvloeden als gevolg van uitkruising van transgenen (aangezien veel soorten gemakkelijk kruisen, het pollen een lange weg kan afleggen en een groot aantal verwante soorten in het wild voorkomt);
- Het effect dat GG-bomen kunnen hebben op biogeochemische processen zoals de decompositie van organisch materiaal (met name door de lange rotatietijd en de toepassing van eigenschappen die de houtsamenstelling wijzigen).

² In lijn met de EU richtlijn 2001/18/EC over het vrijlaten van GG-organismen in het milieu

1 Introduction

Genetic modification is defined as 'the alteration of genetic material in an organism in a way that does not occur naturally by mating and/or natural recombination' (European Commission, 2001). Where traditional breeding programmes involve the crossing of plants of the same species or of closely related species, genetic modification allows scientists to insert genetic material (DNA or RNA) from one species into a completely different species or modify their genetic material. Insertion of a genetic sequence into the genome of the host plant can be done by microinjection (direct transformation), or using vectors such as *Agrobacterium tumefaciens* (indirect transformation).

The number of genetically modified (GM) plant species has been growing rapidly, and has resulted in the commercial deployment of transgenic crops in agriculture (e.g. Bt maize, HT rapeseed). Trees have become subject to genetic modification already in the late 1980s, but GM trees have gained significant interest only recently and they are expected to achieve important economic and environmental benefits. For example, enhancing tree productivity and clonal propagation are activities that are claimed to bear the potential to reduce pressure on native forests because less land would be needed for timber production. Furthermore, genetic modification may reduce environmental pollution by chemicals caused by a change in processing raw material to end products. For instance, improved pulping quality of GM trees will reduce the need of chemicals for bleaching and will cut down waste from pulp factories. However, public concerns on the potential negative environmental effects related to the release of transgenic trees have been increasing. Specific characteristics of trees, such as long distance pollen dispersal, may make it easier for transgenes to spread into the environment. Their longevity may increase the impact of the GM tree on its surroundings which may lead to unintended environmental effects. So far, there is little experience with GM trees in the field.

1.1 Aim and approach of this study

The aim of this project is to review the latest global developments in GM trees and to assess the potential environmental risks related to the release of GM trees in the field. In doing so, we focus on the most important tree species that are being targeted by GM techniques, as well as on the traits most commonly introduced by genetic modification.

There are two main questions we would like to answer: 1) Based on current trends, which development of GM tree species and traits can be expected in the Netherlands in the near future and 2) What are the specific environmental consequences related to the use of transgenic trees compared to transgenic crops, and how should these consequences be addressed in risk assessments?

We have addressed these questions by reviewing scientific literature and relevant policy papers. In addition, several key-players in the field of GM trees have been contacted and asked for the latest developments and new trends in the area of GM trees and risks. Our findings were used to make an overview of the risks that various GM-tree species might pose in the Netherlands. GM-traits that could potentially affect the environment were listed, and potential environmental risks were described according to the structure of the EU directive 2001/18/EC (European Commission, 2001).

This report focuses on both fruit and forest trees, which are here defined as woody perennials which are either being used for fruit production or as forest plantation trees. We compare the GM developments in

trees with those in agricultural crops like corn and soy. In this study, the term 'crop' is used for crop plants that are cultivated as annual plants, in a crop rotation system, irrespective of their natural growth cycle (which may be annual, biannual or perennial). Ethical and economic issues are not regarded as part of this review.

1.2 Structure of the report

In chapter 2 an overview is given of the status and trends of GM trees worldwide, in Europe and the Netherlands. Chapter 3 deals with the potential environmental risks associated with field trials and commercial releases of transgenic trees. Special attention is given to the environmental risks related to transgenic tree species or traits that may be expected in the Netherlands in the near future. Finally, in chapter 4 the conclusions and recommendations of this study are given.

2 Status and trends of genetically modified trees

2.1 Status of genetic modification in trees

Developments in tree genetic modification have been relatively slow compared with those in crop genetic modification, particularly where it concerns GM trees designed for the production of industrial wood (Sedjo, 2005a and 2006). The first regeneration of a genetically modified forest tree was achieved in 1986 in the genus *Populus*. Since then, this genus has become a model for genetic modification and related biotechnology studies in trees. The first attempt to genetically modify a conifer (*Larix*) was reported in 1991 (Huang et al., 1991). Since the publication of the complete genetic sequence of black cottonwood (*Populus trichocarpa*) in September 2004, researchers have discovered that *Populus* trees basically have the same set of genes as herbaceous plants, but that these genes are regulated in different ways in trees (Cseke and Podila, 2004). In particular, the regulation of genes for wood formation and longevity are different. The development of GM trees has been accelerating over the last two decades, which resulted in the first commercial releases (CSL, 2007).

The Food and Agriculture Organisation of the United Nations (FAO) published an extensive review of the availability of genetic modification technology, based on a dataset covering over 2.700 major biotechnology activities in the period 1994-2004 (FAO, 2004). This publication by the FAO is the most recent review currently available containing quantitative and qualitative data³. According to their estimate, genetic modification is available for about thirty tree species, mostly species that are important for clonal propagation in plantation forestry (FAO, 2004).

Genetic modification is often considered to be a tool for improving knowledge of tree biology and gene function, for example for studying cell wall properties and wood formation. Research on the genetic basis of wood quality in forest trees has significantly increased, triggered by the discovery of genes regulating secondary tissue and lignin formation in the annual plant *Arabidopsis thaliana* (Goujon et al., 2003). The most significant breakthroughs in biotechnology are coming from research into the structure of genomes and the genetic mechanisms underlying economically and adaptively important traits (FAO, 2004). The recent completion of a genome sequence for *Populus trichocarpa* (in 2004) is perhaps the most notable accomplishment in forest tree genomics.

Traditionally, tree breeding programmes merely involved the selection of the best individuals of the economically most valuable tree species. Development of horticultural techniques such as propagation by cuttings, controlled pollination, grafting, as well as development of information technology, lead to the implementation of sophisticated tree improvement activities (e.g. Fladung and Ewald, 2006). Conventional long-term tree breeding programmes and large-scale clonal propagation techniques have resulted in, and will continue to provide, significant productivity gains, especially for fast-growing species such as *Eucalyptus* and *Populus*. However, the development of conventional tree breeding has been relatively slow for reasons related to the biology of forest tree species such as their longevity and

³ Several other qualitative reviews have been conducted since then, but these do not provide new quantitative data (e.g. Fladung and Ewald, 2006; Strauss et al., 2009).

outcrossing requirements (Campbell et al., 2003). Many commercial forest trees are outcrossing species with a high level of heterozygosity (Geburek and Turok, 2005). They therefore suffer from a high genetic load, meaning that many recessive deleterious alleles are retained within populations leading to inbreeding depression, which restricts conventional breeding efforts like selfing and backcrossing (Williams and Savolainen, 1996). This means that selecting for a naturally rare recessive allele of commercial interest is very difficult and time consuming with conventional breeding. To avoid this, many efforts to improve tree breeding use clonal (i.e. vegetative) propagation of selected superior individuals. However, this method still requires the selection of superior genotypes, which is often difficult and highly time consuming. Genetic modification offers a means of circumventing this problem as it allows the insertion of a novel trait in any genotype in a single generation, with modest or no modification of its other genetic properties (Campbell et al., 2003). Through genetic modification breeding of forest tree species can therefore be greatly accelerated. This is particularly important because tree breeding has long been hindered by the time trees require to become reproductively mature (Hoenicka et al., 2006). Further potential benefits of GM trees are summarized in Table 2.1.

Table 2.1
Benefits of GM trees (FAO 2004, modified).

Category	Potential benefits
Commercial	increased wood production; improved wood quality; resistance to insects and disease; reduced production and processing costs of wood or chips; and reduced chemical costs for pulping
Environmental	reduced pressure on natural forests; reduced use of chemicals in forests and in processing; phytoremediation and carbon sequestration; increased productivity, adaptation to stresses; reduced erosion; renewable energy
Human health	reduction in pollen and allergy problems; reduced environmental pollution; environmental protection and restoration
Other	potential economic benefits for developing countries and countries in transition; significant acceleration of conventional breeding programmes. Genetic modification could also provide basic biological knowledge and employment

2.2 Global trends in the development of transgenic trees

Modern biotechnology has potentially outstanding applications in the forestry and fruit-tree sector because of the possible genetic gains it could confer, such as new genetic pools and significant reduction in tree selection time (e.g. Frankenhuyzen and Beardmore, 2004; Fladung and Ewald, 2006). It is however difficult to predict the role of GM trees in the future. It has been argued that GM trees will be needed to find solutions for the increased pressure on trees and forests as a result of population growth, climatic change, and fossil fuel shortage (e.g. Strauss et al., 2009). One of the potentials of genetic modification technology might indeed be to reduce pressure on the world's natural forests by creating rapidly growing trees that fulfil the global demand for high quality timber and timber products. Consequently, GM is often considered as the most efficient long-term strategy for increasing forest productivity. In addition, genetic modification may also facilitate tree breeding and could potentially reduce pesticide and fossil fuel use. Genotypes exhibiting resistance to fungal or bacterial diseases could be generated through genetic modification, thereby offering an approach to save endangered species, such as the American chestnut (*Castanea dentata*). At this stage, however, the advantages of GM trees still need to be proved (Sedjo, 2005a).

It is expected that research into genetic modification stimulates the sequencing of the complete genomes in tree genera like *Populus*, *Eucalyptus*, *Pinus*. This type of forest tree genetic research increasingly comes from the public sector, whereas the private sector seems to focus investments more on specific traits of commercial interest (FAO, 2004; Frankenhuyzen and Beardmore, 2004). In member states of the Organisation of Economic Co-operation and Development (OECD), research into genetic modification is increasingly being funded and initiated by the private sector, as opposed to a decade ago, when most GM activities were funded by the public sector.

As underlined by the OECD, and partly confirmed by the results of a brief questionnaire sent to various key-players in the area of GM trees, the following trends are visible in the development of transgenic trees:

- There is a global shift in the forestry sector towards timber production in tree plantations, where the use of GM trees might be considered. Plantation forests are also considered as ideal resources for fibre production (e.g. the enriched-cellulose poplar, of which trials initiated mid-2009 in Belgium).
- There is an increasing interest in breeding new varieties of fast-growing short rotation trees such as pines or eucalypts for wood and fibre in high growth tropical and sub-tropical regions such as Florida, US (Sedjo, 2005; Arundel and Sawaya, 2009).
- The increased global demand for biofuels has recently raised the economic attractiveness of GM trees (e.g. fast growing tree varieties for bioenergy and fibre production). Indeed, volume increase, faster growth, size and stem characteristics (harvest index) are among the traits to be expected in future GM tree research.
- Research into GM trees has a growing focus on traits such as tolerance to biological (plant pathogens or pests) and chemical stresses (use of herbicides) and adaptation to various soils and climates.
- Controlling pollen and gene flow of long-lived species has become more important in the field of GM tree research, especially stimulated by concerns of transgene spread. This comprises the introduction of male or female sterility or reduced flowering into transgenic trees.
- GM tree research is focusing increasingly on modification of rootstocks of fruit trees.
- Recently also the transfer of *Rhizobium* or *Cyanobacterium* N-fixation strategy to trees has become focus of genetic modification in trees (M. Fladung, Institute for Forest Genetics, Germany. pers. comm.).
- According to B. Mezetti (Ancona Marche Polytechnic University, Italy. pers. comm.) most of the genes to be used for genetic modification, will be genes from the own genepool, that is from the species itself and all crossable species. Such events, called cisgenesis, are already available in apple scab resistant plants, albeit not on the commercial market.
- The genetic transformation of rootstocks, for example with genes to improve plant vigour or to increase virus resistance, is another trend in genetic modification research (B. Mezetti, pers. comm.). After grafting, the resistance factors can then transfer via the xylem to the scions.

2.3 Countries active in genetic modification of trees

The review published by the FAO in 2004 revealed that at least 35 countries are involved in genetic modification of forest trees. This includes all registered lab research and field trials. Most of this GM research occurred in North America (48%) and Europe (32%). Asia followed with 14% of the reported activities, Oceania with 5%, South America with 1% and Africa with less than 1% (Figure 2.1a).

Considering the large reforestation programmes in China and the role in global timber production of Brazil, these are the two most likely countries involved in GM tree commercialization (Sedjo, 2005b). European countries currently involved in GM research on trees are Austria, Belgium, Denmark, Finland, France, Germany, Great Britain, the Netherlands, Norway, Portugal, Spain and Sweden (Biotech 2007).

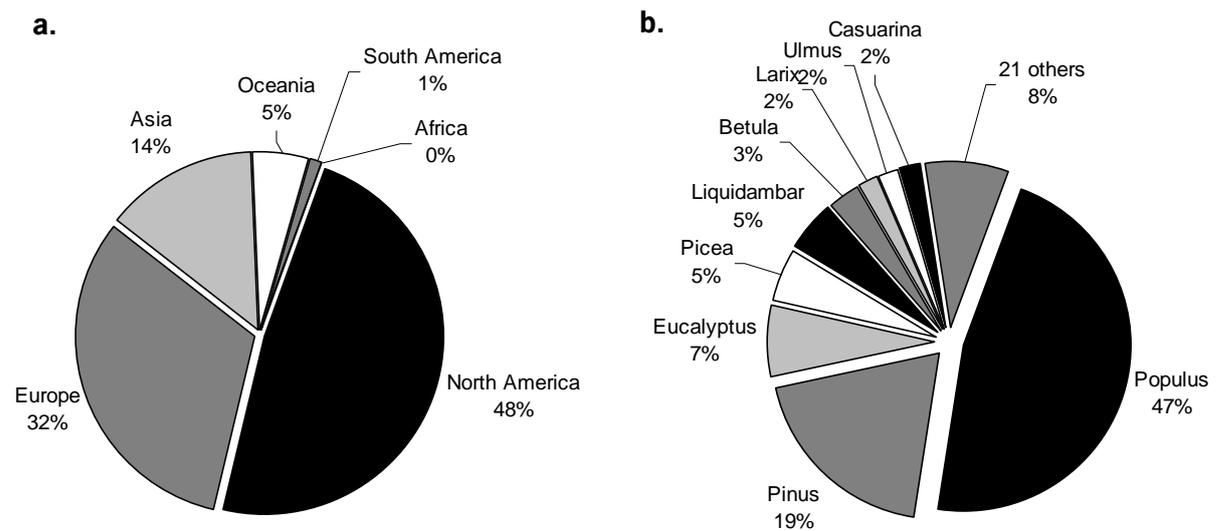


Figure 2.1

Share of different regions of the world active in genetic modification research (a) and share of species of forest trees in lab research and field trials (b) (after: FAO 2004).

2.4 Species used for genetic modification

By 2004, there were genetically transformed tree species in 30 genera (FAO, 2004). In 1986 the production of the first transgenic tree of poplar NC-5339 (*Populus alba* x *P. grandidentata*) was realized in the US (Sederoff, 2007). Worldwide, 80% of the lab research and field trials for forest tree species has been restricted to five genera: *Populus*, *Pinus*, *Eucalyptus*, *Picea* and *Liquidambar* (Figure 2.1b). Of these, *Populus* is the most studied tree genus for genetic modification purposes, accounting for almost half of all genetic engineering activities (FAO, 2004). It has become a model genus for tree genetics in general. The remaining 8% in Figure 2.1b involves the following 21 genera: *Abies*, *Acacia*, *Actinidia*, *Calocedrus*, *Carica*, *Castanea*, *Citrus*, *Diospyros*, *Hevea*, *Juglans*, *Liriodendron*, *Malus*, *Morus*, *Olea*, *Poncirus*, *Prunus*, *Pseudotsuga*, *Pyrus*, *Quercus*, *Robinia*, and *Tsuga*. The number of GM tree species is still increasing and the first commercial releases have already taken place (section 2.7). Appendix 1 gives an overview of transgenic forest and fruit tree species and their characteristic traits, with the number of field trials per country.

2.5 Traits subject to genetic modification

In forest trees genetic modification is mainly focused on herbicide tolerance, insect resistance, low lignin content, fibre production, heavy metal accumulation, and increased growth for biomass production (FAO, 2004). For example, trees with a reduced lignin content are easier to process in the pulp and paper industry and may provide economic and environmental benefits (Chen et al., 2001). In fruit trees, genetic modification is largely focused on pest resistance and herbicide tolerance. In comparison with annual crops, the breeding of trees is strongly limited by their long reproductive cycles. The long life cycle of trees as such is also subject to genetic modification, e.g. by inducing early flowering (Hoenicka and Nowitzki et al., 2006). The main traits subject to genetic modification of both crops and trees in the EU are shown in Appendix 3. In the following paragraphs, we will describe these traits in more detail.

2.5.1 Herbicide tolerance

Tolerance to herbicides such as glyphosate has been a major target for genetic engineering in trees (Strauss et al. 2001). Hybrids with high levels of glyphosate tolerance were created for *Larix decidua* (Shin et al. 1994) and *Eucalyptus grandis* (Jain & Minocha 2000). The first poplar hybrids with high levels of glyphosate tolerance were created in 2002 by insertion of the *CP4* gene (Meilan et al. 2002). In addition, transgenic white poplars have been developed that were completely tolerant to the herbicide phosphinothricin (Basta) under laboratory conditions (Confalonieri et al. 2000). Another way to achieve herbicide tolerance is by modification with a microbial gene encoding an enzyme for the detoxification of the herbicide. For example the *bar* gene from *Streptomyces hygroscopicus* specifies herbicide tolerance in *Pinus radiata* and *Picea abies* (Bishop-Hurley et al. 2001; Vengadesan et al. 2006).

2.5.2 Insect resistance

Damage of forest trees caused by defoliating insects can be significant and may result in substantial economic losses. Infected trees frequently experience limited growth and survival (Pena and Seguin, 2001). Efforts have been made to increase insect resistance through modification of the trees with *cry* genes from the soil bacterium *Bacillus thuringiensis*, coding for delta-endotoxins (Bt toxin). Bt toxins exhibit a specific mode of action against certain insect groups (Lepidoptera, Diptera or Coleoptera). The mechanism of action is that the toxin binds to specific receptors in the intestine and kills the insects due to pore formation. This modification has been successfully tested in poplar (Tian et al., 2000; Hu et al., 2001) and several conifer species (Lachance et al., 2007). Insects are however able to develop resistance to Bt toxins (e.g. MacIntosh, 2009).

For other mechanisms that confer insect resistance, the development of resistant insects seems less likely. One possible method to avoid insect resistance involves the introduction of a gene coding for a protease inhibitor that modifies insect digestion and causes the death of the insect. For example the introduction of potato gene *pin2* into *Populus alba* x *P. grandidentata* through the vector *Agrobacterium tumefaciens* significantly increased resistance to insects, but no resistant insects were detected (Klopfenstein et al., 1991). Another study showed that insect resistance in *Liquidambar styraciflua* increased by the combination of a *Bt* gene with a peroxidase anionic enzyme gene which is involved in cell growth and cell wall development (Sullivan and Lagrimini, 1993).

2.5.3 Resistance to viral, fungal and bacterial pathogens

Genetic engineering has allowed the development of bacterial and fungal resistance in fruit trees, and more recently also in forest trees. A number of genes from various origins has been used in this respect. Insertion of the endochitinase gene (*ech42*) from the fungus *Trichoderma harzianum* in black spruce (*Picea mariana*) and hybrid poplar (*Populus nigra* x *P. maximowiczii*), resulted in increased levels of resistance to several pathogens (Noël et al., 2005). Scab resistance in apples was successfully achieved in apple trees that were modified with a gene encoding an antimicrobial protein (hordothionin) from barley. Field trials with scab resistant apples in the Netherlands showed that the leaves of transgenic apple trees suffered 60% less infection (PRI, 2008).

2.5.4 Increased resistance to abiotic stresses

Some genetic modifications aim to induce tolerance to abiotic stresses like extreme temperatures, drought, salinity or chemical toxicity. Adaptation to cold, salt and frost was achieved recently in a transgenic larch hybrid (*Larix x leptoeuropaea*), which was modified to express a *Vigna aconitifolia* gene for pyrroline 5-carboxylate synthase, the rate-limiting step in proline synthesis. This modification resulted in elevated levels of proline in the plant tissue and this significantly increased resistance to cold, salt and freezing stresses (Gleeson et al., 2005). Successful results are also reported for salt tolerance in transgenic *Populus nigra* (Hu et al., 2005; Junghans et al., 2006) and cold tolerance in eucalypt hybrids (*Eucalyptus grandis* x *E. urophylla*) (FederalRegister, 2007).

2.5.5 Wood and growth characteristics

Wood structure and quality (e.g. fibre and lignin content) are among the most important traits subject to genetic engineering of trees. Wood with reduced lignin content allows less chemicals to be added during pulp processing, which could yield economic and environmental benefits (Chiang, 2006). One way of reducing lignin content is by introducing antisense genes that suppress enzymes that are crucial in lignin biosynthesis, such as 4-coumarate:CoA ligase (4CL) or cinnamyl alcohol dehydrogenase (CAD). Lignin content may be lowered by as much as 45% in transgenic trees (Chiang, 2006). Field trials with this type of GM trees have already been taking place for some years in the EU.

Genetic modification of trees often involves growth enhancement to accelerate the production of woody biomass. Trees are considered a potentially important source of carbon neutral biofuels with potentially high yields. Genetic modification seeks to produce trees with increased relative carbon partitioning to above-ground woody tissues, and increased cellulose availability for enzymatic digestion to release its component, fermentable sugars (Ragauskas et al., 2006). So far, increased growth was observed in transgenic *Populus trichocarpa* that was modified to overexpress glutamine synthetase (GS), a key enzyme in nitrogen assimilation. This modification increased nitrogen use efficiency of the tree and resulted in higher mean growth rates (Fu et al., 2003). Studies on *Jatropha*, another high potential biofuel crop, are underway (Li et al., 2008b).

2.5.6 Flowering modification

Induction of male or female sterility in trees is seen as an important way to reduce gene flow of transgenes into non-transgenic populations. So far, male and female sterility has been achieved in poplar (Li et al., 2000; Hoenicka et al., 2006). Flowering modification is used in another way to reduce generation times by stimulating precocious flowering. An extreme example of this is the ectopic expression of the *BpMADS4* gene in silver birch, in which the earliest flowers were produced already eleven days after rooting, when the seedlings were only three cm high (Elo et al. 2007). Other species in which early flowering was stimulated include citrus, apple and poplar.

2.5.7 Phytoremediation

Phytoremediation refers to the use of plants to remove, sequester or detoxify pollutants, including heavy metals and pesticides. So far transgenic poplars have been engineered with genes to take up heavy metals such as mercury, cadmium, copper and arsenic (Merkle, 2006), and detoxify pesticides and various pollutants like volatile hydrocarbons (e.g. chloroform and benzene) (Doty et al., 2007).

2.6 Field trials

Field trials of transgenic trees are, as opposed to greenhouse trials, considered as deliberate releases into the environment. The first field trial of GM trees worldwide was established in Belgium in 1988, with glyphosate resistant poplars (Frankenhuyzen and Beardmore, 2004). By 2004, tree field trials had taken place in 28 countries. In total, there were 229 field trials of GM trees (forest and fruit species together) with the majority occurring in the US (see Appendix 2). As of May 2009, authorities in the US have approved 187 trials with poplars. Legislation of trials with transgenic plants has become less stringent in the US, meaning that approvals are required only for categories of GM crops or trees and not for individual releases.

Worldwide, field trials for GM forest trees are mainly restricted to *Populus*, *Eucalyptus* and *Pinus*, whereas field trials for fruit trees are primarily restricted to *Malus*, *Vitis*, and *Carica* (Table 2.2 and Table 2.3). Development of GM *Carica* (papaya) mainly takes place in Hawaii, Thailand, Jamaica, Brazil and Venezuela (Gonsalves 2006). In the period 2006-2007 the number of field trials with GM trees increased with 95 new notifications for forest species alone, and with 12 new notifications for fruit trees (CSL, 2007).

Like in the US, *Populus* is the primary genus in field trials with GM trees in the EU. As has been said, the first release trial of transgenic poplars in the EU took place in Belgium (Frankenhuyzen and Beardmore, 2004). Nowadays, trials with transgenic poplars have taken place in France (10), Germany (4), the UK (2), Spain, Sweden, Norway and Belgium. Some 50 field trials of GM trees were approved by national authorities in Europe in the period between 1993 and 2008. In addition to poplars, these include fruit trees such as apples, cherries and plums and forest species such as birch, spruce and pine.

In general, field trials are very small (12-2850 plants) and have short rotations. Many countries oblige to destroy such trials before the reproductive phase. Most of the issues addressed in field trials concern plant growth and performance, gene expression stability and environmental risks. Furthermore, attention is given to horizontal gene transfer, application of herbicides, mycorrhizal status, vegetative propagation and sterility.

Table 2.2

Cumulative number of approved field trials with genetically modified trees in the EU by May 2009 (source: EU, BVL, 2009).

	Species	Species/Genus	Country	Releases	
EU	Fruit trees	Apple	<i>Malus x domestica</i>	UK, Netherlands, Sweden, Belgium, Germany	11
		Cherry	<i>Prunus avium</i>	Italy	3
		Citrange	<i>Citrus sinensis x poncirus trifoliata</i>	Spain	2
		Coffee	<i>Coffea canephora</i> or <i>Coffea arabica</i>	France	1
		Grape vines	<i>Vitis sp.</i>	Finland, Italy, Germany	6
		Kiwi	<i>Actinidia deliciosa</i>	Italy	3
		Lemon	<i>Citrus sp</i>	Italy	1
		Olive	<i>Olea europea</i>	Italy	3
		Orange	<i>Citrus x sinensis</i>	Spain	5
		Pear	<i>Pyrus sp.</i>	Sweden	1
		Plum	<i>Prunus domestica</i>	Spain, Chechia, Romania,	4
	Forest trees	Common Aspen	<i>Populus tremula</i>	Denmark, Norway	3
		Eastern Cottonwood	<i>Populus deltoides</i>	France, Germany, UK	5
		Eucalypt	<i>Eucalyptus sp.</i>	UK, Spain, Portugal	4
		Hybrid Aspen	<i>Populus tremula</i> hybrids	France, Germany, Sweden, UK	12
		Norway Spruce	<i>Picea abies</i>	Finland	2
		Poplar	<i>Populus sp.</i>	France, UK, Germany, Spain, Norway, Sweden, Belgium	20
		Quaking Aspen	<i>Populus tremuloides</i>	France	1
		Scots Pine	<i>Pinus sylvestris</i>	Finland	2
		Silver Birch	<i>Betula pendula</i>	Finland	5

Table 2.3

Cumulative number of approved field trials with genetically modified trees in the US by May 2009 (source: US, ISB 2009).

	Species	Species/Genus	Releases		
US	Fruit trees	Apple	<i>Malus domestica</i>	56	
		Bananas	<i>Musa sp.</i>	5	
		Blue berry	<i>Vaccinium sp.</i>	4	
		Cherry	<i>Prunus domestica</i>	8	
		Chestnut	<i>Castanea dentata</i>	6	
		Citrange	<i>Citrus sinensis</i> x <i>poncirus trifoliata</i>	2	
		Coffee	<i>Coffea canephora</i> or <i>Coffea arabica</i>	3	
		Date plum (kaki)	<i>Diospyros kaki</i>	4	
		Grape fruit	<i>Citrus paradisi</i>	15	
		Grape vines	<i>Vitis sp.</i>	57	
		Lemon	<i>Citrus aurantifolia</i>	2	
		Orange	<i>Citrus</i> x <i>sinensis</i>	1	
		Papaya	<i>Carica papaya</i>	29	
		Pear	<i>Pyrus sp.</i>	5	
		Pine apple	<i>Ananas comosus</i>	9	
		Walnut	<i>Juglans sp.</i>	14	
		Forest trees	Allegheny serviceberry	<i>Amelanchier laevis</i>	1
			American Elm	<i>Ulmus americana</i>	3
			American Sweetgum	<i>Liquidambar sp.</i>	30
	Black Cottonwood		<i>Populus trichocarpa</i>	1	
	Common Aspen		<i>Populus tremula</i>	1	
	Eastern Cottonwood		<i>Populus deltoides</i>	37	
	Eucalypt		<i>Eucalyptus sp.</i>	61	
	Gray Poplar		<i>Populus tremula</i> x <i>P. alba</i>	15	
	Hybrid Aspen		<i>Populus tremula</i> x <i>P. tremuloides</i>	6	
	Hybrid Poplar		<i>Populus</i> x <i>euramerica</i>	3	
	Loblolly Pine		<i>Pinus taeda</i>	94	
	Monterey Pine		<i>Pinus radiata</i>	1	
	Pitch Pine x Loblolly Pine		<i>Pinus rigida</i> x <i>Pinus taeda</i>	45	
	Poplar	<i>Populus sp.</i>	121		
	White Spruce	<i>Picea glauca</i>	1		

2.7 Commercial releases of GM trees

So far, releases of GM trees on the commercial market have been very limited and only a few examples of commercial deployment are reported. The only GM forest trees known to be deployed on a substantial scale are GM poplars in China (Farnum et al., 2007). In 1999, the first pilot plantations of 80 ha on eight sites in China were established with GM insect-resistant *Populus nigra*. In 2002, this was commercialized and 1.4 million trees were planted in a 300-500 ha plantation (FAO, 2004). Of another *Populus* species, the GM hybrid poplar clone 741 (*P. alba* x [*P. davidiana* x *P. simonii*] x *P. tomentosa*), which is resistant to leaf eating insects due to insertion of *Bt cryI* and *API* genes, about 0.4 million cuttings were propagated and planted in China in 2003 (Yang et al. 2003). The only GM fruit tree known to be commercially exploited is the papaya fruit tree line 55-1 (*Carica papaya*) resistant to Papaya

Ringspot Virus (PRSV). This tree line was introduced in Hawaii in 1998 and was later also grown in China.

Considering the current biotechnology research on trees, it can be expected that a limited number of forest tree taxa (*Eucalyptus*, *Pinus taeda*, *P. radiata*, *P. pinaster*, and *Populus*) will be commercially released globally in the near future (FAO, 2004). The use of biotechnology within the global timber industry is unlikely to increase rapidly because of public concerns. Nevertheless, there is an increased global trend in forestry towards production in timber plantations (Carle and Holmgren, 2008; Seppälä, 2007) and there seems to be commercial interest in GM forest trees. For example, the private company ArborGen is focusing on the development of three GM varieties: fast-growing loblolly pine (*Pinus taeda*) for Southern US pine plantations, low-lignin *Eucalyptus* species for use in South America, and cold-hardy *Eucalyptus* species for the Southern U.S (Farnum et al., 2007).

2.8 Possible field release of GM trees in the Netherlands

No GM trees have been approved for commercialisation by the appropriate authorities, so that no commercial release can be expected on short term (B. Mezetti, Ancona Marche Polytechnic University, Italy. Pers.comm.).

At the moment there are no deliberate releases of genetically modified trees in the Netherlands. As in several other EU countries, forest management in the Netherlands has shifted from a timber production focus towards a more nature-oriented focus. FSC-certification has been introduced to enhance sustainable timber production. This trend may slow down the (commercial) interest in GM forest trees, because the FSC certification scheme does not allow for the use of transgenic trees (FSC, 2000). In the Netherlands, field trials with GM apple trees, *Malus domestica* (COGEM, 2004) have ended in 2008. Recently, a field trial has been approved for female 717-1-B4 poplar field trials in the province of Zeeland. Based on climatic requirements, commercial attractiveness, trends observed elsewhere in the world, and existing knowledge about genetic modification, few other GM trees could be expected in the Netherlands in the near future (Table 2.4 and Table 2.5). For example, Silver Birch (*Betula pendula*) is not commercially attractive in the Netherlands, and consequently it seems unlikely that field trials for genetically modified *B. pendula* will be requested here in the coming years. A large group of tree species used for genetic modification worldwide, naturally occurs in warmer climates (e.g. *Eucalyptus* sp., *Pinus radiata*, *P. taeda*). The unsuitable Dutch climate makes it unlikely that these will be planted in GM field trials in the Netherlands, at least on the short term. Some *Populus* species used to be commercially attractive in the Netherlands, but the decreasing sales of poplar trees over the last decade (P. Jansen, Probos. pers. comm.) indicate that they may be losing their commercial attractiveness. However, several species within the genus *Populus* may still be expected for GM field research in the Netherlands, mainly because *Populus* is the most common tree genus used for genetic modification, and because many *Populus* species grow well in the Dutch climate.

A genus that is gaining interest for commercial use is Willow (*Salix* sp.). In the Netherlands, *Salix* sp. has been planted on a small scale for biomass production to generate bioenergy. So far, this genus has not been used for genetic modification, and it seems unlikely that it will be in the near future (W. Boerjan, VIB Department of Plant Systems Biology, University Gent, Belgium. pers. comm.). Many of the fruit tree species that have been genetically modified are not suited for the Dutch climate (e.g. *Musa* sp., *Mangifera* sp., *Citrus* sp., and *Olea europea*). Fruit trees that are expected, based on their commercial attractiveness, are *Malus domestica*, *Prunus domestica*, and *Pyrus pyraeaster*. Currently, GM research into fruit trees is taking place in the Netherlands, and scab-resistant GM apple varieties have already been tested in field trials. In addition, wine production is an upcoming sector in mid-European countries

including the Netherlands. Consequently, GM grapevine (*Vitis sp.*), for example modified to improve cold resistance, forms one of the new genera to be expected for field trial requests.

Table 2.4

Likelihood of field trials with GM forest tree genera and species in the Netherlands based on three criteria: 1 = Commercially attractive in the Netherlands; 2 = Already commercialized in some countries; 3 = Climate suitability in the Netherlands. Only trees that have already been released in field trials are mentioned.

GM forest tree species	Common name	Country of previous releases	Criteria			Requests in NL
			1	2	3	
<i>Betula pendula</i>	Silver Birch	Finland	-	-	+	unlikely
<i>Eucalyptus camaldulensis</i>	River Red Gum	US	-	-	-	unlikely
<i>Eucalyptus globulus</i>	Tasmanian Blue Gum	Portugal	-	-	-	unlikely
<i>Eucalyptus grandis</i>	Rose Gum	UK, US	-	-	-	unlikely
<i>Eucalyptus urophylla</i>	Timor White Gum	US	-	-	-	unlikely
<i>Liquidambar sp.</i>	American Sweetgum	US	-	-	+	unlikely
<i>Picea abies</i>	Norway Spruce	Finland	-	-	+	unlikely
<i>Picea glauca</i>	White Spruce	US	-	-	+	unlikely
<i>Pinus radiata</i>	Monterey Pine	US	-	-	-	unlikely
<i>Pinus rigida</i> x <i>P. taeda</i>	hybrid Pine	US	-	-	-	unlikely
<i>Pinus sylvestris</i>	Scots Pine	Finland	+	-	+	likely
<i>Pinus taeda</i>	Loblolly Pine	US	-	-	-	unlikely
<i>Populus alba</i> x [[<i>P. davidiana</i> x <i>P. simonii</i>] x <i>P. tomentosa</i>	hybrid Poplar	China	-	+	+	likely
<i>Populus deltoides</i>	Eastern Cottonwood	US	+	-	+	likely
<i>Populus nigra</i>	Black Poplar	China	+	+	+	likely
<i>Populus tremula</i>	Common Aspen	Germany, US, Norway	-	-	+	unlikely
<i>Populus tremula</i> x <i>P. alba</i>	Gray poplar	France, Sweden, Spain, UK, US	-	-	+	unlikely

Table 2.5

Likelihood of field trials with GM fruit tree genera and species in the Netherlands. Criteria as in Table 2.4. Only trees that have already been released in field trials have been included.

GM fruit tree species	Common name	Country	Criteria			Requests in NL
			1	2	3	
<i>Actinidia deliciosa</i>	Kiwi	Italy	-	-	-	unlikely
<i>Amelanchier laevis</i>	Allegheny serviceberry	US	-	-	+	unlikely
<i>Carica papaya</i>	Papaya	Thailand, Brazil, US, Venezuela	-	+	-	unlikely
<i>Castanea dentata</i>	Chestnut	US	-	-	+	unlikely
<i>Citrus paradisi</i>	Grape fruit	US	-	-	-	unlikely
<i>Citrus sinensis</i>	Orange	Spain	-	-	-	unlikely
<i>Citrus sinensis</i> x <i>poncirus trifoliata</i>	Citrange	Spain	-	-	-	unlikely
<i>Citrus sinensis</i> x <i>poncirus trifoliata</i>	Citrange	US	-	-	-	unlikely
<i>Citrus</i> sp.	Lemon	Italy	-	-	-	unlikely
<i>Citrus</i> sp./ <i>Citrus aurantifolia</i>	Lemon	US	-	-	-	unlikely
<i>Coffea canephora</i> or <i>Coffea arabica</i>	Coffee	France, US	-	-	-	unlikely
<i>Diospyros</i> sp.	Date Plum (Kaki)	US	-	-	-	unlikely
<i>Juglans</i> sp.	Walnut	US	-	-	+	unlikely
<i>Malus domestica</i> / <i>M. pumila</i>	Apple / Paradise apple	Belgium, Germany, Netherlands, Sweden, US	+	-	+	likely
<i>Mangifera</i> sp.	Mango	Worldwide	-	-	-	unlikely
<i>Musa</i> sp.	Bananas	US	-	-	-	unlikely
<i>Olea europea</i>	Olive	Italy	-	-	-	unlikely
<i>Prunus avium</i>	Cherry	Italy	+	-	+	likely
<i>Prunus domestica</i>	European plum	Czech Republic, Romania, Spain, US	+	-	+	likely
<i>Pyrus pyraister</i>	Pear	Sweden, US	+	-	+	likely
<i>Vitis berlandieri</i> x <i>riparia</i>	Grape	France	+	-	+	likely
<i>Vitis vinifera</i> x <i>berlandieri</i>	Grape	France	+	-	+	likely
other <i>Vitis</i> sp.	Grape	France, Germany, Italy, US	+	-	+	likely

3 Potential environmental risks specific for GM trees, with special emphasis for the situation in the Netherlands

As for other GM crops, the potential environmental risks of introduction of GM trees have to be assessed before GM trees can be cultivated in the EU. However, differences in the biology of tree species relative to crops may imply different environmental risks. For example, trees have a long life-cycle which might have a longer-spanning impact on ecosystem functioning, and many trees also have a potential long-distance dispersal of pollen and seeds. So far, little research has been conducted on the environmental impacts of GM trees. Farnum et al. (2007) underlined that knowledge and relevant scientific data regarding the potential behaviour of transgenic trees in the environment are still lacking. Predictive models may be used to extrapolate the results of short term empirical studies (DiFazio, 2002; Kuparinen and Schurr, 2007). These models include factors such as mating success, pollen movement, seed and vegetative propagule establishment, and the competitiveness of hybrid seedlings in the wild.

3.1 Environmental risk assessment

Based on the EU directive 2001/18/EC (European Commission, 2001) on the deliberate release of genetically modified organisms into the environment, the environmental risk assessment considering the release of GMOs (including trees) should address the following nine aspects:

- Likelihood of the GM tree becoming more persistent than the recipient or parental trees in agricultural habitats or more invasive in natural habitats.
- Any selective advantage or disadvantage conferred by the GM tree.
- Potential for gene transfer to the same or other sexually compatible tree species under conditions of planting the GM tree and any selective advantage or disadvantage conferred to those tree species.
- Possible immediate and/or delayed environmental impact resulting from direct and indirect interactions between the GM tree and target organisms, such as predators, parasitoids, and pathogens (if applicable).
- Possible immediate and/or delayed environmental impact resulting from direct and indirect interactions of the GM tree with non-target organisms, (also taking into account organisms which interact with target organisms), including impact on population levels of competitors, herbivores, symbionts (where applicable), parasites and pathogens.
- Possible immediate and/or delayed effects on human health resulting from potential direct and indirect interactions of the GM tree and persons working with, coming into contact with or in the vicinity of the GM tree release(s).
- Possible immediate and/or delayed effects on animal health and consequences for the feed/food chain resulting from consumption of the GMO and any products derived from it, if it is intended to be used as animal feed.
- Possible immediate and/or delayed effects on biogeochemical processes resulting from potential direct and indirect interactions of the GMO and target and non-target organisms in the vicinity of the GMO release(s).

- Possible immediate and/or delayed, direct and indirect environmental impacts of the specific cultivation, management and harvesting techniques used for the GM tree where these are different from those used for non-GM trees.

3.1.1 Persistence and invasiveness

Invasions of introduced tree species have been common and several tree species have the potential to become invasive, by seed dispersal or vegetative spread. A well-known example from Dutch forests is black cherry (*Prunus serotina*), a species of which seedling establishment in its native habitat is regulated by soil-borne pathogens that are apparently lacking in the Netherlands (especially *Pythium* spp.) (Packer and Clay, 2003). Its rapid growth and ability to alter soil properties increase its potential invasiveness.

Potential hazards

Transgenes may be successfully transferred and established into wild populations of the same or related species. This is possible only when the transgene itself results in a selective advantage or is coupled to alleles that result in a selective advantage of a certain genotype above other genotypes. If, on the other hand, a transgene has a negative effect when it is outcrossed into wild populations (deleterious genes) it is likely to get lost from the population over time. Genes that increase fitness are in general more likely to support persistence of the transgene in the genome of the recipient population. However, traits leading to invasiveness are generally not based on a single gene, which implies that (many) different genes would have to be altered for a species to become invasive (Irwin and Jones, 2006). Importantly, the potential to become invasive also depends on the environmental conditions, such as the availability of suitable ecological niches. Because invasiveness is linked to environmental stochasticity it is hard to predict invasive behaviour of plants.

Crops versus trees

Both crops and trees have the potential to become invasive in suitable environments where, for example, natural enemies are lacking. Contrary to trees, annual crops distribute their pollen in the very year of planting, whereas trees may do so only after they have reached reproductive maturity. The age of reproductive maturity varies between species. Most poplars begin flowering between 10-15 years of age, although flowering in *P. deltoides* may occur as early as age four (OECD, 2000). It therefore seems obvious that until reproductive maturity is reached, trees possess a limited risk to become invasive. Only vegetative reproduction could enhance the spread of trees in this period. Vegetative reproduction forms an important mechanism of spread for most species in the genus *Populus*, which are known for their vigorous sprouting and sucker development. The longer life cycle of trees makes that a seed source persists on the same spot whereas crop farming usually only returns to the same patch of farm land (crop rotation) after three or four years. Furthermore, many tree species are known for their long-distance pollen dispersal, which can range up to several kilometres for temperate conifers (O'Connell et al., 2007), and their copious production of seeds, especially in mast fruiting years. Like certain crops, most pioneer trees (with the exception of *Populus*) also possess very high seed longevity, so that transgenes may potentially be stored for decennia in the seedbank.

Potential invasiveness in the Netherlands

Whether transgenic trees introduced in the Netherlands will become invasive depends on the new traits obtained as a consequence of the genetic modification, and the location of plantings, i.e. the distance to conspecific populations or wild populations of related species with which they can outcross and the presence of suitable habitat. Based on their climatic requirements and potential commercial interest, poplars (*Populus* sp.) are the most likely GM forest tree species to be introduced in the Netherlands (Table 2.4). They do indeed possess traits which may stimulate invasiveness, such as fast growth and

reproduction, long distance pollen dispersal, and extensive seed production. Poplar is one of the most common tree genera in the Netherlands and the reproductive barriers between related poplar species are very weak (Vanden Broeck et al., 2004). When these trees would become more tolerant to abiotic and biotic stress than other tree species, they could become invasive over time. Indeed, some invasive poplar species have been observed in acidifying dune valleys (*P. x canescens*) and former heathlands and desiccating peat areas (*P. tremula* and hybrids) in the Netherlands (A. Corporaal, ESG-Wageningen UR, pers. comm.). Nevertheless, whether increased fitness indeed leads to invasiveness depends on the physical environment resulting in altering biotic interactions. For example, changes in soil nitrogen concentration may favour some species above others.

At the moment it seems that there is not much interest in the commercial application of GM forest tree genera other than *Populus* (see paragraph 2.8). Considering the increasing economic importance of Christmas tree plantations throughout Europe, however (Venhorst, 2009), an increase in size and numbers of these plantations may also be expected in the Netherlands. This would include the genera *Abies* and *Picea*. These genera have long distance pollen dispersal, but growth and reproduction is slower compared to other pioneer species like pine or birch. Potential invasiveness of fruit trees may seem less likely because fruit trees tend to grow and reproduce relatively slowly. However, their pollen can be dispersed over long distances via wind or insects and may outcross with wild populations. Apple (*Malus domestica*), pear (*Pyrus domestica*), plum (*Prunus domestica*) and cherry (*Prunus avium*) are all represented outside orchards by escaped domesticated individuals. Also, there are numerous apple hybrids and wild apple populations (*Malus sylvestris*) in the Netherlands (Van der Meijden et al., 2009). A study with microsatellite markers indeed indicated that gene flow and introgression between *M. domestica* and *M. sylvestris* is occurring in the Netherlands (Koopman et al., 2007).

3.1.2 Selective advantages

Selective advantages refer to increased fitness through an increase in e.g. survival and reproductive capacity. Depending on the trait, genetic modification could lead to increased fitness for example by increased tolerance to biotic and abiotic stress and improved seed production.

Potential hazards

It is not unlikely that transgenic trees which are transformed to increase resistance to unfavourable biotic or physical conditions have a selective advantage above wild relatives lacking these traits. Genes conferring resistance to insects, fungi, herbicides, or abiotic stress can greatly increase competitive advantage of the transgenic crop or tree. For example, the glyphosate tolerance gene is likely to result in a selective advantage compared to non-resistant relatives, but only in fields where glyphosate is applied. This may lead to increased reproduction and further spread of the resistance gene. Spread of glyphosate resistance into wild populations of creeping bentgrass (*Agrostis stolonifera*) was among the first cases of an observed spread of a transgene into a wild population (Reichman et al., 2006). So far, the spread of glyphosate resistance is not considered an environmental risk.

Moreover, several of the traits that are used in GM trees do not appear to result in any selective advantage, but rather in a reduced fitness. For example, low lignin trees are more susceptible to windthrow, stem breakage, and pathogen attack compared to their non-transgenic relatives (Fink, 1999). A study on perennial plants showed that genes controlling lignin biosynthesis are physically linked with genes that influence winter survival (Casler et al., 2002). Suppression of lignin biosynthesis could potentially result in lower winter survival in these plants.

Crops versus trees

Selective advantages are relatively easy to observe in annual crops. Indications for increased fitness in transgenic plants were first documented for crop/wild hybrids (backcrosses) of Bt sunflower, which had a higher survival and enhanced fecundity (higher seed production) when compared to the non-transgenic control plants under field conditions, due to reduced herbivory (Snow et al., 2003). For trees, however, there is no such evidence so far, but traits conferring tolerance to physical and biotic stresses may enhance their fitness in the field. Major differences between selective advantages of crops and trees seem not to be present.

Potential selective advantages in the Netherlands

Currently, there is no commercial use of GM trees in the Netherlands. GM research focuses on biomass production (short rotation trees) and adjusted wood chemical composition in forest tree species. These traits are not likely to result in selective advantages for the trees. On the other hand, pest resistance may be introduced in both forest and fruit trees. This trait may increase the selective advantage of GM trees compared to non-transgenic relatives. In addition, forest and fruit trees that are tolerant to herbicides are also being developed. Herbicide tolerance may increase the selective advantages under cultivated conditions, where herbicides are applied. However, this is not specific for trees.

3.1.3 Outcrossing (and horizontal gene transfer)

Outcrossing of transgenes to non-transgenic relatives potentially increases the area on which any possible adverse effects related to the genetic modification can occur. Establishment of the new trait via sexual reproduction in trees (i.e. vertical gene transfer) requires the successful dispersal of pollen and development of seed. The transgenes might be transferred to non-transgenic relatives or to wild relatives through out-crossing. Transgene flow is sometimes predicted with simulation models such as STEVE (DiFazio, 2002) and AMELIE (Kuparinen and Schurr, 2007). Another transfer mechanism of genes is horizontal gene transfer (HGT). This is the non-sexual transfer of genes between species, for instance between tree roots and soil micro-organisms. Below we will discuss the potential hazards, crops vs trees, and potential outcrossing for the Netherlands for vertical gene transfer and horizontal gene transfer separately.

Vertical gene transfer (outcrossing)

Potential hazards

Most tree species used in forest plantations are undomesticated outcrossers that, depending on their biology, may readily interbreed with related species (Irwin and Jones, 2006). It is well known that many native eucalypt species easily hybridize in the wild (Florence, 1996). A study of white spruce (*Picea glauca*) in the US revealed that self-fertilization rates were very low and 96% of seeds were sired by immigrant pollen (O'Connell et al., 2007). In addition, many trees have copious production of wind-borne pollen that may travel long distances and still be viable, which amplifies the likelihood of vertical gene transfer through pollination of non-transgenic trees. Although no evidence exists of gene transfer from transgenic to non-transgenic trees, introgression of genes of a cultivated poplar species (*Populus deltoides*) has been observed in the offspring of natural *Populus nigra* populations (Vanden Broeck et al., 2004). Along the Dutch river Rhine, wild *Populus nigra* was observed to hybridize with *P. x canadensis*⁴

⁴ A hybrid between *P. nigra* x *P. deltoides*

and offspring of hybrid cultivated poplars competed for the same ecological niche as native Black poplars (Smulders et al., 2008).

Nevertheless, outcrossing is not a hazard in its own right. The environmental hazard associated with outcrossing depends on the transgenic trait that is transferred by outcrossing, and the properties of the resulting GM tree.

Crops versus trees

As stated above most tree species used in forestry are from wild populations, so are relatively undomesticated, and consequently individuals easily interbreed. Long distance dispersal of pollen is shared among many tree species and plays a primary role in maintaining low genetic differentiation among natural populations. In contrast, many agricultural crops have been cultivated and selected for thousands of years, and interbreeding is often prevented by (man-made) barriers, such as strongly increased selfing and/or the creation of polyploid species hybrids that poorly hybridize with the parental species (if still known to exist at all). This may also hold true for fruit trees which like agricultural crops have also been cultivated for a longer time than most forest trees.

Potential outcrossing of transgenes in the Netherlands

The surface area over which potential effects can occur may be extensive since transfer of transgenes to crossable relatives through vertical gene transfer is highly likely for flowering trees. Poplar trees are common and widely distributed in the Netherlands, which induces the chance of outcrossing of transgenes compared to less common species or species that hybridize less easily. Some wild *Prunus* and *Malus* trees are also common throughout the Netherlands and hybridization with these wild relatives may occur. To overcome the potential of vertical gene transfer in a current request for poplar (*P. x canescens* (*P. tremula* x *P. alba*)) field trials in the Netherlands, only male sterile trees are used and flowers are actively removed before seed formation (VROM, 2009).

Horizontal Gene Transfer

Potential hazards

HGT of transgenes from crops or trees to soil micro-organisms has never been shown to occur in the field (Nielsen et al., 2000, Nehls et al., 2006). However, some recent studies may give some phylogenetic evidence of past horizontal gene transfer between plants possibly mediated by fungi (Davis et al., 2005) and between parasitic plants and their host (Davis and Wurdack 2004; Park et al., 2007). Several studies indicate that antibiotic resistance genes, such as the *nptII* gene, could be transferred from transgenic plants to soil microbes (Nielsen et al., 1998; Nielsen et al., 2000; De Vries et al., 2001), albeit at extremely low frequencies and up till now only confirmed under laboratory conditions only (e.g. Schluter et al., 1995; Nielsen et al., 2000).

Crops versus trees

The chance of HGT is possibly higher in trees compared to annual crops, considering the intensive and long time interaction of tree roots with soil fungi, and the extensive root system of trees.

Potential HGT of transgenes in the Netherlands

There are no specific aspects for GM trees in the Netherlands.

3.1.4 Impact on target organisms

Genetic modifications may aim to affect physical and physiological properties of the GM tree itself or to affect the interaction of the GM tree with target organisms such as herbivorous insects, fungi and

weeds. The disappearance of these target organisms may result in shifting populations of associated organisms that are for example preying on OF predators of the target organism.

Potential hazards

Effective control of harmful insects may disrupt a food chain, negatively affecting closely associated insect or insect-eating populations that are beneficial to the crop. A study on the effects of Bt crops revealed that the loss of target organisms could be contributing to the decrease of predators (Wolfenbarger et al. 2008), but this is a usual ('predictable') consequence of any introduction of resistance against specific target organisms. The elimination of certain target pests potentially leads to shifting communities, with new pest organisms replacing them. Emergence of new pests following GM trees deployment has been observed in China in Bt poplar plantations. Reports of the Chinese Institute for Forestry state that some of the transgenic poplars that proved successful against target insects, were attacked by pest insects that were previously unknown in non-transgenic poplars (Rautner, 2001). Problems associated with new secondary pests are also known for Bt (Bollgard) cotton resistant to bollworms (Bachelor, 2000). It was found that the transgenic fields sustained 59% less boll damage from bollworms compared to fields planted with conventional cotton (1.61% vs. 3.93% damage, respectively). However, stink bug damage to bolls was approximately 4-fold higher in the transgenic fields (2.58% vs. 0.61%) (Bachelor, 2000). This would however be interpreted as an economic loss, not an environmentally adverse effect.

Crops versus trees

Trees bear large, long-lived structures that provide numerous ecological niches for several species groups (Brändle and Brandl, 2001). They are for instance habitat for many infant forms of terrestrial arthropods such as beetle larvae and caterpillars. Brändle and Brandl, 2001) found between 9 to 728 phytophagous insects and mites to be associated with common tree species in Europe; most species were found on *Salix* (728), *Quercus* (699), *Betula* (499), *Populus* (470) and *Prunus* (436) (Figure 3.1). Insect numbers associated with agricultural crops are generally lower; for instance Nuessly et al. (1994) found 61 herbivore and 32 predator insect species associated with faba bean. For sugar cane, 36 associated insect species were detected by Meagher et al (1993).

Shifts in populations of species associated with trees, or the replacement of target pests with other pest-forming organisms could potentially be enhanced by longevity of trees compared to annual crops, but interpreting such results is complicated due to the complexity of the insect communities hosted on trees (Shelton et al., 2002).

Potential impact on target organisms in the Netherlands

There are no specific aspects for GM trees in the Netherlands.

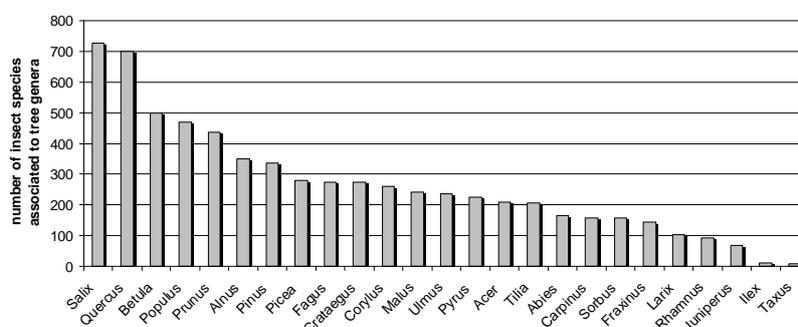


Figure 3.1

Phytophagous insect and mite species associated with different tree genera (after: Brändle and Brandl, 2001).

3.1.5 Impact on non-target organisms

Making trees resistant to certain insect pests may have impacts on other insect groups that are closely related. Examples can be mortality of important pollinators or seed dispersers. The introduction through GM of antifungal proteins (e.g. chitinases) may interrupt beneficial plant-fungal relationships. Additionally, animals that depend on pollen, seeds or fruits may be affected by flowering control or sterility, both being traits that can be introduced through genetic modification. As the number of insects feeding on trees may be very large (e.g. Figure 3.1) these potential hazards have to be taken into account in the environmental risk assessment.

Potential hazards

Organisms closely associated with trees (e.g. pollinators, herbivores and detritivores) and the predators of these organisms can be affected by the new trait of the transgenic trees. An observed effect on non-target organisms is that altered plant hormone levels obtained through GM can critically affect the formation of mycorrhizal partnerships that are crucial for the plant's phosphorus uptake (Barker and Tagu, 2000). Kaldorf et al., (2002) found that mycorrhization did not differ between transgenic aspen, transformed with a *rolC* gene, and non-transgenic trees. The only statistically significant difference found between one of the transgenic clones and the control was in the composition of the mycorrhizal community: one of the four commonly occurring morphotypes was missing in this case. This effect was, however, specific for one *rolC* clone, and mycorrhizal diversity in general did not differ between transgenic and non-transgenic trees.

While lignin reduction and enhanced growth are both of significant commercial value, the altered secondary metabolism, specifically leaf chemistry, may have ecological impacts. This is because altered leaf chemistry could alter the evolved chemical defence system of aspen against herbivory. The possible occurrence of pleiotropic effects due to the genetic modification are not specific for trees.

Crops versus trees

Insect numbers associated with agricultural crops are generally lower than insect numbers associated with trees (see Paragraph 3.1.4). The longevity, larger size and complex architecture of trees imply that more organisms are associated with trees compared to crops. For instance, a pine tree provides more micro-habitats than a maize plant and tree plantations accumulate organic matter in their litter and soil over the years, whereas, depending on tillage, soil formation hardly occurs under annual crops). Therefore, if an effect is expected as a result of the genetic modification, more non-target organisms may be affected in trees than in crops.

Potential impact on non-target organisms in the Netherlands

There are no specific aspects for GM trees in the Netherlands with respect to non-target effects. However, if an effect is expected as a result of the genetic modification, more non-target organisms may be affected in trees than in crops.

3.1.6 Effects on human health

Potential hazards

Effects of genetic modification in trees on human health for tree species may consist of pollen allergies, such as increased incidence of hay fever or may occur through consumption of parts of transgenic trees. This would play a role when, as a consequence of the genetic modification, pollen production and/or the number of allergic substances is increased. Modifications to reduce pollen production (sterility) could on the other hand reduce this effect. Possible risks to human health caused by incidental consumption of transgenic tree parts would be by incidental consumption of parts of the transgenic tree

itself or by consumption of parts of other trees that have hybridized with the transgenic tree and have introgressed the transgenes that lead to the production of toxic or allergic substances.

Crops versus trees

So far, there is no reason to assume that human health risks through incidental consumption of transgenic tree parts are greater in tree species compared to annual or perennial crops. On the other hand, the incidence of allergies through pollen dispersal may be greater in trees compared to crops, as many tree species possess copious production of pollen and long-distance pollen dispersal observed in many tree species may cause a broader range of allergy incidence. Nevertheless, increased pollen production is not among the traits that are being modified in GM trees. In contrast, male sterility or the exclusive use of female trees are among the more likely traits for GM trees. In addition, grasses and some agricultural crops are also notorious for their copious pollen production and long-distance pollen dispersal.

Potential impact on human health in the Netherlands

There are no specific aspects for GM trees in the Netherlands with respect to human health. However, if an effect is expected as a consequence of the genetic modification, the incidence of allergies through pollen dispersal may be greater in trees compared to crops, as many tree species possess copious production of pollen and long-distance pollen dispersal observed in trees may cause a broader range of allergy incidence.

3.1.7 Effects on the feed chain

Potential hazards

The chances of illness caused by the consumption of products of transgenic trees by animals depend on whether the consumed material has toxic or allergic characteristics, or is anti-nutritional.

Crops versus trees

Animal fodder consists for the majority of crops like soy and maize. Forest trees are not primarily processed to animal fodder and potential effects occurring through fodder are therefore negligible. Fast growing poplars can however disperse into grazing fields and their seedlings (or sprouts) can be eaten by grazing animals. Waste fruit that is not suitable for human consumption potentially ends up as animal fodder. In addition, animals grazing in fruit orchards may repeatedly ingest fruits or parts of the fruit trees.

Potential effects on the feed/food chain in the Netherlands

Field release of transgenic trees in the Netherlands can also affect the feed chain through the above mentioned pathways. The short rotations of trees for biomass or pulp production will suppress regeneration (no flowering events) and thereby limit incidental consumption of seedlings by grazing animals. This is however not specific for the Netherlands.

3.1.8 Effects on biogeochemical cycles

The introduction of transgenic trees could directly or indirectly alter biogeochemical cycles by expressing the new trait. For example, the production of anti-microbial proteins by GM trees in order to make trees resistant to disease may affect (micro-)organisms involved in nutrient cycling.

Potential hazards

The chemical composition of wood or other tree parts may change as a consequence of genetic modification. Trees have, for example, been modified to increase growth rates, impede lignin biosynthesis or enhance uptake of heavy metals or other pollutants. These modifications may influence biogeochemical cycles mainly through decomposition of litter and dead wood. For instance, modified nitrogen efficiency for faster growth leads to alterations in the wood structure and composition which affects the decomposition rate and alters soil structure and nutrient availability (Irwin and Jones, 2006). Also trees modified to reduce lignin concentrations in their wood produce litter which can be rapidly decomposed, thereby altering microbial population dynamics (Talukder, 2006) and possibly resulting in higher CO₂ emissions from litter decomposition (Pilate et al., 2002).

Wei et al., (2006b) found that populations of bacteria, actinomycetes, and fungi differed significantly between soils planted with transgenic papaya⁵ and non-transgenic papaya or no papaya, after cultivation for nine months. Alterations in rhizosphere communities are also described for *Picea glauca* that was genetically modified by insertion of the *cryIA(b)*, *uidA* (beta-glucuronidase), and *nptII* genes (LeBlanc et al., 2007). It is however not clear whether these changes have any environmentally significant consequences and how these observations relate to variability in soil communities between non-transgenic varieties in general.

GM trees modified to absorb or detoxify contaminants from the soil substrate are claimed to have positive effects on biogeochemical cycles, as they help restore the original soil composition. The use of GM trees for phytoremediation seems promising as they may be effective in taking up contaminants, such as toxic heavy metals (Merkle, 2006). On the other hand, accumulation of contaminants in trees could lead to toxic trees that potentially affect associated organisms, for example through decomposition of litter.

Crops versus trees

Because of the longevity of trees it can be speculated that compounds produced by trees and entering the soil ecosystem by active secretion or by leaching are present in the soil over a longer period than in crops. Depending on the degradation rate of the compound, this could also lead to accumulation of the product. Depending on the product, this could lead to an impact on the soil microflora. Also interactions with soil microbial fungi are different and longer-existing in trees compared to crops. Furthermore, build-up of litter and soil formation occur under trees but less so in annual crop systems, unless non-tillage systems are used. Therefore these aspects should be taken into account specifically in the risk assessment for GM trees.

The baseline information available for trees shows that these types of effects may be extensive. An example is nitrogen fixation by trees that have a symbiosis with *Rhizobium* (for *Fabaceae*), or *Frankia* (which has a symbiosis with a large number of trees). These long term interactions may result in important effects on the soil ecosystem (e.g. eutrophication through continuous nitrogen fixation). Therefore these aspects should be taken into the account specifically in the risk assessment for GM trees.

⁵ The transgenic papaya in this study contained a replicase (RP) mutant gene of the papaya ringspot virus (PRSV), which conferred resistance to the virus, the neomycin phosphotransferase II (NPT II) marker gene, which conferred Km resistance, and a cauliflower mosaic virus 35S promoter (CaMV 35S).

Potential impact on biogeochemical cycles in The Netherlands

Traits that can be expected in field trials in the Netherlands are increased growth, reduced lignin content and female/male sterility in timber or pulp plantations, and increased tolerance to herbicides, pathogens and parasites, early flowering and female/male sterility in fruit tree plantations. Increased growth and reduced lignin content cause alterations in wood/leaf composition and quality that might lead to shifts in the decomposition of organic material, possibly resulting in altered levels of nutrients released to the soil or in gaseous form to the atmosphere. So far, it is unknown to what extent these potential alterations may occur. Faster decomposition of low-lignin wood indeed could cause higher CO₂ emissions, but the faster growth of these trees, on the other hand, enhances the rate of carbon uptake by the tree. So, alterations of biogeochemical cycles may occur as a consequence of the use of GM trees, but whether these alterations have positive or negative impacts depends on the context (e.g. carbon storage, phytoremediation) and the extent of change.

3.1.9 Effects of cultivation and management

The commercial use of transgenic trees may require alternative management compared to plantings of non-transgenic trees. The application of low-lignin trees with increased incidence of stem breakage for example, may require adjustment of regular harvesting methods, and the application of herbicide-tolerant trees leads to alternative herbicide use compared to non-tolerant trees. Some forest trees, such as *Pinus radiata* and *Picea abies* have been modified to tolerate the application of commercial rates of Buster (Bishop-Hurley et al., 2001). Also the application of transgenic trees modified to kill pest insects, for instance through Bt genes, may lead to a change in insecticide use.

Potential hazards

The use of pest-resistant GM trees may result in a decrease in pesticide use which can be assumed to be beneficial for the environment. For example, Hu et al. (2001) found that the number of pupae and the leaf damage on transgenic Bt *Pinus nigra* were reduced to the extent that chemical protection measures were not needed. Transgenic trees that produce chemical substances may lead to more extensive management, but the release of these substances may in turn affect the biodiversity in the surrounding vegetation. Additionally, potential spread of transgenic trees could lead to increased management efforts needed in surrounding vegetation to eliminate the invasives.

Crops versus trees

Agricultural commodities have already seen significantly improved yields and management efficiencies with the commercialization of genetically engineered varieties. Unlike crops, no GM trees have yet been approved for use in commercial forestry operations in the Europe and the US⁶. Therefore, there is little experience in management effects of transgenic compared to non-transgenic tree plantations. However, the complex associations of trees with numerous organisms implies that incidence of cascading ecological effects of e.g. herbicide or insecticide use may be more prominent for GM trees than for GM crops because the trees are long-lived and not harvested annually.

Potential impact of cultivation and management of GM trees in the Netherlands

Herbicide-tolerant trees could have negative environmental effects through increased use of herbicides, whereas pest-tolerant trees may have positive environmental effects through the reduced use of pesticides. Resistance to insects or harmful micro-organisms potentially increases the commercial

⁶ It is known that there are field trials in China, but these are not well documented.

attractiveness of certain GM trees in the Netherlands, especially fruit trees and grape vines. So far, examples of management effects of such GM crops are scarce and, although it is a complex and long term issue, environmental risk assessment requires to identify these potential effects of altered management.

3.1.10 Summarizing overview

In summary, the specific potential environmental risks associated with the use of GM trees, in relation to other GM crops, are briefly summarized in Table 3.1.

Table 3.1

Summary of potential environmental risks as identified by the EU directive 2001/18/EC. Differences between GM trees and crops, and the perceived impact of GM tree release in the Netherlands are summarised. See text for further explanation.

Environmental risk (see section 3.1)	Crops versus trees
Persistence and invasiveness	Risks are smaller in trees until reproductive maturity is reached; risks are potentially higher in reproductive trees (e.g. through mast fruiting)
Selective (dis)advantage	No clear difference; maybe smaller risks in trees until reproductive maturity is reached, and potentially higher in reproductive trees
Outcrossing and HGT	No clear difference; varies widely between species and genera; Potential for horizontal gene transfer (HGT) higher in trees than in crops
Impact on target organisms	Risk potentially higher in trees due to large number of associated organisms (that potentially replace lost target organisms) and longevity (permanent transgene exposure)
Impact on non-target organisms	Risks potentially higher in trees due to large number of associated organisms
Effects on human health	No clear difference, but potential larger risk of pollen allergies in trees (high pollen production and long-distance dispersal since the majority of trees have wind dispersed pollination)
Effects on the feed chain	Risks potentially higher in crops as most animal fodder consists of annual crops like soy and maize
Effects on biogeochemical cycles	Risks potentially higher in trees due to longer lasting interactions with soil fauna. Build-up of litter and soil much lower (negligible) in annual crops
Effects of cultivation and management	No clear difference; potentially higher in trees due to large number of associated organisms (higher incidence of cascading ecological effects), but could also be smaller in trees due to less intensive management

3.2 General concerns related to genetic modification

Some general issues related to genetic modification (the instability of transgenes, pleiotropy, resistance development and non-targeted characteristics) are always considered in the environmental risk assessment of GMs but may need more attention in GM trees. These issues are described below.

Transgene instability

Transgene expression may be reduced or lost through transgene silencing or transgene loss. Silencing is the process through which foreign DNA is recognized and inactivated by the host plant. It may result from interactions between homologous genes (Kumar and Fladung, 2001), mutations in genes and their

promoters, environmental changes such as exposure of the host plant to high temperatures, or dormancy of the seed (Scorza, et al., 2001). Transgene silencing might become a problem in GM trees when, for example, engineered sterility due to gene silencing is lost, allowing the transgenic tree to reproduce again. It has been suggested that the long life cycles, difficult transformation, and general absence of sexual propagation before field deployment makes trees particularly prone to instable gene expression (Li et al., 2009). Indeed, phenotypic instability of the *rolC* gene was shown for transgenic aspens (*Populus tremuloides*) (Kumar and Fladung, 2001). Nevertheless, perennial plants - including poplars - have very high levels of transgenic stability (Li et al., 2009). For example, Li et al., 2008a) reported high stability of herbicide tolerance transgenic poplars (*Populus* sp) in an eight-year field trial and (Hawkins et al., 2003) showed long-term stability of expression of the GUS gene in poplars. Also in conifers, stable transfer of a transgene is possible: a green fluorescent protein (GFP) was successfully transferred into the Christmas tree species *Pinus virginiana* and the gene appeared stable in the next generation (Tang and Newton, 2005). They indicated that other traits may be inserted in Christmas trees as well, such as frost resistance, early flowering (for rapid reproduction) and longevity of needles (not dropping off). So far, no differences in transgene instability between trees and crops have been identified.

Pleiotropic effects

Pleiotropy occurs when a single gene influences multiple phenotypic traits. One of the possible underlying mechanisms is that the gene codes for a product that is used by various cells, or has a signalling function on various targets. These 'pleiotropic effects' may not be observed for many years, as they may be dependent on the growth stage of the tree, or may become apparent in response to varying environmental conditions (Irwin and Jones, 2006). It is likely that pleiotropy will be a common feature. For instance, increasing cellulose content may have possible undesired pleiotropic effects related to lignin reduction in transgenic trees (Fladung and Ewald, 2006). Pleiotropic effects of inserted genes may also result in effects on non-target organisms. GM aspens (*Populus tremuloides*) with reduced lignin levels, were observed to exhibit a range of pleiotropic side effects, including enhanced growth and altered secondary metabolism (Brodeur-Campbell et al., 2009). One of these low lignin aspen lines experienced reduced insect herbivory, possibly as an indirect consequence of genetic modification resulting from the insertion point of the antisense *PtACL* gene in the genome (which in fact is a position effect).

The occurrence of pleiotropy effects does not seem to be specific for GM trees in comparison to GM crops.

Resistance development

Insects may evolve resistance to insecticides when they are regularly exposed to them. Similarly, insects may become resistant to the toxins produced by transgenes. Genissel et al., 2003) showed for forest populations of the insect *Chrysomela tremulae* that recessive alleles conferring resistance to Bt toxin can occur at a rate close to the value theoretically expected before the actual implementation of Bt poplars. Despite their large-scale cultivation, development of resistance in plague insects has rarely been found in agricultural crops up till now (see e.g. Naranjo, 2009). It seems likely that the likelihood of resistance development is higher in Bt trees than in Bt crops, considering the longevity of trees.

Engineered sterility

Management of GM trees in field trials or commercial plantations may require specific measurements to eliminate potential environmental effects: so-called containment strategies. Examples are the removal of floral buds to prevent flowering and subsequent pollen dispersal and the removal of vegetative propagules from the site. To reduce potential environmental impacts as a consequence of transgene dispersal from trees, genetically engineered reproductive sterility may be applied (Skinner et al., 2000). Sterility has been applied already in fruit trees and poplar (Brunner et al., 1998; Li et al., 2000; Wei et

al., 2006a). Normally small scale field trials are intensively managed and monitored. GM trees are only allowed to be commercially grown after an extensive safety evaluation, therefore management and monitoring takes place on different levels. In some cases, sterility of trees can be applied as a management measure. Engineered sterility can never fully prevent outcrossing of transgenes owing to spontaneous reversions and possible gene silencing phenomena (Van Frankenhuyzen and Beardmore, 2004). In addition, the introduction of floral promoters to abolish flowering may have negative side effects such as decreased vegetative growth (Skinner et al., 2000). The issue of sterility and stability of engineered sterility is especially important in trees in the light of their long life cycle. However, a recent literature review showed that most of the containment strategies for transgenic crops are as yet insufficiently studied or proven (De Maagd and Boutilier, 2009).

4 Conclusion

4.1 Status

Field tests with transgenic forest and fruit trees have been conducted all over the world, but transgenic trees are not yet commercialized, with the exception of insect-resistant poplar in China and virus (PRSV)-resistant papaya in China and the US, respectively. The main traits subject to genetic modification in forest tree breeding include herbicide tolerance, resistance to insects, viral, fungal, or bacterial pathogens and to abiotic stresses, wood and growth characteristics, flowering modification, and phytoremediation. Worldwide, the genetic modification activities for forest tree species have been restricted largely to five genera: *Populus*, *Pinus*, *Eucalyptus*, *Picea* and *Liquidambar*. Currently, *Populus* is by far the most studied tree genus for genetic modification purposes, but the number of GM tree species is still increasing. Field trials for GM forest trees have mainly been restricted to *Populus*, *Eucalyptus* and *Pinus*, whereas field trials for fruit trees have primarily been restricted to *Malus*, *Vitis* sp. and *Carica papaya*.

4.2 Trends

It is expected that genetic modification research stimulates the sequencing of genomes and identification of gene functioning in tree genera like *Populus*, *Pinus* and *Eucalyptus*. Only a few of the mentioned tree species and traits are likely to be expected in the Netherlands. Most likely are apples and plums that are resistant to common pests like scab or rust, and poplar species with improved growth characteristics. Traits subject to genetic modification in the Netherlands are resistance to insects and diseases, pesticide tolerance; faster growth for biomass production, adaptation to various soils and climates, and controlling pollen/gene flow, including modified sterility.

4.3 Environmental risk assessment

The deliberate release of GM trees into the environment, either in field trials or commercially, may entail potential environmental risks that are to be assessed as part of the environmental risk assessment. Potential environmental risks are always assessed on a case-by-case basis and considered within the ecological context of the particular organism, trait and environment. Some aspects of the environmental assessment may be different for trees compared to annual crops, because of their differences in biology and role in ecosystems. Trees are generally seen as the drivers of terrestrial biodiversity due to the high number of organisms associated with them.

Aspects that are specific for the environmental risk analysis⁷ of GM trees (in comparison to transgenic crops) are:

- Potential environmental impacts may increase or be longer lasting (due to the longevity of trees);

⁷ Following the EU directive 2001/18/EC on the release of GM organisms in the environment.

- The impacts on large groups of tree-associated organisms that are potentially exposed to transgenic trees (e.g. insects, birds, litter biota, symbiotic fungi);
- The potential environmental impact as a result of outcrossing of transgenes among trees (considering the ease with which some species hybridize, and the long distance dispersal of pollen, and the high number of wild relatives in many tree species);
- The potential impact that GM trees may have on long-term biogeochemical processes like the decomposition of organic material (due to the long rotation time and the use of traits that alter the wood composition).

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Appendix 1 List of GM *forest* tree species per country, per traits with the number of trial fields and their source in EU member states and the USA

Country	GM forest tree species	Common name	Period	Traits	# Field trials	Source
Finland	<i>Betula pendula</i>	Silver birch	1996-2005	marker system; resistance to fungi (non-specified); resistance to insects (non-specified); synthesis of chitinase; synthesis of glucanase; restoration of male sterility/fertility; synthesis of nitrate reductase	5	1
Finland	<i>Picea abies</i>	Norway spruce	1996, 1997	marker system	2	1
Finland	<i>Pinus sylvestris</i>	Scots Pine	1996, 1997	marker system	2	1
France	<i>Populus x canescens</i> (= <i>Populus tremula</i> x <i>P. alba</i>)	Grey poplar	1996	alteration of lignin biosynthesis; down regulation of cinnamoyl CoA reductase; marker system	1	2
France	<i>Populus</i>	Poplar	1993-1995	resistance to insects (Bt-derived); restoration of male sterility/fertility; tolerance to glufosinate	2	2
France	<i>Populus x canescens</i> (= <i>Populus tremula</i> x <i>P. alba</i>)	Grey poplar	1995-2007	alteration of lignin biosynthesis; downregulation of cinnamoyl CoA reductase; downregulation of o-methyl transferase; restoration of male sterility/fertility; downregulation of the floral homeotic gene to induce sterility; improvement of paper production; improvement of wood quality; testing of gene expression; testing of gene stability	7	2
Germany	<i>Populus</i>	Common Aspen	1996-2000	monitoring transgene flow; tolerance to glufosinate; synthesis of rol gene product(s); tolerance to glyphosate	2	3
Germany	<i>Populus</i>	Poplar	2001, 2002	phytoremediation of soils; removal of heavy metals	2	3
Norway	<i>Populus tremula</i>	Common Aspen	1999	synthesis of phytochrome A	1	4
Portugal	<i>Eucalyptus globulus</i>	Tasmanian Blue Gum	1997	tolerance to cold, lignin content, markers	1	5,6
Spain	<i>Eucalyptus</i>	Eucalyptus	1994	tolerance to cold, lignin content, markers	1	6,7

Country	GM forest tree species	Common name	Period	Traits	# Field trials	Source
Spain	<i>Populus x canescens</i> (= <i>Populus tremula</i> x <i>P. alba</i>)	Grey poplar	1998	stimulation of growth rate; synthesis of glutamine synthetase; testing of gene stability	1	7
Sweden	<i>Populus tremula</i> x ...	Hybrid Aspen	2004	alteration of photosynthetic properties; inhibition of photosynthetic functions; inhibition of photosynthetic proteins	1	8
Sweden	<i>Populus</i>	Poplar	2008	resistance to insects (Bt-derived)	1	8
UK	<i>Eucalyptus</i>	Eucalyptus	1993-1998	tolerance to cold, lignin content; marker system; testing of gene stability	2	6,9
UK	<i>Eucalyptus grandis</i>	Rose Gum	1995	tolerance to glyphosate	1	9
UK	<i>Populus</i>	Poplar	1996	alteration of lignin biosynthesis; downregulation of cinnamoyl CoA reductase; downregulation of o-methyl transferase	1	9
UK	<i>Populus x canescens</i> (= <i>Populus tremula</i> x <i>P. alba</i>)	Grey poplar	1995	alteration of lignin biosynthesis; downregulation of cinnamoyl CoA reductase; downregulation of o-methyl transferase	1	9
US	<i>Amelanchier laevis</i>	Allegheny serviceberry	1987-2008		1	6,10
US	<i>Eucalyptus camaldulensis</i>	River Red Gum	2002-2007		5	6,10
US	<i>Eucalyptus grandis</i>	Rose Gum	1987-2008	lysine levels, fertility, lignin levels, and growth rate	32	6,10
US	<i>Eucalyptus sp</i>	Eucalyptus	1987-2008		23	6,10
US	<i>Eucalyptus urophylla</i>	Timor White Gum	1987-2008	fertility and growth rate	1	10
US	<i>Liquidambar sp.</i>	American Sweetgum	1987-2008		30	10
US	<i>Pinus radiata</i>	Monterey Pine	1987-2008	growth rate, lignin levels, and fertility	1	10
US	<i>Pinus rigida</i> x <i>Pinus taeda</i>	Pitch Pine x Loblolly Pine	1987-2008	growth rate, lignin levels, and fertility	45	10
US	<i>Pinus taeda</i>	Loblolly Pine	1987-2008		94	10
US	<i>Pinus taeda</i> / <i>Pinus rigida</i> x <i>Pinus taeda</i>	Loblolly Pine/Pitch Pine x Loblolly Pine	1987-2008		2	10
US	<i>Pinus sp.</i>	Hybrid Pine	1987-2008		2	10
US	<i>Populus sp.</i>	Poplar	1987-2008	growth rate, fertility, flowering time, light response, uptake of heavy metals, <i>Coleopteran</i> resistance, expression of a visual marker	121	10,11
US	<i>Populus deltoides</i>	Eastern Cottonwood	1987-2008		37	10
US	<i>Populus tremula</i>	Common Aspen	1987-2008		1	10
US	<i>Populus x canescens</i> (= <i>Populus tremula</i> x <i>P. alba</i>)	Grey poplar	1987-2008		15	10

Country	GM forest tree species	Common name	Period	Traits	# Field trials	Source
US	<i>Populus tremula</i> x <i>P. tremuloides</i>	Hybrid Aspen	1987-2008		6	10
US	<i>Populus trichocarpa</i>	Black Cottonwood	1987-2008		1	10
US	<i>Populus x euramerica</i>	Hybrid Poplar	1987-2008		3	10
US	<i>Picea glauca</i>	White Spruce	1987-2008	growth rate, lignin levels, and fertility	1	10
US	<i>Populus, Picea glauca</i>	Poplar, White Spruce	1987-2008		1	10
US	<i>Ulmus americana</i>	American Elm	1987-2008	Dutch elm disease resistance	3	10
Total						

Appendix 2 List of GM *fruit* tree species per country, per traits with the number of trial fields and their source in EU member states and the USA

Country	GM fruit tree species	Common name	Period	Traits	# Field trials	Source
Belgium	<i>Malus domestica</i>	Apple	1989-2008	resistance fungus, bacteria, modified rooting, flower formation	2	12,13
Czechia	<i>Prunus domestica/Prunus sp</i>	Plum	2006-2007	virus resistance	1	14
France	<i>Coffea canephora/Coffea arabica</i>	Coffee	1998	herbicide tolerance, insect resistance, reduced caffeine content	1	15
France	<i>Vitis sp. (berlandieri x riparia)</i>	Grape	1994	virus resistance (grapevine fanleaf nepovirus)	2	2,16
France	<i>Vitis sp. (vinifera x berlandieri)</i>	Grape	1994-2004		3	2,16
France	<i>Vitis sp. (sand grape)</i>	Grape	1994		1	2,16
France	<i>Vitis sp.</i>	Grape	1996	virus resistance (grapevine fanleaf nepovirus)	1	2
Germany	<i>Malus domestica</i>	Apple	2003	resistance to bacteria (expression of T4 lysozyme); resistance to bacteria (not specified); resistance to fungi (not specified)	1	3,12
Germany	<i>Vitis sp.</i>	Grape	1998	resistance to fungi (not specified); synthesis of chitinase; synthesis of glucanase; synthesis of ribosomal inactivating protein	1	3,16
Italy	<i>Actinidia deliciosa</i>	Kiwi	1998	resistance to fungi (not specified); synthesis of chitinase; synthesis of glucanase; synthesis of ribosomal inactivating protein	3	17,18
Italy	<i>Citrus sp</i>	Lemon	2004	resistance to fungi, characteristics of flowering and fruiting;	1	18,19

Country	GM fruit tree species	Common name	Period	Traits	# Field trials	Source
Italy	<i>Olea europaea</i>	Olive	1998	synthesis of osmotin; synthesis of pathogenesis related proteins; synthesis of rol gene product(s)	3	18
Italy	<i>Prunus avium</i> .	Cherry	1998	modified root systems; synthesis of rol gene product(s)	3	18,20
Italy	<i>Vitis sp</i>	Grape	1999	synthesis of auxin; synthesis of tryptophan-2-monoxygenase	1	16,20
Netherlands	<i>Malus domestica</i> (incl. <i>Malus pumila</i>)	Apple (incl. Paradise apple)	1998- 2007	resistance fungus, bacteria, modified rooting, flower formation	4	12,21
Romania	<i>Prunus domestica/Prunus sp</i>	European plum	2004	virus resistance (plum pox potyvirus)	1	14,22
Spain	<i>Citrus sinensis</i>	Orange	1996-2009	induction of early flowering; stimulation of growth rate; marker system; Modification of fruit aroma; resistance to fungi (<i>Phytophthora citrophthora</i>)	6	7
Spain	<i>Citrus sinensis x poncirus trifoliata</i>	Citrange	2004 -2008	modification of plant architecture, flowering and fruiting behaviour; induction of early flowering	3	7,19
Spain	<i>Prunus domestica/Prunus sp</i>	European plum	2006-2007	virus resistance (plum pox potyvirus)	2	14,19
Sweden	<i>Malus domestica</i>	Apple	1999, 2004	improvement of the rooting ability; synthesis of rol gene product(s)	2	12
Sweden	<i>Pyrus pyraister</i>	Pear	2004	synthesis of rol gene product(s)	1	8
US	<i>Carica papaya</i>	Papaya	1987-2008		29	10
US	<i>Castanea dentata</i>	American Chestnut	1987-2008	resistance to fungi and herbicides	6	10
US	<i>Citrus x sinensis en Citrus paradisi</i>	Orange	1987-2008		1	10
US	<i>Citrus paradisi</i>	Grape fruit	1987-2008		15	10
US	<i>Citrus sinensis x poncirus trifoliata</i>	Citrange	1987-2008	resistance to fungi, characteristics of flowering and fruiting	2	10,19
US	<i>Citrus sp./Citrus aurantifolia</i>	Lemon	1987-2008	resistance to fungi, characteristics of flowering and fruiting	2	10
US	<i>Coffea canephora or Coffea arabica</i>	Coffee	1999	herbicide tolerance, insect resistance, reduced caffeine content	3	15
US	<i>Diospyros sp.</i>	Date Plum (Kaki)	1987-2008	cold tolerance, drought tolerance, and <i>Lepidopteran</i> resistance	5	10
US	<i>Juglans sp.</i>	Walnut	1987-2008	rooting, changed flowering time, insect resistance	15	10,23

Country	GM fruit tree species	Common name	Period	Traits	# Field trials	Source
US	<i>Malus domestica</i>	Apple	1987-2008	polyphenol oxidase levels, sugar alcohol levels, brown spot resistance, fruit ripening, and fire blight resistance	48	10,11
US	<i>Musa sp.</i>	Banana	1987-2008	resistance to fungi, nematodes and bacteria	5	10,24
US	<i>Prunus domestica/Prunus sp</i>	European plum	1992-2008	virus resistance	8	10,14
US	<i>Pyrus sp</i>	Pear	1987-2008	bacterial resistance, delayed maturity, fruit ripening	5	10,25
US	<i>Vitis sp.</i>	Grape Vine	1987-2008	resistance to fungi, characteristics of flowering and fruiting (grape size)	57	10,16

Sources:

1. <http://mbg.jrc.ec.europa.eu/deliberate/Fl.asp>
2. <http://mbg.jrc.ec.europa.eu/deliberate/FR.asp>
3. <http://mbg.jrc.ec.europa.eu/deliberate/DE.asp>
4. <http://mbg.jrc.ec.europa.eu/deliberate/NO.asp>
5. <http://mbg.jrc.ec.europa.eu/deliberate/PT.asp>
6. <http://www.gmo-compass.org/eng/database/plants/34.eucalyptus.html>
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13. <http://www.gmo-compass.org/eng/database/plants/61.plum.html>
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15. http://www.gmo-compass.org/eng/database/plants/73.grape_vine.html
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18. http://www.gmo-compass.org/eng/database/plants/305.citrus_fruits.html
19. <http://www.gmo-compass.org/eng/database/plants/285.cherry.html>
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21. <http://mbg.jrc.ec.europa.eu/deliberate/RO.asp>
22. <http://www.gmo-compass.org/eng/database/plants/71.walnut.html>
23. <http://www.gmo-compass.org/eng/database/plants/20.banana.html>
24. <http://www.gmo-compass.org/eng/database/plants/22.pear.html>

Appendix 3. Traits subject to GM in crops and trees in EU field trials in the period 1992-2008.

(Source: http://www.gmo-compass.org/eng/agri_biotechnology/field_trials/)

Genetically Modified Traits in GM crops and trees in EU field trials (1992-2008)

