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NINA • NIKU
PROJECT REPORT

Long-term environmental impacts
of release of transgenic
Norway spruce (*Picea abies*)

Bjørn Åge Tømmerås
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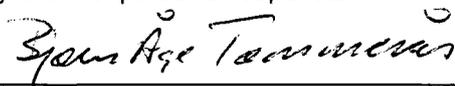
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Abstract

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The aim of the present project is to develop methods and strategies for assessing ecological impacts of introduced transgenic individuals of a species which plays a key role in natural ecosystems. Norway spruce (*Picea abies*) plays such a role in the boreal zone from Norway to Siberia and is an economically important species. A large part of the boreal Norway spruce forests is commercially utilised and managed with varying levels of intensity in forest management. The spruce forest areas are fragmented in domesticated and natural forests. An hypothetical release of transgenic Norway spruce is selected for this case-study exercise.

The knowledge of cultivating spruce to the forest industry and its importance to boreal forest ecosystems, which house thousands of species of flora and fauna, urgently point out the need of broad scientific impact analysis when release of genetically modified spruce (GM-spruce) is considered. The combination of natural and introduced spruce has resulted in a variety of ecological impacts related to forestry, many of them rather intensively studied. This knowledge is essential as a basis to predict long-term environmental effects of released GM-spruce.

Norway spruce is distributed across regions and country borders. It is, therefore, necessary to consider the topic of release of GM-spruce in an international forum. There is a broad international agreement that the environmental effects of genetically engineered organisms must be evaluated on a case by case basis and using a stepwise procedure that includes fully enclosed pilot studies. In widespread organisms experience from field trials with transgenic plants in one region, cannot be applied to risk assessments of release in other regions without explicit tests of the effect of environmental and genetic variation on the outcome of the release. This is a formidable task which will have to be carried out by scientists with proper knowledge specific to the local biodiversity and ecosystem functions.

The impact assessment principles evolved in this report build on existing knowledge. There is lack of knowledge especially on topics related to the whole biodiversity and ecosystem functions. In addition, many short-term and especially long-term effects, are more or less unpredictable due to stochasticity in

climatic and biological conditions and the specific conditions in the area at the time of release.

We have reviewed the status and possible progress in producing GM-spruce intending to improve tree phenotypes. At the moment there is no GM-spruce on the market ready to be released.

A monography including both natural and domesticated spruce biology is presented to make a basis for discussion of the possible responses of existing ecosystems to the release of GM-trees.

Norway spruce migrated to Norway through Finland and Sweden. The species established in Norway approx. 500 BC, and spread at an estimated speed of 9 km per 10 years. The species now covers most of the boreal zone in Norway. Photoperiod is the environmental factor that initiates the cessation of growth and development of frost hardiness, but with some modifications caused by temperature. These important limiting factors affect various provenances different.

Spruce seeds are mostly produced by outcrossing, but some self-fertilisation occurs. Sexual maturity generally is reached at an age of 20-30 years or even earlier from planting. Flowering occurs often more than once a decade but with considerable geographical variation. Seeds are dispersed mainly by wind and rather few are spread over larger distances. The gene flow by means of pollen dispersal is quite effective and plays an essential role for the population genetic structure of Norway spruce.

Some hybridisation occurs between Norway spruce and other *Picea* species, but rather few potential hybrid crossing combinations have been attempted.

The overall principles for environmental risk assessment involve the evaluation of genetically engineered spruce according to the biological properties (phenotype). Due to uncertainties about stability of a GMO in general, gene expression and gene transfer to other organisms (horizontal gene transfer), these problems also have to be addressed in one specific impact assessment.

The intended effects of releases of transgenic organisms are usually specific with regard to species and environment, and limited in time. Evaluations of environmental effects must, however, also include other species and environments, because both the transgenic organisms and their genes can spread after deliberate or accidental release. Moreover, genetically modified organisms are exposed to natural selection and may evolve better adaptations to the environment over time.

General considerations of invasion potential and gene flow to related populations make it clear that Norway spruce is a high-risk organism. Among a selected set of attributes, the most important ones for grading transgenic spruce at the high-risk end of the spectrum are (1) the essentially self-propagating characteristics of Norway spruce, (2) the broad geographic range it occupies, (3) the high level of gene flow among populations/neighbourhoods and the proximity of cultured (transgenic) stands to wild populations, (4) the important role that Norway spruce plays in the structure and function of boreal ecosystems, (5) the virtual impossibility of simulating realistic ecological conditions in the laboratory or in field test orchards, and (6) the typically uncontrolled access for the public to test sites.

To develop adequate tools for risk assessment of GM-spruce and to predict risks when gene transfer occurs, some mathematical models for gene dispersal are presented. Such models can be helpful especially when an ecological selective benefit or disadvantage of a certain GM-tree can be estimated. Even though the eventual fate of the transgenes differs a lot between those that are selected for vs those selected against, it may take many spruce generations before a selective benefit or disadvantage can be determined.

Evaluation of specific GM-spruce must also include the conservation of biodiversity dependent of the species Norway spruce. There are differences in the risk assessment between various potential types of genetically modified Norway spruce. For some GM-types the main risk is invasion to new areas (i.e. frost tolerance) while other types affect the forest where spruce already is the dominating species (i.e. enhanced pathogen resistance). Finally, both today's Norway spruce areas and new potential invasive areas can be affected (i.e. enhanced drought resistance).

A type of GM-spruce able to resist or change the decomposition of wood (i.e. insect resistance) are considered as specifically questionable due to threat to natural ecosystem function, habitats and species. The impact of using GM-spruce with enhanced fungi resistance is also uncertain, and can seriously affect mycorrhiza systems which are indispensable for ecosystem functioning.

By releasing GM-spruce in an area where the inserted genes have the possibility to spread throughout the natural (native) range of the species, great impact on the genetic resources must be expected. Due to the uncertainties about the probability for potentially irreversible impacts, independent of the intended benefit from the genetic modification, we recommend no release of genetically modified Norway spruce.

Key words: *Picea abies*, spruce biology, release of genetically modified organism (GMO), risk assessment, ecological impacts, key species, genetic variability, gene flow

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Preface

OECD has initiated efforts to develop harmonisation of the regulatory oversight in biotechnology. This includes identification and analysis of issues relevant to applications involving releases of genetically engineered species into the environment. In the present project the impacts of releasing a semidomesticated species into environments where the species naturally occurs, have been analysed. The tree species Norway spruce is selected in this case study. To fulfill the objective of this project, six scientist from the Norwegian Institute for Nature Research and Norwegian Forest Research Institute co-operated.

The scope and coverage of this report have stressed the benefits of crossdisciplinary co-operation. The combination of knowledge about general ecology, genetics, modelling and forest and forestry science have been a great inspiration and a necessity for developing this report.

The Directorate for Nature Management (DN) in Norway represented by Norunn Sæther Myklebust, has followed the working process and has given valuable contributions in discussions and on manuscript versions of this report. We acknowledge the comments of Thomas Geburek, Federal Forest Research Centre in Austria. In addition many scientists have participated in helpful discussions on different topics. Especially Odd Arne Rognli and Minna Nurminiemi (The Agricultural University of Norway) and Odd Terje Sandlund (NINA) are acknowledged for important contributions.

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Trondheim, May 1996

Bjørn Åge Tømmerås
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1 Introduction

The production and use of genetically modified plants in agriculture are increasing rapidly. The potential of improving productivity and quality of products is thought to be the most important incentive for the application of genetic modification. The release of genetically modified plants to man-made or natural environments represents a challenge to risk assessments of environmental effects. The scientific discussion about assessing ecological risks is far from consensused not only regarding the risk assessment itself, but also procedures for how it should be carried out.

The aim of the present project is to develop methods and strategies for assessing ecological impacts of transgenic trees using Norway spruce (*Picea abies*) as a model. Norway spruce is an economically important species of geographically large areas. It plays a key role in natural ecosystems in the boreal zone from Norway and Middle Europe to Siberia. The species shows interbreeding between cultured and natural populations. Thus, Norway spruce is a good model system for evaluating environmental impacts of genetic modification of a key species which features both cultured and natural populations.

Development of genetically modified trees is still in its infancy. We assume, however, that within few years the release of genetically modified forest trees (GM-trees) will be considered. The GM-trees may be released within the natural range of the species. Such a release necessitates risk assessments which include evaluation of potential changes of the tree as a key species in the habitat as well as the possibility of gene transfer to natural tree populations.

1.1 General background

Semi-domesticated species are often major components of large ecosystems. At the same time, the natural populations present in these ecosystems are important sources for the continuous development of new cultivars. In the future, we may see the development of new transgenic cultivars. This raises questions of both the long-term effect of gene-flow into natural populations from the transgenic plant and changes in occurrence, distribution and performance of the plant in its natural centre of genetic diversity.

Regions with high genetic diversity may include indigenous populations, cultivars and landraces which can hybridise with introduced forms. The effect of hybridisation on indigenous populations depends on many factors such as the characteristics of the transgenic organism including its inserted genes and

the function of the indigenous species in the ecosystem. A pertinent example is new tree cultivars which exhibit increased frost-tolerance, which may enhance the competitive abilities of the cultivar in the wild. The potential risk to natural ecosystems is large and has to be evaluated on a *case by case* basis and using a stepwise procedure that includes fully enclosed pilot studies.

Before simplified procedures for risk assessment can be developed, the general validity of risk assessments conducted in a restricted number of environments must be demonstrated. In widespread organisms experience from field trials with transgenic plants in one region, cannot be applied to risk assessments of release in other regions without explicit tests of the effect of environmental and genetic variation on the outcome of the release. This is a formidable task which will have to be carried out by scientists with proper knowledge specific to the local biodiversity and ecosystem functions.

Many species, like Norway spruce, are distributed across regions and country borders. The assessments of potential impacts due to the release of transgenic organisms should therefore involve more than one country and the topic must be considered in an international forum.

1.2 Topics for this case study

There is no established definition of «long-term» in connection with assessing environmental impacts. We have followed a pragmatic definition of 10 to 100 generations as a guideline (Crawley 1995). The generation time for Norway spruce is approximately 25 years, and the time scale for long-term environmental impacts of release of transgenic trees can therefore be hundreds of years.

The knowledge of cultivating spruce to the forest industry and its importance to boreal forest ecosystems, which house thousands of species of flora and fauna, urgently point to the need for a broad scientific analysis of potential risks involved in releasing transgenic spruce to the environment. Norway spruce exists in natural ecosystems, as well as in large areas of managed forest. The combination of natural and introduced spruce has resulted in a variety of ecological impacts related to forestry, many of them rather intensively studied. This knowledge is essential as a basis for predicting long-term environmental effects of releasing genetically engineered spruce.

The risk assessment principles evolved in this report build on existing knowledge. But, even for a well known species as Norway spruce there is lack of

knowledge especially on topics related to the whole biodiversity and function of forest ecosystems. The lack of knowledge is partly responsible for the uncertainties in predicting impacts by releasing transgenic spruce. Moreover, many short-term and especially long-term effects are unpredictable due to stochasticity in climatic and biological conditions and the specific conditions in the area at the time of release.

An understanding of spruce biology is needed in order to evaluate the different characteristics of GM-spruce, and to outline the possible responses of existing ecosystems to the release of transgenic trees. Thus, a monography of Norway spruce is included (Chapter 2) which follows the traditional concept for description of taxonomic status, dispersal of the tree and genes, establishment of the tree and interactions between the tree and the environment. In addition, the role of spruce as a key factor in the structuring and dynamics of boreal forests is reviewed.

The forest in the boreal zone comprises both natural as well as managed spruce forest areas. Domestication of Norway spruce has a significant impact on the forest ecosystems, and information of management activities is included in this report (Chapter 3) to give a background understanding of why genetic engineering has been brought up as a means to improve production and quality of forest products.

A review of the «useful» genes as well as how they are proposed to improve the tree phenotype is presented (Chapter 4). The information is needed to evaluate possible long-term environmental impacts of GM-spruce on different ecosystems. Pure «science fiction» is omitted, however, and the problem of transgene inactivation is discussed in relation to the idea to produce sterile transgenic trees to prevent uncontrolled spread of alien genes to wild populations of the tree species.

General principles and questions for risk assessment of GMOs are reviewed in Chapter 5. Norway spruce is then discussed in this general context as a host organism for genetic modification.

Taking into account the importance of spruce for boreal ecosystems and the rather intensive forestry in boreal forests, the effects on the whole flora and fauna must be evaluated. The possible impacts are both connected to changes in spruce performance as a species and to changes of ecosystem structure and dynamics. As spruce individuals form habitats for other organisms, both as growing tree and decaying wood, a variety of environmental consequences are reviewed.

Mathematical models for gene dispersal and selection are presented as tools helping to predict risks when gene transfer occurs. Such models can be helpful especially when a selective benefit or disadvantage of a certain GM-tree can be estimated.

The way we have addressed the necessary topics regarding release of GM-spruce, is more or less independent of the inserted gene(s). To present a closer view on some specific GM-spruce we have discussed in detail GM-spruce with enhanced resistance to pathogens, insect resistance, frost tolerance during active growth and increased drought resistance (Chapter 6).

2 Spruce biology

This chapter reviews the biology of the species, *Picea abies* (L.) Karst. Special emphasis has been placed on knowledge that is essential to predict potential long-term environmental effects of releasing genetically engineered spruce. Knowledge particularly relevant for the species grown under Norwegian and Nordic conditions is stressed.

The most comprehensive treatise of the biology of Norway spruce, including its use as a semi-domesticated tree species, has been given by Schmidt-Vogt (1977, 1986, 1989, 1991). This magnificent work contains a wealth of knowledge on Norway spruce and is indispensable to anyone who wants to study the biology of the species in depth.

2.1 Taxonomy and natural distribution

2.1.1 Taxonomy

Norway spruce (*Picea abies* (L.) Karst.) belongs to the genus *Picea* which includes at least 36 different species that all have their distribution in the northern hemisphere (Schmidt-Vogt 1977). The species *P. abies* has been designated by a large number of synonyms, partly caused by classification to different species or subspecies due to its great number of varieties and forms. The best known of these is the classification of the Siberian spruce in a separate species *P. obovata*, based on the shape of its cone scales. These variations, however, should be considered as normal patterns of variation within a widespread species and should at most be used to designate different climatic varieties (Schmidt-Vogt 1977, 1978).

2.1.2 Number of chromosomes

The number of chromosomes of Norway spruce is $2n = 24$ (Schmidt-Vogt 1977 and references cited there). Tetraploidy has occasionally been observed in Swedish and German provenances (Kiellander 1970) and has also been induced by colchicine treatments (Johnsson 1975). Trees with irregular chromosome numbers seem in general to have reduced fitness.

2.1.3 Natural distribution and origin

The natural distribution of *P. abies* can, according to Schmidt-Vogt (1977), be divided into three areas: Central and Southeast European, Northeast European and Siberian spruce area (**Figure 2.1**). The first area covers mainly mountainous and subalpine regions and is separated from the second one by a spruceless area in Poland. The Northeast European area includes both the Baltic, the Nordic and the Russian spruce. The Siberian area covers all spruce east of the Ural mountains.

The total natural distribution of *P. abies* covers 31 degrees of latitude from the Balkan Peninsula (latitude 41°27'N) to its northernmost extension near the Chatanga River, Siberia (latitude 72°15'N). Longitudinal range is from 5°27'E in the French Alps to 154°E at the Sea of Okhotsk in Eastern Siberia. The vertical distribution is from sea level and to altitudes above 2 300 m in the Italian Alps. Outside this area the species has been widely planted, in particular in Central Europe and in Scandinavia.

Most likely *P. abies* had its prehistoric origin in East Asia and migrated to Europe through Siberia and the Ural Mountains (Schmidt-Vogt 1977). During the last ice age it is assumed to have survived in Europe in refugia in four regions: North-central Russia, the Carpathian and Transylvanian Alps, the Dinarian Alps and the Appenninian Peninsula. From these refugia it migrated into its present natural areas (**Figure 2.2**). The Central and Southeast European spruce originate from the last three refugia, while the Northeast European spruce migrated from Russia.

2.1.4 Migration through Finland and Sweden and establishment in Norway

The migration of Norway spruce into Fennoscandia took place from the Russian refugium. It advanced through the forest area at the present border between Russia and Finland and passed through Finland and northern Sweden into Norway in the period 3 500-500 BC (Moe 1970, Schmidt-Vogt 1977, Hafsten 1991, 1992a, b). The advance from the northern part of

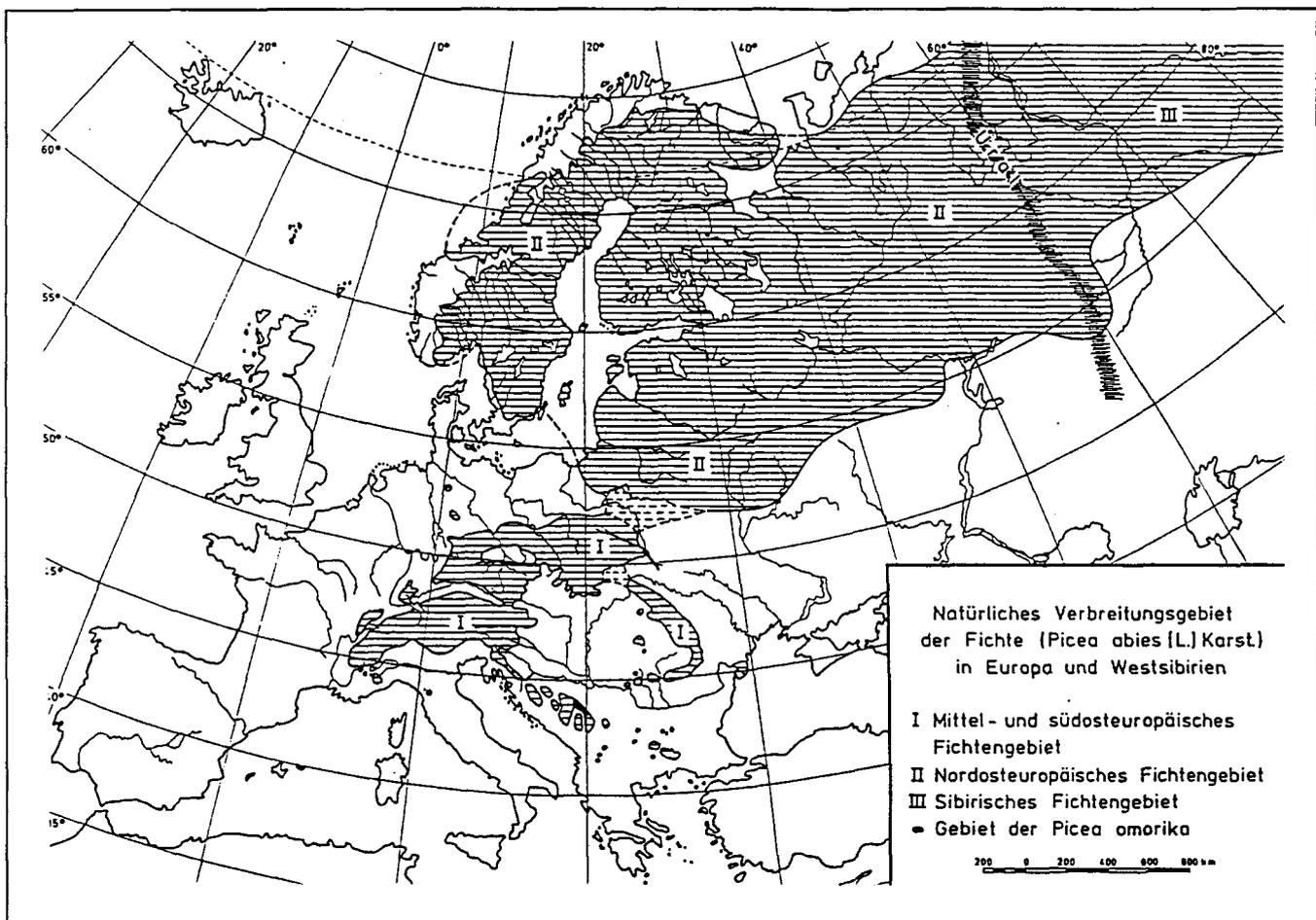


Figure 2.1. The natural distribution of *Picea abies* in Europe and West Siberia and its subdivision into the three main regions. From Schmidt-Vogt (1977).

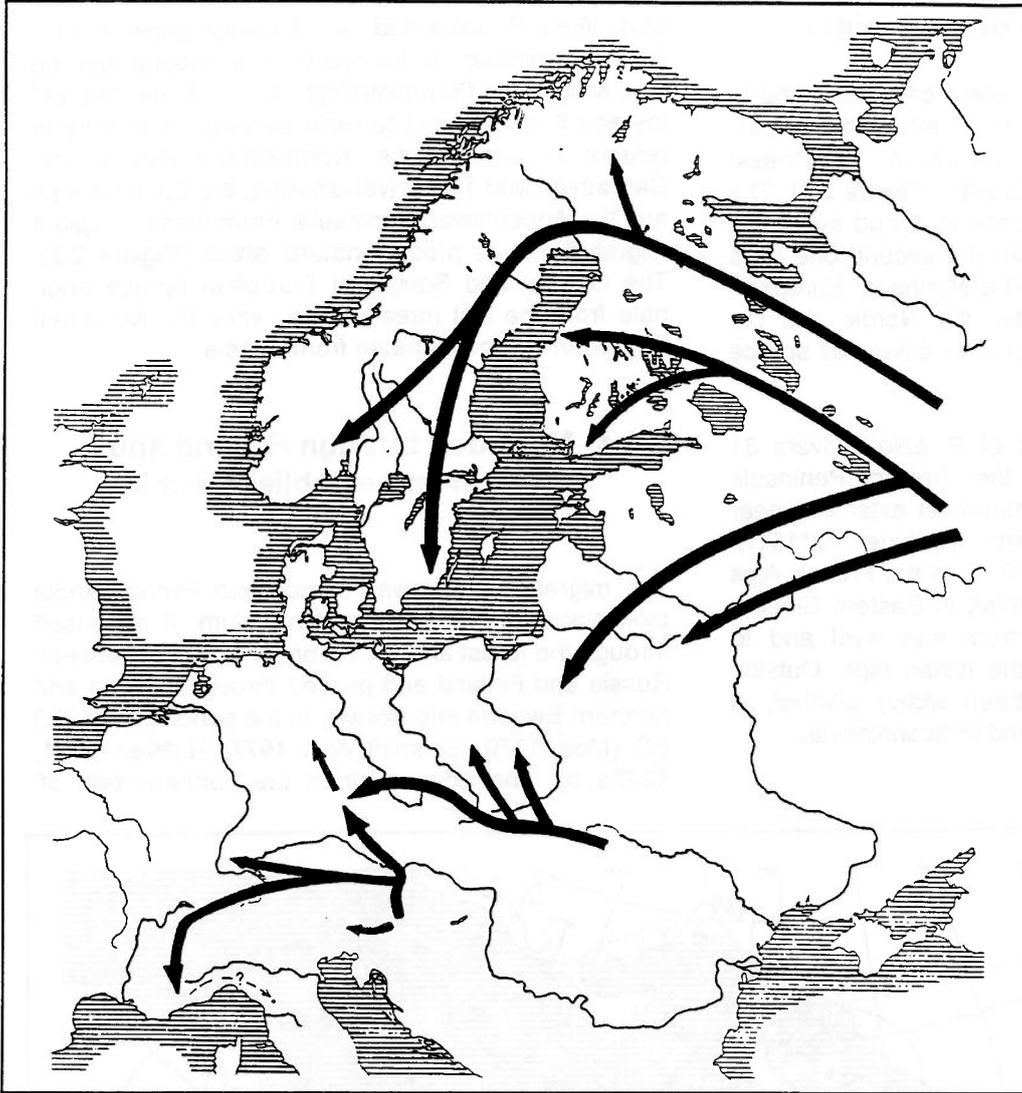


Figure 2.2. The migration of *Picea abies* in Europe after the last ice age. From Schmidt-Vogt (1986).

Sweden and southwards was rather fast; it has been estimated at an average speed of 9 km per 10 years (Moe 1970). The border area between Sweden and Norway in Central Norway was crossed approx. 500 BC, most likely northeast in Lierne (Hafsten 1992a). The species spread fast in the north-eastern area of Central Norway, but delayed its establishment in the southwestern area of the region till the period AD 800-1200. In southern Norway, the earliest immigration took place in the area along the present border between Norway and Sweden in the years 400-200 BC. Eight hundred years later, it was established in the southeastern lowland area. The migration into the valleys to the species' present altitudinal boundaries was not completed until the period AD 1 000-1 300. In the southwestern part of the range of the species, the populations are very young, less than a hundred years old, and are still migrating westwards (Gløersen 1941). The species never established naturally in western Norway, except for a few scattered populations, which have a late establishment, and most likely are spread from the nearest source stands east of the mountain range (Hafsten

1991). Norway spruce thus has a wide distribution in Norway and is a common and dominant species in particular in the southeastern part of the country, in Central Norway and in North Norway as far north as the Arctic Circle. It occurs scanty or is lacking in the very dry areas in eastern Norway. The maximum altitude of the alpine timber line of Norway spruce in the country is 900-1 000 m (in central parts of south-eastern Norway), in Central Norway 500-600 m and in North Norway at its northernmost natural extension approximately 250 m (Børset 1985). It must be considered a young species in Norway, establishing during a long time period and still advancing naturally into new regions of the country.

2.2 Reproductive biology

2.2.1 Sexual reproduction

P. abies is monoecious, having both male and female flowers on the same individuals, but on separate organs. The male flowers are in most cases located at the base of the preceding year's shoot, while the female strobili are at the tip of the shoot, directed upwards. The reproductive buds are initiated during the growth season the year before.

Male meiosis and pollen development take place after the winter dormancy in late winter or early spring and is regulated by the temperature conditions (Loumajoki 1993). A male flower may contain as many as 600 000 pollen grains which are released at anthesis and dispersed by wind.

Female meiosis usually starts before the female strobili become receptive (Sarvas 1968) and occurs close to or during the period of pollination. Pollen grains are accommodated in a pollen chamber which has a limited volume and on average contains 3-5 pollen grains. They germinate and pollen tubes grow towards the female gametophyte (egg-cell) in the ovule where the fusion of the male and female gametes takes place. Under natural conditions this normally occurs 5-6 weeks after pollination. The fertilised egg develops rapidly into a seed. Several egg-cells may be fertilised in each ovule, but only one will develop into a seed. Empty seeds may be caused by lack of pollination or be due to abortion.

The climatic conditions play an important role during several stages of the reproductive process. High temperature during the growth season is one of the main factors favouring floral initiation and development of reproductive buds which will flower the next spring. Dry conditions and moderate to high temperatures during the flowering period the following year are necessary to obtain a sufficient pollination. Seed development and maturation require high accumulated temperature sums during the summer and early autumn. Such specific weather patterns during two successive years occur rather seldom, in particular far north and at high latitudes, and may explain why seed crops are both rare and irregular in these areas.

2.2.2 Mating system and gene flow

The mating system of Norway spruce falls into the mixed mating category. That is, some proportion of seeds is produced by self-fertilisations and the rest by outcrossing. The cross fertilisations are both between trees standing close in the stand and among distant individuals in the same or in nearby stands.

The actual rate of self-fertilisations in natural populations may vary considerably between trees, and was found by Müller (1977) to vary between 7 % and 18 % in a study of five trees. Koski (1971) estimated the mean proportion of self-fertilisations to 10 %, but concluded that only 1 % of the filled seeds originate from the self-fertilisations.

Spruce pollen is able to move over long distances. Andersson (1955) found that the amount of pollen at 2 500 m distance was 47 % of the amount at the edge of the forest in one stand, and 15 % in another stand. In two Norway spruce stands in Finland Koski (1970) estimated the background pollination to be approximately 60 % of the total pollen catch. Lindgren et al. (1991) reported results from studies of the dispersal of Norway spruce pollen being artificially released in seed orchards outside the local pollen shedding season. Their results of pollen deposition at different distances from the pollen source show the rapid drop-off of the pollen dispersion curve with the largest amount of pollen being deposited less than 50 meters away from the pollen source. However, large variation patterns most likely due to wind turbulence were found between pollen catches in individual days, indicating that a curve showing a smooth movement in the wind direction is not a good model for individual pollen dispersion events (Lindgren et al. 1991).

Both local and long-range pollen movements will determine the natural pollination patterns and actual gene flow in Norway spruce. On average, most pollinations will be with local pollen or pollen from nearby populations (Koski 1970), but with exceptions in some years. In an allozyme marker genetic study in an experimental Norway spruce plantation, Xie & Knowles (1994) estimated the proportion of ovules fertilised with own pollen, pollen from trees within the plantation and from trees outside the plantation to be 0.09, 0.75 and 0.16, respectively. The proportions varied considerably among parent trees. Estimates of the number of migrants per generation calculated from genetic diversity parameters, show that wind pollinated forest trees have a high number of migrants per generation compared to other plant species (Govindaraju 1988, 1989). For Norway spruce, an estimate of approx. 4.5 migrants per generation can be calculated from data presented by Lagercranz & Ryman (1990), compared to estimates of approx. 3 and 1.2 for wind and insect pollinated plants, respectively. The conclusion is therefore that the gene flow by means of pollen dispersal is extremely effective and plays an essential role for the population genetic structure of Norway spruce (Koski 1970).

2.2.3 Inbreeding depression

Most spruce trees will produce some filled seed after self pollinations, but the seed yield is strongly reduced (Skrøppa & Tho 1990). The main reason for this is embryo abortions caused by lethal or deleterious genes that become harmful when they occur as recessive homozygotes in the selfed individuals (Koski 1971). Inbred spruce trees generally have reduced fitness compared to their outbred relatives. They have a lower survival in the field, a shorter growth season and grow more slowly (Langlet 1941, Eriksson et al. 1973, Skrøppa 1996). The inbreeding depression varies both among populations and among individuals within the same population (Skrøppa 1996). For a trait such as height growth at age 10 years, it may vary in the range from 10 to 50 % among selfed offspring of trees from the same population.

2.2.4 The seed crop

Norway spruce trees undergo a rather long juvenile period under which they will not flower and set seeds. In the open stand sexual maturity will generally be reached at an age of 20-30 years, while it occurs later in the closed stand (Schmidt-Vogt 1978). However, a substantial flowering and seed set may occur considerably earlier than 20 years from planting (Skrøppa, unpublished) if the conditions are favourable for floral induction. In exceptional years, female flowering has been observed on 8-10 and male flowering on 12-15 year old Norway spruce trees.

Under Central European conditions Norway spruce will flower several times in a decade (Schmidt-Vogt 1978). In the boreal forest cone harvests occur less frequently and at irregular intervals. In the lowlands of southern Norway there were no cone crops between 1976 and 1983. Since then, however, both substantial flowering and seed crops occurred in the years 1983, 1987, 1989, 1993 and 1995. In northern Norway, on the contrary, successful seed harvests have been obtained only three times during the last 40 years (1958, 1970 and 1981).

2.2.5 Natural regeneration

The Norway spruce seeds are dispersed mainly by wind and partly by birds and animals (Sokolov et al. 1977). Most seeds will be dispersed close to the mother tree, but some may also be dispersed over larger distances.

To provide germination and establishment, the access to soil moisture seems to be the most critical factor. The natural regeneration also very much depends on the species composition of the bottom and field layer. The most productive spruce forest seems to be the most problematic for natural regeneration, for example in "Spruce forest with tall hubs" (*Melico-Piceetum aconitetosum*) and "Spruce forest with small ferns" (*Eu-Piceetum athyrietosum*). The optimal habitat as to natural regeneration is "Spruce forest with small ferns" (*Eu-Piceetum dryopteridetosum*). The regeneration in our most common and widespread spruce community "Spruce forest with bilberry" (*Eu-Piceetum myrtilletosum*), is very much hampered by a thick raw humus layer, especially at higher altitudinal levels (Mork 1944, 1945, 1968).

The Norway spruce seedlings are very shade-tolerant and can survive for decades under a closed canopy (Siren 1955). They grow slowly during the first years, and the height growth increases after 5-10 years (Sokolov et al. 1977, Nikolov & Helmisaari 1992). *P. abies* is often associated with grey alder (*Alnus incana*) on river plains in the boreal zone and may tolerate occasional flooding (Sokolov et al. 1977).

Dynamics of regeneration

Boreal forests which are undisturbed from human activity have dynamic properties with complex succession stages which influence habitats and create possibilities for regeneration. The most important disturbance regimes consist of fire, storms and pathogens like bark beetles (Tømmerås 1994). The fire tolerance of *P. abies* is very poor (Drakenberg 1981). Mainly due to its shallow root system, spruce is intolerant to windthrow (Sokolov et al. 1977). Storms can blow down many trees, in particular in wind exposed areas, where occasionally almost all trees in a stand may blow down. Bark beetles (*Ips typographus*) can from time to time turn to pest and kill trees (Christiansen & Bakke 1988). Together with gaps created by the death of individual old trees, these disturbances open up the canopy and lay the foundation for regeneration.

2.2.6 Vegetative reproduction in nature

Norway spruce will under certain conditions naturally reproduce vegetatively through the lowest branches which may come into contact with the soil and differentiate roots and new shoots (layering). This occurs in particular in alpine areas where the climatic conditions prohibit sexual reproduction and is there important for the distribution of the species (Skoklefeld 1993). Examples of such vegetative reproduction can be seen above the timberline where scattered clonal groups occur.

2.2.7 Crossability with other species

Crossability of spruce species can be judged both from introgressive hybridisation and from attempted controlled hybridisation. The only known natural hybrids involving Norway spruce are *P. abies* var. *obovata* with *P. jenoensis* and *P. koraiensis* (Schmidt-Vogt 1977). Successful artificial hybridisations are reported with eight other spruce species (*P. asperata*, *P. glauca*, *P. mariana*, *P. montigena*, *P. omorica*, *P. orientalis*, *P. rubens* and *P. sitchensis*) (Kleinschmit 1979). Rather few of the potential hybrid crossing combinations have been attempted.

2.3 Genetic variability

The genetic variability of Norway spruce has been studied by a large number of methods and at different genetic levels. More than 100 years ago experiments replicated at several locations with seed lots from different origins (provenances) were planted in Austria, Germany and Switzerland (Langlet 1971). In these first geneecological experiments traits such as height increment, needle morphology, growth habit, time of growth initiation and frost damage were studied. Later, both national and international provenance experiments were established, some of these organised by the International Union of Forest Research Organisations (IUFRO), and often including large numbers of provenances planted on multiple test sites in several countries (Krutzsich 1992). The traits studied were in particular related to forestry cultivation of Norway spruce and characterise climatic adaptation, growth potential and quality. They are typically quantitative, having a continuous phenotypic distribution and are strongly influenced by the environment. The same type of traits has been measured in numerous experiments with offspring from both natural and artificial populations, in most cases established with the intention of testing breeding materials. The field trials have been supplemented by tests under more controlled conditions in which variability in specific physiological traits has been studied. After biochemical markers became available, several population genetic studies have characterised the intra- and interpopulation variation and genetic structure of the species (e.g. Lagercrantz & Ryman 1990, Müller-Starck et al. 1992). Recently, molecular DNA marker techniques have been developed for Norway spruce (Bucci & Menozzi 1993, Binelli & Bucci 1994).

2.3.1 Overall variability

A large genetic variability exists within the extensive range of the natural distribution of Norway spruce. The most pronounced adaptive patterns relate to the

responses of populations to the climatic conditions. Across the European range of the species these patterns of variability can often be related to latitude and altitude of origin and with degree of continentality, and will sometimes vary clinally. In Central Europe, however, differences among populations from the same geographic region are in many cases large and reflect several generations of Norway spruce cultivation. This fact often blurs patterns of variation of adaptive traits. Traits that characterise the annual growth cycle, in particular the onset of growth in the spring and the termination of growth and development of frost hardiness in late summer, show the most pronounced provenance variability. However, observations of these and of growth performance traits are made in common garden field tests comparing provenances that are transferred unequal distances. Such provenance transfers may affect provenances differently. Provenance differences must therefore always be interpreted relative to the planting site conditions.

Populations with an early growth start, often expressed as bud burst or bud flushing, originate from high latitudes in northern Scandinavia, Finland and Siberia and from high altitudes in the Central European Alps (Krutzsich 1975). The eastern and more continental provenances generally have a late growth start, and the latest flushing populations come from White Russia, Northeast Poland and the interior of the Baltic Republics. The variation in bud flushing and initiation of shoot growth of Norway spruce provenances is assumed to be regulated both by differential responses to accumulated temperature sums in the spring and by the conditions during acclimation the preceding year (Heide 1974b, Schmidt-Vogt 1977, Dormling 1982).

Photoperiod is the environmental factor that initiates the cessation of growth and development of frost hardiness (Dormling 1973), but with some modifications caused by temperature (Heide 1974a). Under growing conditions in climate chamber, seedlings of provenances from the northernmost latitudes will respond with a terminal bud set at a night length of two to three hours compared to eight to nine hours of darkness for south-western European origins (Dormling 1973). The northern Scandinavian and Finnish provenances and those from high altitudes in the Alps have the earliest cessation of shoot growth. The latest growth cessation occurs in provenances from South Poland and the East Carpathian Mountains. These variation patterns in the timing and duration of the shoot growth period are shown in **Figure 2.3** for two provenances (Poland and Finland).

At the provenance level, strong relationships are generally present between traits that characterise the timing and duration of the growth period, the lignifi-

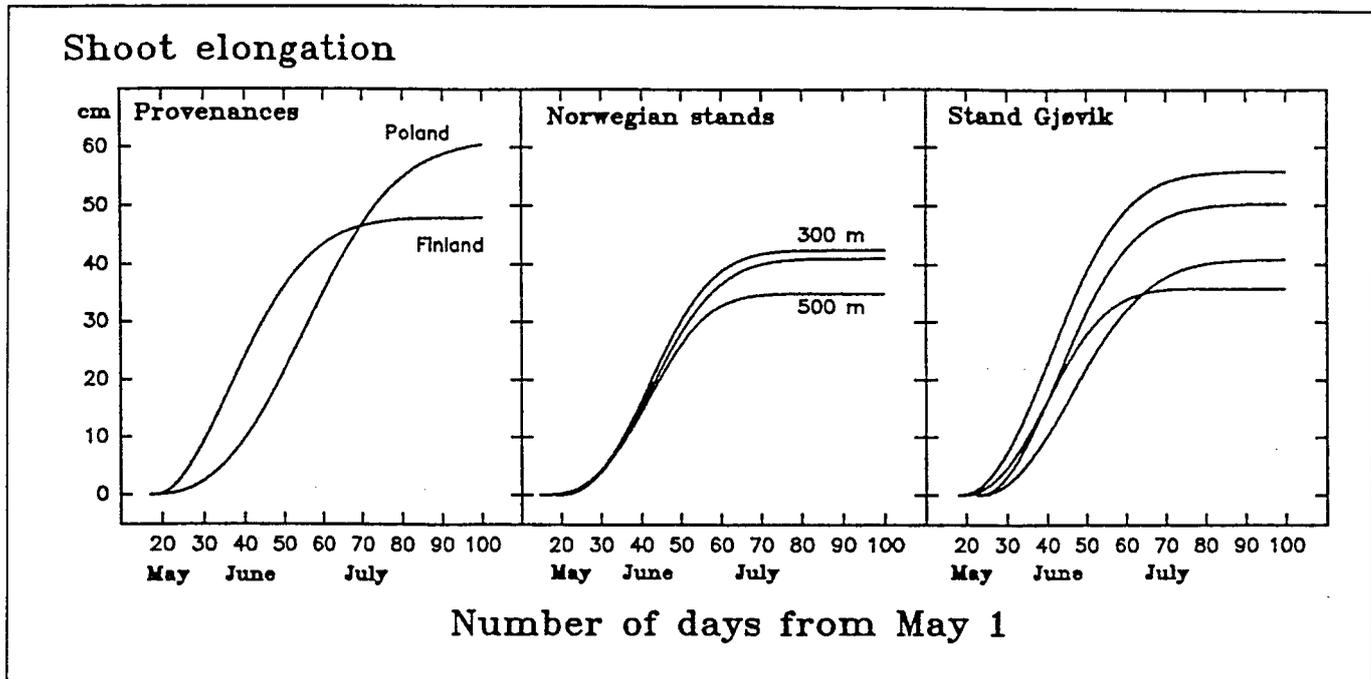


Figure 2.3. Estimated mean shoot elongation curves of eight-year-old Norway spruce trees grown in the same experiment at Ås, Norway (Skrøppa 1982a). The trees are from two provenances (Poland and Finland), three Norwegian stands less than 50 kilometers apart and four controlled cross full-sib families from the same stand. There is large variation both in timing and duration of the shoot elongation period at all three genetic levels.

cation of the annual ring and the development of autumn frost hardiness (Skrøppa & Magnussen 1993). These traits are components of an annual sequence of developmental events which describe the inherent annual rhythm of the trees of the same provenance (Sarvas 1972, Skrøppa & Magnussen 1993).

Resistance to late spring frost is closely related to the time of growth start; the late flushing provenances from eastern Europe suffer less damage than native Nordic provenances during spring frost events in Scandinavia (Werner et al. 1991). A similar, but somewhat weaker relationship is present between the timing of growth cessation and the resistance to autumn frost. Provenances with an early termination of growth will normally enter dormancy (develop frost hardiness) earlier than those extending their growth period late in the summer and thus get less damaged by early autumn frosts.

The growth capacity of different provenances is closely related to the duration of their growth period (Skrøppa & Magnussen 1993). Northern provenances or those from high altitudes that are adapted to a short growth season will therefore have a poorer growth potential than those adapted to a longer season. Two provenance regions of particular high growth potential have been identified (Schmidt-Vogt 1978). One covers parts of the postglacial advance of spruce from the Russian refugium including the Baltic Republics, Northeast Poland and northern parts of

White Russia. The other region covers the eastern Carpathian and Bihor mountains and parts of the Beskides. However, the growth potential of a specific provenance at a given site is also influenced by the latitudinal distance of provenance transfer due to the change in photoperiod (Magnesen 1972, Skrøppa & Magnussen 1993).

Provenance variation has been shown for a number of other traits, such as nutrient demands, respiration activity and shade tolerance (Schmidt-Vogt 1977). Provenance differences are present in crown form related to resistance against snow and ice break, with an increasing resistance with the altitude (Schmidt-Vogt 1977).

Enzyme genetic marker studies reveal a great genetic variability within Norway spruce populations (Müller-Starck et al. 1992), also in populations that are close to the climatic margin of the species (Tigerstedt 1973, 1979). In the most comprehensive isozyme study in Norway spruce (Lagercrantz & Ryman 1990), only 5 % of the total genetic diversity was explained by differences among provenances. Some differentiation occur among populations that are derived from different glacial refugia and seem to reflect their post-glacial evolutionary history (Lagercrantz & Ryman 1990). Populations from the same region show little genetic differentiation (Bergmann 1973, Lundkvist & Rudin 1977, Lundkvist 1979). The central European provenances seem to contain a reduced genetic diversity, expressed by a reduced

level of average heterozygosity, compared to those from eastern Europe and Scandinavia (Lagercrantz & Ryman 1990).

2.3.2 Genetic variability within Norway

Studies of Norwegian spruce provenances have demonstrated a clinal variation corresponding to latitude and altitude for traits related to the annual growth cycle. This is in particular true for traits characterizing the cessation of growth, lignification of the annual ring and development of autumn frost hardiness. In an artificial freezing test experiment comprising 30 provenances from most of the natural distribution of the species in Norway, a continuous variation in autumn frost hardiness was found with a continuous increase in hardiness from south to north and from low to high altitudes (Dæhlen et al. 1995). The most frost hardy provenances originated from 200-400 m in the interior part of Nordland county in northern Norway. In this study, as much as 87 % of the variation in hardiness could be explained by the latitude and altitude of the seed stand. For the initiation of growth in the spring, geographic patterns of variability are likewise present (Dietrichson 1971). In southern Norway, the eastern provenances, originating from a more continental climate, have a later growth start than those from the more coastal regions. Provenances from the highest altitudes in northern Norway have an earlier initiation of growth than those from the low altitudes. A large number of traits were measured in experiments with trees from seed after crosses made in two populations 15 kilometers apart: Veldre (altitude 500 m) and Gjøvik (altitude 270 m) (Skrøppa 1982a, Skrøppa unpublished). The trees of the Veldre population had the shortest growth period and the lowest rate of growth (**Figure 2.3**), and were after ten years 20 % shorter than those originating from Gjøvik.

The southern and low altitude provenances have the longest duration of the growth season, and, as a consequence, the highest growth potential. A clinal variation in growth performance is present within Norway, related to latitude and altitude.

The patterns of provenance variation show geographic variability on a large scale. However, genetic variation may be present between offspring from populations within the same provenance, and this variation is often larger than the variability between provenances. Dietrichson (1973) sampled three populations from each of the same altitudinal zone (620-750 m) in each of five provenances covering an area of 200 km north-south and 250 km west-east in southern Norway. Measurements were made of heights at two and four years, growth initiation and cessation, and lignification of the annual ring. Several

of the traits showed a larger variation among populations within provenance than among provenances.

2.3.3 Variability within populations

The large genetic within-population variation that has been demonstrated by genetic markers, has been confirmed in studies of quantitative traits. Genetic variability has been found within all natural Norway spruce populations studied, also for traits that shown clinal variation at the provenance level (Dietrichson 1971, 1973, Eriksson 1982, Skrøppa 1982a, Ekberg et al. 1985, 1991). Traits that show such variation characterise germination, early and later height and diameter growth, the timing and duration of the shoot growth period, autumn frost hardiness, survival in the field and branch, crown form, stem and wood quality (Schmidt-vogt 1977, Skrøppa 1991, 1993). **Figure 2.3** shows the variability found in the timing and the duration of the shoot growth period between different families from the same population. The range of variation may sometimes be as large as that found between geographically distant provenances.

Genetic relationships that are present among traits comparing geographically distant provenances may not be similar when comparing families from the same population (Ekberg et al. 1994, Skrøppa unpublished). For provenances, more than 80 % of the total variation in height growth can be attributed to the duration of the growth period (Skrøppa & Magnussen 1993). For families from the same population, however, the duration of the growth period and the intensity of the growth are equally important (Skrøppa, unpubl).

2.3.4 Resistance to fungi and insects

Studies have been made to investigate the resistance of Norway spruce against root rot (*Heterobasidion annosum*) both at the provenance and clonal level. Treschow (1958) found no variation in growth of *H. annosum* among trees of different provenances. In an inoculation experiment with *H. annosum* on 98 Norway spruce clones difference among clones were found in lesion length and fungal growth in sapwood (Swedjemark et al. 1995). This indicates the presence of genetic variation among individual clones in degree of resistance to the fungus and that progress in resistance can be achieved by selection. Similar variation among clones has been found in resistance to bark beetle-fungus infection (*Ceratocystis polonica*) (Christiansen & Berryman 1995).

Very little information is available on genetic variation in resistance to attacks by insect species.

2.3.5 Organisation of the genetic variability

Studies of progenies from controlled crosses show a prevalence of additive genetic effects and rather low or non-existing non-additive genetic effects for most of the quantitative traits studied (Eriksson et al. 1978; Karlsson & Danell 1989, Skrøppa 1993). This fact is confirmed in studies of interprovenance hybrids which have failed to demonstrate provenance heterosis effects (Ekberg et al. 1982, Kaya & Lindgren 1992).

Maternal and reciprocal effects only seem to be present for traits expressed at the very juvenile stage (Skrøppa 1993).

2.3.6 Factors influencing the genetic variability

The large genetic variability of the Norway spruce forests is influenced by a large number of factors: ancient origin and immigration history, natural selection, an extensive gene flow caused by pollen dispersal, genetic drift due to small population size and human activities. Different factors may cause specific variation patterns to be present in parts of the range of the species. As an example, in some areas the existence of frost pockets or different slopes and exposure aspects may have resulted in selection of different annual growth rhythms. In Central Europe, the species has been cultivated for more than 300 years, partly with seed material that has been transferred from other regions. Differences in performance between provenances from the same region may therefore not exclusively express adaptational differences. Recent experimental results indicate that phenotypic provenance variation in traits characterizing climatic adaptation is not only regulated by classical (Mendelian) gene frequency differences, but also by other mechanisms (e. g. gene regulation). These mechanisms seem to be triggered by environmental influences during the generative reproductive process (Skrøppa & Johnsen 1994, Johnsen et al. 1995, 1996).

2.4 Synecology and associated species

The forests of *P. abies* in Norway belong to three associations according to Kielland-Lund (1981, 1994). These are: 1. *Eu-Piceetum* (EP). This is the most common forest association in eastern Norway, and probably Trøndelag. EP is the typical climax community on medium rich, podzolic soil types. EP is subdivided into subassociations e.g. *myrtilletosum* (= «Spruce forest with bilberry»), *dryopteridetosum*

(= «Spruce forest with small ferns»). EP is most frequent in the middle boreal and lower part of the north boreal zone in eastern Norway and Trøndelag, that is, in the hill districts and lower mountains (Dahl et al. 1986). 2. *Melico-Piceetum* (MP). According to Kielland-Lund (1994), MP (= «Spruce forest with low herbs») occurs in the eastern parts of the country, on warmer localities and more calcareous soils than *Eu-Piceetum*, mainly in the boreonemoral and south boreal zones. MP has three main subassociations: *pinetosum* on dry limestone soils, *typicum* (= «Spruce forest with low herbs») and *aconitetosum* (= «Spruce forest with tall herbs»). Most *P. abies* forests have a trivial vascular plant flora, except *Melico-Piceetum pinetosum* which houses, among others, rare and in some areas threatened orchids, e.g. *Ophrys insectifera* and *Cypripedium calceolus*. Kielland-Lund (1981, 1994) has described the association *Chamaemoro-Piceetum* on clay or thin organic soils. This community is called 'spruce swamp forest' and is known from East Norway in its typical form.

The Norway spruce forest communities in the superhumid parts of Central Norway, differ from those described from Southeast Norway (Kielland-Lund, 1981, 1994) in having frequent oceanic species, in the field layer e.g. *Blechnum spicant* («bjønnekam») and *Cornus suecica* («skrubber»), in the bottom layer the suboceanic bryophytes *Plagiothecium undulatum* and *Rhytidiadelphus loreus*.

In oceanic parts of Western Norway *P. abies* is replaced by birch (*Betula pubescens*). Aune (1973) has described the association *Corna-Betuletum pubescentis* from western Central Norway. This association can be regarded as an oceanic vicariant of the *Eu-Piceetum* from East Norway.

2.4.1 Norway spruce as a key species

The following reasons are important in the considerations of Norway spruce as a key species in boreal forests:

- 1 Norway spruce stands make a characteristic microclimate due to the ability to grow in low light levels.
- 2 Norway spruce is an active podzolic soil producer, due to the fairly low pH of the litter and the yearly high quantity of the litter.

Norway spruce has the ability to create forests with one dominating tree species, and both characteristics make it a very competitive species on mesic ground of high to medium acidity in boreal climates of fairly high humidity.

2.4.2 Plant and animal species in spruce forests

The importance of Norway spruce for a very high number of species is due to the ability of spruce to change the soil, create essential structures by the size and distribution of individuals into large continuous forests, and the dominance in creating dynamics in the landscape. In addition Norway spruce as growing tree and decaying wood is directly «home and food» for hundreds of species. In Norway, an estimated 20 000 species (~ half of the number of species in the country) are connected to forests.

Almost half of the threatened species in Norway, Sweden and Finland are living in forests (Data from Nord 1994):

- Norway	1839 species on Red list	898 in forests	(49 %)
- Sweden	1336	695	(43 %)
- Finland	1692	727	(43 %)

The threatened (Red list) species occur in different forest categories:

- Oldgrowth natural forests	20 %
- Humid forests	28 %
- Old deciduous forests	23 %
- Fire areas	4 %

2.4.3 Elements of boreal forest ecosystems

Tree species

Normally, different tree species are dominant during different succession stages. There are exceptions, however, where the same tree species are both pioneer and climax tree on a few vegetation types in the boreal zone. Spruce forests have a deciduous stage (mainly birch, rowen, aspen, *Salix* sp and alder) as the first step before spruce become dominant. The biodiversity of the spruce forest is obviously dependent of successional stages (reviewed in Tømmerås 1994).

Deciduous trees in conifer forests

The deciduous trees lose their dominance in spruce forests after the pioneer period, but there is always some deciduous trees as part of the forest stand. The richer vegetation type, the more deciduous trees are present. Old deciduous trees and dead big trees are very important for numerous lichens, insects and birds.

Period of rotation

The different parts of a succession in forests have naturally given possibilities for plant and animal species to adapt to many niches in a complicated pattern. Specialist species are dependent on specific

conditions such as burned trees, dead wood, small seedlings or old but living trees. Effects on disturbances in the rotation cycle on species diversity are not well known (cf Hansson 1992).

Layers

More vertical layers in the forests are from an ecological point of view one of the foundations for the total biodiversity in an area. This layer structure is most dominant late in the pioneer stage and under late successional stages.

Old trees

In natural forests spruce may reach an age of 2-300 years. Usually some individuals become old and of big dimensions. These trees are important for a lot of species, e.g. many woodpeckers, lichens, bryophytes and insects.

Dead trees

Many different processes result in dead wood, storm felling, fire, pathogens and normal ageing. This leads to a wide spectrum of types of dead wood in a spruce forest. More than one thousand plant and animal species take part in the decomposition process of dead spruce, a large proportion are specialists on a specific stage. It is estimated that more than 700 Norwegian beetle species are dependent on dead wood of boreal forest tree species (Tømmerås, unpubl).

Continuity

Some areas in boreal forests are free from natural disturbances such as fire and storm felling. These forest areas can cover from 20-40 % of a forest landscape, and are often widespread on humid soil and steep valley areas. Many cryptogams and invertebrates are dependent on continuous forest structure.

Special lichens

Due to the continuous damp and mild climate of western parts of the counties Sør-Trøndelag, Nord-Trøndelag and Nordland, a special type of spruce forest called 'boreal rain forest' or 'coastal rain forest' has been developed. The coastal rain forest is very rich in mosses, fungi and lichens. The latter group includes 40-50 species belonging to the so-called 'Trøndelag element'. The lichen species in the Trøndelag element have their only known occurrence in Europe in this forest type, or they have their main occurrence here. A good indicator group for the occurrence of rare epiphytic lichens in the coastal rain forest is the *Lobarion* community, for which *Lobaria pulmonaria* is a characteristic species. In the alliance *Lobarion* the epiphytes *Pseudocyphellaria crocata* and *Ramalina thrausta* are regarded as vulnerable among the Red list species in the coastal rain forest, whereas *Pannaria ahlneri* and 'trønderliav' (*Erioderma pedicellatum*) are highly endangered.

'Trønderlav' was until summer 1994 classified as extinct from Europe. Summer 1994 it was found in two extremely small populations in Nord-Trøndelag (Holien et al. in press).

The very specialised rain forest lichen element in Central Norway depends on long continuity with high and stable humidity. The best habitats of rare epiphytic lichens in this element are in narrow ravines facing north and north-east, where they are protected towards desiccating winds and strong direct sunlight. The rarest species are only found below 200 m, primarily in rich and damp spruce forests.

Special bryophytes

Norway spruce forests have a wide range of microclimatic and edaphic niches for bryophytes. Decaying logs of Norway spruce at various decaying stages are habitats for a lot of more or less specialised and very often rare liverwort communities and species. The occurrence of liverworts on logs of Norway spruce depends mainly on two factors:

- 1 The decaying stage (or the time since the tree felled), and
- 2 The size of the log.

The different species have different preference as to the above-mentioned factors. Some species only occur on young logs. These species will disappear after some time. A larger group of species prefer old logs. In the late stage of decay, common forest floor mosses increase in abundance, e.g. *Hylocomium splendens* and *Rhytidiadelphus* spp.

As to the size of the logs, the bigger logs have more species, and with larger populations. Typical Red list species on decaying logs are *Lophozia ascendens* and *Calypogeia suecica*. The former is a vulnerable species with a preference for big and young logs, the latter for old (heavily decayed) and big logs. Both have their main occurrence in Central Norway. Liverworts on decaying logs of Norway spruce have characteristic asexual reproduction, via gemmae. The number of liverworts on decaying logs is highest in rich and damp forests. The oceanic liverwort *Nowellia curvifolia* has its northernmost occurrence in the world on decaying logs of Norway spruce in coastal rain forest in Central Norway (T. Prestø, NTNU, in prep. Log decay and log size preferences of epiphytic bryophytes in Central Norway).

Structure and dynamics for plants and animals

The natural dynamics of the spruce forests creating a special mosaic landscape changing over time, leave a forest with some parts very little affected by disturbance regimes (Continuous forest) and other parts dominated by e.g. fires at frequency of 100 years. Plants and animals are adapted to these conditions. Many species are dependent on the stable

structural conditions in a continuous forest while other species need the disturbed conditions such as fire (i.e. the bird Hortulan, many insects and fungi) and storm felling. And finally many species are dependent on the mosaic combination at the landscape level, e.g. the three species of forest hens (black grouse, woodgrouse and hazel grouse).

2.4.4 Interaction between Norway spruce forests and other forest types

The general consequences of artificial introduction of *P. abies* into deciduous forests are rather well known, especially from West Norway (Fylkesmannen i Rogaland 1993) The high shade-tolerance of *P. abies* makes it very competitive towards nearly all deciduous tree species and the ground flora. The new microclimate after introducing *P. abies*, is a more humid and oceanic one. In addition the humus quality will change towards a more acid raw humus. The changes in microclimatic and edaphic conditions cause a sparse ground flora and fauna (Børset 1985).

In East Norway and Trøndelag *P. abies* interacts with tree species from principally four different habitats.

- 1 Along the main rivercourses in the southern boreal and middle boreal (500-800 m) zones interaction with grey alder (*Alnus incana*) and *Salix* spp.
- 2 On poor, dry and shallow habitats interaction with Scots pine (*Pinus sylvestris*).
- 3 On mesic and warm habitats in the boreo-nemoral (below 200 m a.s.l.) and southern boreal (200-400 m a.s.l.) zones interaction with elm (*Ulmus glabra*), hazel (*Corylus avellana*), lime (*Tilia cordata*) and beech (*Fagus sylvatica*).
- 4 A weaker interaction exists towards Scots pine (*Pinus sylvestris*) in depressions or boggy areas with more or less water-logged situations.

Under natural conditions there exists a balanced dynamic in the competition between *P. abies* and the above-mentioned neighbouring trees and communities (Figure 2.4).

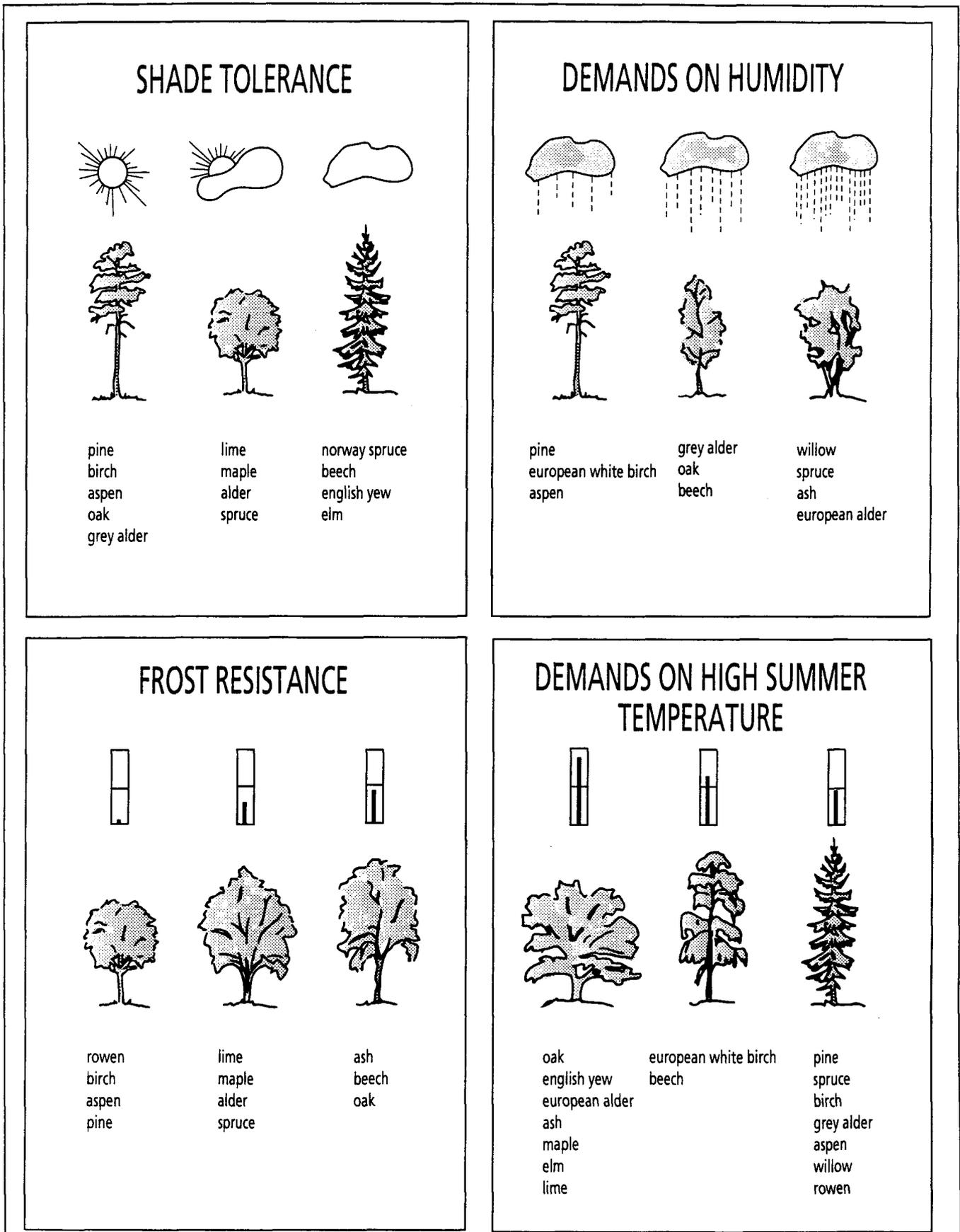


Figure 2.4. A schematic overview over some different tree species' preference for light, humidity, temperature and resistance against frost. Modified from Tømmerås 1994.

3 Domestication of Norway spruce

Norway spruce is the economically most important conifer tree species in Europe. It has a long history of cultivation in Central Europe and has been seeded and planted very intensely since the middle of the 19th century. This has changed natural forests into artificial forests and has led to the introduction of the species far outside its natural range. It has been planted on new types of sites in countries where it occurs naturally, e.g. in Germany and Norway, and has been introduced to new countries such as Denmark, Belgium and Ireland. It has to some extent also been planted in North-America.

Norway spruce has shown good yield and quality performance on very different site conditions, and this favoured the species over a long period. In some areas where maladapted provenances have been used, damage and reduced yield have occurred. In the last two decades it has suffered severely during the forest decline in Central Europe (Vancura 1995, Wolf 1995). This, together with some unwanted ecological effects and the reduced possibilities for recreation in young spruce stands, has to some extent reduced its popularity in reforestation, in particular outside its natural range.

3.1 Provenance transfers

The first provenance transfers were more or less random, with the results that in some areas in Central Europe and southern Scandinavia most of the spruce stands are of unknown origin. Later, provenance trials have provided information about the effects of transferring provenances from different parts of the range of the species and have identified provenance areas which generally have a high growth capacity. Examples of this are provenances from southern Poland and Romania, which with success have been planted in several countries.

Provenance transfers have been used in several cases where materials with specific adaptive properties have been sought. An example of this is the transfer of late flushing Norway spruce provenances from White Russia and the Baltic countries to sites exposed to late spring frosts in southern Sweden. These provenance transfers have reduced frost damage and improved growth (Werner et al. 1991). In other cases, however, maladaptation has occurred. In southeastern Norway, spruce provenances from Austria and southern Germany were extensively planted during a 20-year period starting in the 1950's. A survey of 79 planted stands of Central European origin and 21 of local origin, all at the age of 27 years,

was recently made in Østfold County, Norway (Skrøppa et al. 1993). In the stands of local origin, 30 % of the trees were classified as having saw timber qualities, whilst only 7 % of the trees in the Central European stands obtained the same quality classification. This provenance transfer generally had a negative effect on timber quality and a small positive effect on volume production, even if a few of the stands planted with southern provenances had both high volume production and good stem quality. In addition, the pollen cloud from these stands may reduce the fitness of offspring from the seed in neighbouring stands of local origin.

3.2 Reproductive methods

3.2.1 Flower induction

Application of growth regulators, primarily giberellins, has become an efficient method of regulating flowering in forest trees. With Norway spruce grafts, treatments during the shoot growth period with both giberellic acid alone and in combination with heat have been efficient in promoting female flowering (Johnsen et al. 1994). Heat treatment alone will induce male flowering. The problem of shortening the juvenile phase and obtain regular flowering at an early age still remains unsolved (Chalupka 1991).

3.2.2 Vegetative propagation

Norway spruce has for decades been propagated by grafting. Scions to be grafted are taken from the crown of mature trees and are grafted onto a rootstock which is a young spruce seedling. This technique is being used for the establishment of clonal seed orchards and clonal archives.

Clonal propagation with rooted cuttings is easily achieved with young spruce seedlings. The propagation can be made either early in the spring before bud flushing or after the termination of shoot growth at the end of the summer. Both the rooting capacity and the growth form of the cutting depends on the age of the cutting donor, but can be improved in a proper rooting environment. A large genetic variation has been observed in rooting capacity and subsequent growth habit of the cutting (Johnsen & Skrøppa 1992).

Cell and tissue culture techniques for micropropagation have recently been developed for conifers. The most promising method for Norway spruce seem to be somatic embryogenesis, where somatic cells are stimulated to develop into somatic embryos in a similar way as a zygotic embryo (Mo 1993). The somatic embryo can be stimulated to mature and develop into a plant which will be of the same

genotype as the mother tree. The somatic embryos can be propagated in unlimited numbers on a large scale and can be formed as artificial seeds. This propagation method has an enormous potential both for scientific and commercial purposes. Further developments are needed before somatic embryogenesis can be used for practical purposes (Mo 1993).

3.3 Breeding of Norway spruce

Breeding of Norway spruce was initiated in several European countries in the 1950's. The work started with the selection of phenotypically superior trees ("plus trees") in natural stands. Mature trees that had superior height and diameter growth and stem and branch quality compared to neighbouring trees in the stand were selected. They were grafted in multiple copies on rootstocks in clonal archives or seed orchards. Each grafted seed orchard is composed of a rather large number of selected clones (50-500) with the intention of seed production for one geographic region. The seed orchard will generally start to flower 10-15 years after grafting. However, both the regularity and amount of flowering are very much dependent on the climatic conditions at the orchard site. To promote flowering, seed orchards have often been located to sites with a warmer climate than from where the selected parents originate and where the orchard seed is intended for use.

It was soon realised that the selection of plus trees in natural Norway spruce stands is not an efficient method to select superior genotypes. It is necessary to test the genetic value of each parent, based on an evaluation of their offspring. This is done in progeny tests planted at several sites where measurements are made of survival, height and diameter growth and quality traits. The progeny tests are sometimes supplemented with tests where seedlings are grown under controlled conditions in growth chambers and measurements are made of physiological traits. On the basis of several traits a subset of the original parents is selected for further breeding. Seeds for practical planting can be collected in the orchard on the selected parents, the orchard can be thinned or a new orchard can be established based on the selected group.

Breeding programs have been developed for Norway spruce in different countries, taking both short and long term considerations into account (e.g. Birot 1982, Skrøppa 1982b, Danell 1991, Kleinschmit 1993). Most of these programs have been aimed at seed production in orchards, but some have also been for mass production of rooted cuttings of tested and selected clones. Major concerns in the breeding strategies have been the breeding objectives, the

sizes of the breeding and of the seed producing populations in order to maintain genetic diversity, design and efficiency in testing, and identification of the proper region where the orchard seed should be recommended for use. Gains from breeding have been estimated to 6 % genetic improvement in growth from the original plus tree selection (Danell 1991). An additional 10 % advance in the selected trait can be expected after progeny testing.

In Norway, more than 3 000 selected spruce trees have been grafted in seed orchards or clonal archives. A large number of these have been progeny tested. Most seed orchards, however, consist of the original set of non-tested parents. In some orchards selective harvests have been made on the basis of progeny test results. The main selection criteria have been traits characterizing the annual growth rhythm, height growth and the occurrence of damage in field tests. Artificial freezing tests are being used to test autumn frost hardiness, in particular for materials intended to be planted at high altitudes or far north. Each seed orchard is intended to produce seed for a specific region which is determined by the origin of the parents and the growth rhythm and hardiness performance of the orchard offspring.

3.4 Reproductive materials used

The largest proportion of Norway spruce seeds being used world-wide are collected in natural or planted stands. Each seed lot should be identified by the geographic origin of the stand. In several countries it is required that the seed stand should be selected for superior performance (EU requirement). The relative amounts of stand and seed orchard seed being used vary considerably between countries and regions within countries. In several countries, the regeneration of Norway spruce forests is based on a combination of natural regeneration and planting, often with emphasis on natural regeneration where that is a feasible method. Clonal forestry based on rooted cuttings was initiated to some extent in the 1980s in Germany and Sweden, but occurs at present only on a small scale.

In the Nordic countries, stand seed accounts for the largest proportion of seed used. The percentages of stand seed used were in 1994: 89 % (Denmark), 84 % (Sweden), 81 % (Norway) and 80 % (Finland) (Personal info. Bjerne Ditlefsen, Lennart Ackzell, Gunnar Haug, Hannu Kokkonen). The percentage from seed orchards varied between 10 to 20 %. Sweden has the highest import of spruce seed with 35 % (stand seed). Rooted cuttings account for less than 1 % of the total number of Norway spruce plants.

3.5 Conservation of Norway spruce genetic resources

The combination of natural regeneration and planting of materials with a broad genetic diversity are in many countries the basis for the conservation of the genetic resources of Norway spruce. In addition, several specific conservation measures are taken. In situ activities include national parks, nature reserves and specific conservation or gene reserve forests. The grafted clone banks and seed orchards and provenance and progeny tests constitute the ex situ gene conservation together with long term seed storage. Co-operation at the European level is achieved through the recently established European Forest Genetic Resources Programme (EUFORGEN). Norway spruce is one of the species for which a co-operative network has been formed (Turok et al. 1995).

4 Potential use of genetic engineering in forest trees

4.1 Molecular biology and genetic engineering in forest research and tree improvement

Biotechnology is a collection of techniques that can be used to increase the understanding of biological processes and to enhance the impact of biological and improvement programs. Cell biology and molecular genetics are today used to provide new basic knowledge about structure, regulation and function of genes. This work includes isolation and characterisation of specific genes activated in a process, description of the gene products, regulation of the gene products (promoter characterisation) and their effects on the phenotype in heterologous or homologous transgenic systems. Many important genes controlling biologically and often economically important processes have been cloned and characterised, and are now being used to improve crop plants (Stitt & Sonnewald 1995). The acquired knowledge can be used to identify superior or interesting genotypes in wild or synthetic populations, or more specifically by producing varieties modified through genetic engineering.

Genetic engineering allows the transfer of traits from one species (plants, animals, bacteria, virus) into the genome of plants of other species, with the preservation of the intrinsic properties of the acceptor plants. Selective improvement of a single trait, while still retaining all of the desired traits in the target plants, is an attractive option not easily available in conventional tree breeding. Thus, genetic engineering has the potential to supplement traditional breeding programs in forest trees, which are hindered by the long generation time and large areas needed and the long time required for progeny testing.

In both basic molecular research and in practical improvement, extensive testing of transgenic plants is needed. Many of these tests can be performed in isolated devices such as climate chambers, phytotron rooms or greenhouses where uncontrolled spread of pollen and seeds to the natural environment can be prevented. A lot of relevant and significant information can be extracted from tests under such artificial conditions. However, testing of transgenic plants in the field is necessary if they are to be used commercially as crop varieties. Since 1987, the Animal and Plant Health Inspection Service (APHIS) has approved 1818 field trials at 6762 field sites in USA (last update: September 1995). These trials comprise 40 different

plant species with a wide variety of modifications (Schechtman 1995). The number of Summary Notification Informats distributed in EEC indicate that 341 field trials have been established in Europe (last update February 1995; O.A. Rognli pers. comm. 1996). No summary statistics of field trials with transgenic forest trees are available, but at least 10 trials with transgenic poplar have been established world-wide (Rogers & Parkers 1995). In Europe, transgenic poplar are currently field tested in France and Sweden, transgenic birch is tested in Finland and transgenic eucalyptus species are tested in France and UK (O. Olsson pers. comm. 1996). Only one transgenic conifer species (*Picea glauca*) is currently in field test to our knowledge (Ellis et al. 1995).

4.2 Gene transfer and production of transgenic plants using selectable marker genes

Genetic engineering of plants usually implies direct genetic manipulation of plant cells at the cellular or molecular level, adding new genes to the plant's genome or replacing certain genes with other genes from other sources using recombinant DNA technology. The modern techniques developed for genetic engineering include vector mediated systems based on *Agrobacterium* plasmids, and non-vector systems (direct gene transfer) such as biolistics (particle bombardment) microinjection, electroporation (electric pulse treatment), chemical poration (polyethylene glycol-mediated osmotic DNA insertion), liposome fusion and protoplast fusion (Potrykus 1991). The two most widespread techniques are *Agrobacterium*-mediated gene transfer and particle bombardment (biolistics).

The *Agrobacterium* - mediated gene transformation system relies on pathogenic strains of *Agrobacterium tumefaciens* and *A. rhizogenes*, which are capable to insert DNA fragments from their Ti (tumour-inducing) or Ri (root-inducing) plasmids into the plant chromosomes. To develop the *Agrobacterium* plasmids as gene vectors, the genes causing tumorous phenotype are removed and replaced with "useful" or interesting genes without the loss of DNA transfer- and integration functions (Grant et al. 1991). Successful gene transfer and production of transgenic poplar trees by use of *A. tumefaciens* engineered Ti-plasmid vectors have been reported (Parsons et al. 1986, Fillatti et al. 1987, Nilsson et al. 1992). Transgenic larch plants have been produced using a Ri-plasmid vector of *A. rhizogenes* (Huang et al. 1993, Shin et al. 1994).

Limitation of the host range of *Agrobacterium* restricts its use in many cases, particularly when dealing with monocots (Binns 1990, Shimamoto 1994). One reason

for host-range limitations is the different wound response of various plants and tissues (Grant et al. 1991, Potrykus 1991). Extensive efforts have been made to develop other systems for the delivery of free DNA into these plant species (see above). The most successful method is particle bombardment of embryogenic calli or developing embryos (Vasil et al. 1992). Stable transformation and production of transgenic plants of *Picea glauca* by particle acceleration have been reported (Ellis et al. 1993).

When these techniques are combined with a tissue culture system, it is possible to produce and clonally propagate transgenic plants from genetically modified plant cells. Because only a small proportion of cells are transformed using these gene transfer techniques, it is important, in addition to the genes in interest, to transfer a selectable marker gene that enables transformed tissue to grow and propagate when non-transformed cells do not. Commonly used marker genes conferring resistance to antibiotics are *npt II* (encoding neomycin phosphotransferase; resistance to kanamycin, neomycin, G418, paromycin), *hpt* or *aph IV* (encoding hygromycin phosphotransferase; resistance to hygromycin), *dhfr* (dihydrofolate reductase; resistance to methotrexate), and marker genes used to confer herbicide resistance are *bar* (phosphinotricin acetyltransferase; resistance to phosphinothricin), *aro A* (glyphosate resistance) and modified *als* genes (chlorosulfuron or imidazolanones resistance) (Lindsey & Jones 1989).

4.3 Promoters

Different genes of a genome are not all expressed in a given time or a specific tissue. The activity of a gene is regulated by a DNA sequence named promoter. A lot of different promoters have been characterised, and can be grouped according to their functions (Lindsey & Jones 1989, Stitt & Sonnewald 1995). Some promoters give a constitutive expression of the genes, others are expressed in specific tissues or organs and the third group are induced by external or internal signals or stress.

4.4 Transgene inactivation

The production of transgenic plants is rapidly becoming routine for a number of crop species. It is now possible to create plants that are for example resistant to insect pests, which have multiple or novel disease resistance genes or which are adapted to specific soil or environmental conditions. Such transgenic crops plants will only be of value if their engineered phenotype is faithfully transmitted through subsequent generations in a predictable manner (e.g. Gasser & Fraley 1989, Batraw & Hall 1992). However, desirable new pheno-

types created by the introduction of foreign DNA into plants are frequently unstable following propagation, leading to loss of the newly acquired traits. This genetic instability is not caused by deletion or mutation of the introduced DNA but rather by the inactivation of the transgene (Matzke & Matzke 1994, Finnegan and McElroy 1994, Dougherty & Parks 1995, Matzke & Matzke 1995a,b, ten Lohuis et al. 1995). Inactivation of gene expression is viewed as a consequence of the interaction of multiple homologous sequences that result in events including chromatin restructuring, DNA methylation and the inhibition of mRNA processing, transport, export or translation (Flawell 1994). Thus, the approach towards stabilising expression would be to select and/or screen for those transgenic plants which contain a single intact copy of the transgene (Finnegan & McElroy 1994, Meyer et al. 1992). The use of *Agrobacterium* usually results in single copy insert, but occasionally up to 50 inserts of a transgene have been reported (Dale et al. 1993). Direct DNA transfer, such as the use of biolistics, often results in multiple insertions (both *cis* and *trans*) (e.g. 3-5 copies in *Picea glauca*; Ellis et al. 1993). However, even in situations with a single copy inserted, extensive methylation of the promoter sequence occurred (Meyer et al. 1992). Thus, silencing of genes may represent more than just an unwanted response to foreign genes. The phenomenon could rather reflect a natural use of homologous or complementary nucleic acid sequences to modify gene expression. The altered gene expression could both operate in the nucleus at the DNA level and in the nucleus or cytoplasm as a means to control excess production of mRNA or replication of RNA pathogens (Matzke & Matzke 1995a).

4.5 Proposed traits to be improved or introduced in forest trees

Several important characteristics are being studied using molecular methods and biotechnology on trees today, and some are suggested as possible candidates to be modified by genetic engineering in the future. These are resistance to herbicides, insects (Raffa 1989, Cheliak & Rogers 1990, Bradshaw et al. 1991, Strauss et al. 1991, Huang et al. 1993, Ellis et al. 1993, Shin et al. 1994), fungi and virus (von Arnold et al. 1990, Chet et al. 1991, Huang et al. 1993 Schuerman & Dandekar 1993, Hubbes 1987, 1993), frost and drought resistance (Ahuja 1988, von Arnold et al. 1990, Newton et al. 1991, Huang et al. 1993, Haines 1994), alteration of wood properties to improve the economical value for pulp (Whetten and Sederoff 1991, Dean & Eriksson 1992), and modulation of growth by altered levels of phytohormones (Olsson 1993) or by transgenes coding for phytochrome (Junttila 1993). In

order to minimise the risk for dispersal of the foreign gene into natural forest ecosystems, introduction of sterility by means of genetic engineering has been proposed (Strauss et al. 1995).

4.5.1 Herbicide resistance

Four different types of herbicide resistance mechanisms are known (Comain and Stalker 1986, Mullineaux 1993): 1) An overexpression of the target protein in the plants upon which the herbicide acts, results in a tolerance of low amounts of herbicide present. 2) Mutation in the gene coding the target protein may result in change in binding site of the herbicide without any loss of enzymatic activity. 3) Genes from bacteria produce enzymes which detoxify the herbicide. 4) Genes from plants coding for enzymes which detoxify the herbicide.

Transgenic trees carrying the *aroA* gene (glyphosate resistance) have been produced in *Populus* (Fillatti et al. 1987) and in *Larix decidua* (Shin et al. 1994). Development of trees that are tolerant to herbicides is thought to be an important component in weed control in intensively managed forests and arboreta.

4.5.2 Insect resistance

Two different strategies have been used to develop transgenic plants with insect resistance. The first one involves proteinase inhibitor genes isolated from plants (e.g. Hilder et al. 1987, 1989, Gatehouse et al. 1993). These inhibitors are natural compounds affecting enzymes in the digestive track of the insects, leading to reduced or blocked uptake of nutrients. The inhibitors are effective to a great variety of insect species. The second involves genes from *Bacillus thuringiensis* (*B.t*) (e.g. Perlak et al. 1990, 1991). Different strains of the bacteria can produce different crystalline proteins. The crystalline only becomes toxic after exposure to proteinases in the digestive track of the insect so that the protein fragment interacts with the epithelial cells that line the midgut. The cells if the epithelium disrupt, and the insect is killed. The *B.t* toxins are not toxic to humans or mammals. The various endotoxins affect insects of Lepidoptera, Diptera and also Coleoptera. Genetically engineered trees of *Populus* and *Larix* containing *B.t* genes have been produced (Huang et al. 1993). Transgenic trees with insect resistance are thought to contribute to both tree improvement and pest management programs.

4.5.3 Fungal disease resistance

Plants respond to attack by pathogenic microorganisms by producing a diversity of proteins, many of which are believed to have a role in defence. Among those proteins are hydrolytic enzymes such as chitinases and β -1-3 glucanases, which degrade the fungal cell walls (Keen 1992, Ohashi & Ohshima 1992, Hubbes 1993, Sharma et al. 1993). In addition, many plants respond to pathogen invasion with an altered pattern of protein synthesis. Some of these proteins are involved in the synthesis of low molecular weight compounds such as phytoalexins, phenols, lignins, tannins, and melanins (Bell 1981, Børja et al. 1995). Phytoalexins are often synthesised locally and accumulate after exposure to pathogens. In some cases, a correlation has been found between concentration of phytoalexins and the resistance to specific pathogens (Ebel 1986, Keen 1990). By altering the levels of some of these low or high molecular compounds in transgenic systems, an increased disease resistance may be expected. Transgenic tobacco and rape plants containing a bean chitinase gene with a constitutive promoter have been shown to exhibit higher basal levels of chitinases and concomitant increased resistance to *Rhizoctonia solani* when compared to control plants (Brogli et al. 1991). Transgenic trees with disease resistance might be of great economic importance in the future.

4.5.4 Viral disease resistance

Resistance against viral infections is possible by incorporating antiviral elements or antiviral genes into plants and trees (Schuerman & Dandekar 1993). Advances in the understanding of the structure and function of viral nucleic acid and in the understanding of the host-pathogen relationship have given rise to the development of new strategies for engineering virus resistance in plants. These are 1) using antisense viral RNA, 2) cross-protection using genes coding for the viral coat-protein and 3) expression of the satellite RNA which can modify the virulence phenotype of their companion virus. Expression of these genes in transgenic plants can reduce or delay the viral symptoms, and reduce the number of virus after inoculation (Hemenway et al. 1988, Lawson et al. 1990, Gerlach et al. 1987, Harrison et al. 1987). Very little is known about viral diseases in conifers, but viral resistance might become important in some angiosperm trees species.

4.5.5 Frost resistance

Engineering trees or plants to withstand environmental stresses will be a long-term strategy. The plant responses to stress factors are complex processes

which may involve the expression of many genes. Cold tolerant genes are likely to be of commercial value for some tree species, in particular in eucalyptus (Haines 1994). Genes encoding proteins based on antifreeze activity found in arctic flounder have been introduced into tobacco, tomato and poplar (Hightower et al. 1991, Kenward et al. 1993, Olsson 1993) and some of these experiments show results indicating a slight increase in freezing tolerance, but others do not. However, extracellular antifreeze proteins have also been found in plants when these are exposed to cold hardening treatments (Hon et al. 1994, 1995). Cold specific genes are currently being isolated from several plant species (Guy 1990), and an increased molecular understanding of cold acclimation will most likely become a reality in the future. An over-expression of antifreeze proteins in the intercellular space in transgenic plants might be a solution to improve frost tolerance in frost sensitive species. Moreover, species with high levels of frost hardiness in autumn, winter and spring, are often very sensitive to frost during active growth (shoot elongation). If it is possible to combine active growth with increased tolerance to frosts by means of an over-expression of antifreeze proteins in transgenic plants, this will be of great importance to improve quality of trees growing in frost pockets.

4.5.6 Drought resistance

Response mechanisms to drought stress have cellular and metabolic components (Newton et al. 1991). Plants adapt to tolerate drought stress through response mechanisms such as reduction in DNA content, changes in proteins, and synthesis of abscisic acid (ABA). Gene responses have been observed by the detection of unique proteins in plant tissue that are subjected to a variety of stresses including heat-shock, salinity, cold and drought. Some stress-related proteins are unique to a particular stress, and osmoregulatory genes may be good candidates for conferring tolerance to drought/salt stress. To offset drought-induced damage to forests, it is imperative to understand the molecular and physiological responses of trees to water deficits. Better understanding is needed of how gene responses to drought are integrated through cellular, organ, tree and population levels. This understanding is necessary before we can manipulate the genome to genetic engineering.

4.5.7 Wood quality

Modification of lignin structure, composition and content to improve wood quality is being studied in several laboratories. The general strategy is to isolate and clone several genes coding for a series of enzymes involved in the lignin biosynthesis in trees (Bugos et al. 1991, Dean & Eriksson 1992, Whetten &

Sederoff 1994) Modifications that lead to more efficient pulping of wood for paper may become an important future. Other potential areas of application include fiber morphology, wood density, extractive contents, biomass-utilization efficiency and dimensional stability. However, a better understanding of the developmental process of wood formation is needed before genetic engineering can be applied conifer trees in this respect will help to meet the demand for wood and paper products in the next century.

4.5.8 Modulation of growth by phytohormones

Plant growth is affected by various phytohormones. Molecular characterisation of plant growth regulation is currently being investigated in transgenic systems by direct over-production and/or inhibition of endogenous plant hormones or indirectly by introducing genes for phytochrome in several plant species (e.g. Oeller et al. 1991, Boylan and Quail 1989, 1991), including hybrid aspen (*Populus tremula x tremuloides*) (Olsson 1993, Junttila 1993). Although these research activities are mainly motivated by the wish to increase the basic knowledge of growth regulation in plants and trees, one cannot exclude the possibility that transgenic trees with modified growth pattern will be launched as candidates for practical and commercial use in the future.

4.5.9 Sterile transgenic plants

Prevention of the escape of genes into wild populations is likely to become an important task in the future. Introducing sterility (male or complete sterility) to transgenic trees is regarded as a general safety measure in biotechnology to avoid undesirable ecological effects if certain transgenes become widely dispersed (Strauss et al. 1995). Moreover, a complete lack of sexual reproduction is regarded beneficial for wood and biomass production «make wood not sex». Male sterility is thought to improve progress in breeding, by preventing self-pollination in conventional seed orchards and to facilitate controlled crossings in genetic research (von Arnold et al. 1990). Male sterile plants can be formed by suppression of the synthesis of key enzymes in the anthers. Methods are available to shut off genes by using antisense techniques. Alternatively, pollen development can be aborted by local over-production of ribonucleases in transgenic tissue in the anthers. A molecular understanding of the flowering process in the angiosperms is increasing, but basic knowledge about flowering is scarce in conifers. Thus, information of floral gene sequences and expression is needed before male or total sterility can be introduced by the use of genetic engineering in conifer trees.

4.6 Release of transgenic Norway spruce

No releases of transgenic Norway spruce into natural habitats are currently being planned in Norway. Whether or not transgenes will be planted in the future depend on the progress in biotechnology of forest trees, knowledge about risks involved, on public opinion and political decisions. Release with the knowledge we have today would be highly premature. Producing sterile transgenic spruce along the lines suggested is dubious as long as we cannot guarantee that antisense or ribonuclease genes will be stably inherited and activated in the transgenic plants. Thus, there could be a risk that presumed sterile transgenic spruce trees could flower under favourable conditions due to transgene inactivation (silencing of inserted genes). Moreover, we do not know how transposable elements operate in Norway spruce. Tissue specific, wound specific, or age specific promoters coupled with structural genes coding for insect or fungal resistance will probably be conditionally expressed in the first generation of transgenic trees, but if transposons are active, a constitutive production in the whole tree could be the result in advanced generations. However, if all these problems are solved in the future, experimental field release of transgenic Norway spruce can be discussed in relation to the ecological risks involved.

5 Describing potential impacts

The intended effects of releases of transgenic organisms are usually specific with regard to species and environment, and limited in time. Evaluations of environmental effects must, however, also include other species and environments, because both the transgenic organisms and their genes can spread after deliberate or accidental release. Moreover, genetically modified organisms are exposed to natural selection and may evolve better adaptations to the environment over time. It will therefore always be a formidable task to provide documentation on the environmental effects of a release, and almost impossible to predict them.

In this section we shall discuss some real or potential dangers following the release of genetically modified organisms, outline a set of questions which need to be addressed in order to evaluate these dangers and place Norway spruce in a general context of risk evaluation. In the next chapter (6), we return to these general questions to speculate about the possible environmental effects of transgenic Norway spruce under specific scenarios combining the genetic modifications and release environments.

5.1 Principles for risk assessment

The Ecological Society of America has proposed the following principle for risk evaluations when genetically modified organisms are released to the environment (Tiedje et al. 1989):

"Genetically modified organisms should be evaluated and regulated according to their biological properties (phenotypes), rather than according to the genetic techniques used to produce them."

This means that the release of genetically modified organisms needs not differ from the release of other organisms, as regards their threat to the environment. Others have pointed out that the technique used in the genetic engineering must also be an object for assessment. There is, for example, little control on the final location of a gene that is inserted in the genome of animals and plants. This can have consequences for the stability of the genome, for example with respect to regulating gene expression (see chapter 4).

There will always be an element of biological gambling involved in the release of genetically modified and other (non-modified) organisms. Our genetic and ecological knowledge is inadequate to allow us to give *a priori*, precise risk assessments for releases

(Simonsen & Levin 1988, Drake et al. 1989, Ryman et al. 1995). We can provide general guidelines on how to assess environmental effects, but we are unlikely to obtain sufficient knowledge of the ecological interplays within and among species to provide precise predictions. This is because we neither know the genes that are important for adaptations to the environment nor the selective forces acting on those genes. Other information which is necessary for making precise genetic and ecological predictions, including population size, migration of individuals and genes between populations, the evolutionary history of the species, its nutritional requirements and natural enemies, are also unknown quantities in nature - even among well-studied organisms.

We are unlikely to obtain the necessary ecological and genetic knowledge to make precise risk assessments within the foreseeable future. Hence, the best we can do is to base our opinions on experience and give as precise *a posteriori* risk assessments as possible. For transgenic organisms whose phenotypes lie within the range expressed by commonly cultured varieties, this experience should provide valuable insight into possible environmental effects of new releases (See chapter 3.1 concerning experiences from spruce culture plantings).

An important principle for risk assessment is that the environmental effects of genetically modified organisms must be evaluated on a case-by-case basis through a stepwise procedure that includes fully enclosed pilot studies (OECD 1986). There is broad international agreement about this principle (Royal Commission 1989, Tiedje et al. 1989). But even a risk assessment based on the principles of "case-by-case" and "step-by-step" has limitations such that (1) we have to use short-term experience to assess long-term impacts, (2) we can only test the possible problems we are aware of, and (3) an inability to prove any impact can be caused by problems with the method or that the investigation does not cover all the relevant effects. Moreover, it has been shown in many cases of deliberate releases that the persistence of the released population largely depends upon how many individuals were released (Griffith et al. 1989). Therefore, the results of small-scale experiments need not translate directly to large-scale releases.

Collectively, these considerations mandate a way of thinking about releases that must incorporate the 'precautionary principle' as a guideline for management.

5.2 Consideration of environmental effects

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

For each of these categories, which in the following will be called «invasion potential», «gene flow» and «altered use», respectively, some combinations of genetic modifications, source populations and release environments will clearly be more hazardous to the environment than others. Tiedje et al. (1989) have provided a detailed list of questions which can be used as a checklist for identifying releases on a subjective scale from low to high risk. Rather than repeating that list, we discuss below some general considerations which must be evaluated if we are to predict effects of releases with any confidence. In the next sub-chapter we evaluate Norway spruce and the boreal environment as candidates for genetic modification in the same general context. Some specific cases, where possible traits for modification are identified, are discussed in more detail in chapter 6.

5.2.1 Invasion potential

Some key questions regarding the invasion potential of a genetically modified organism are: (1) is the expression of the genetic modification well documented and understood, (2) do the genetically modified trait represent an ecological novelty, (3) to what degree is the host organism domesticated, (4) is the organism a key species in the environment, (5) is the release environment «contained» by natural barriers for spread of the organism, or can the spread be controlled by human intervention, and (6) how well can the dynamics of the release environment be simulated prior to, and monitored after, release?

Transgene expression

Increased predictability of the phenotypic expression of the genetic modification means safer to the environment. Some factors affecting the stability of transgene expression were reviewed in sub-chapter 4.4.

Ecological novelties

Transgenic organisms will be likely to pose greater ecological risks than conventionally cultured organisms, particularly if the transgene has not previously been tested against the genetic and environmental

background of the organism (e.g., a flounder gene introduced to spruce). In the words of Tiedje et al. (1989): "organisms with novel combinations of traits are more likely to play novel ecological roles, on average, than are organisms produced by recombining genetic information existing within a single evolutionary lineage".

Other transgenic traits that are potentially hazardous, include those which increase the environmental tolerance of the species. This tolerance can relate to both the abiotic (e.g., climate) and biotic environment (e.g., competing species), and have effects on the abundance (e.g., carrying capacity) or distribution of the organism.

Domestication

The degree of domestication of an organism often reflects how dependent it is on human support for survival and reproduction. Fully domesticated species are expected to pose little risk to the environment, because any unwanted effects can be controlled by human intervention.

The semi-domesticated species Norway spruce grows both in natural stands and in plantations. Both types of stands may be managed to different degrees. After the reproductive stage is reached at an age of 15-30 years no control of the spread of genes from any type of stand is possible.

Key species

Some species are ecologically more important than others. It is clear that genetically modified varieties of these species will have greater ecological impacts than modified species which play a lesser ecological role. Ecologically important species have been termed «keystone species» which may be defined as: *a species is keystone if its experimental removal (or introduction) causes major ecosystem-level changes in structure, dynamics or nutrient flows* (Crawley 1995).

Natural spread and its control

Releases of genetically modified organisms are considered safer if the organism has a small area within which it can survive and reproduce, and if limited dispersal capability (or an environmental barrier) prevents it from reaching other such areas, should they exist. Greater potential risks would be posed by organisms which exist naturally in large areas, and for which dispersal capabilities (or lack of environmental barriers) make rapid range expansion possible following, say, genetic modification or environmental change. In the latter case, a pertinent question is whether the spread of the transgenic organism can be controlled by human intervention.

Simulation and monitoring

The ecosystem performance of many small, short-lived organisms can be simulated with good ecological realism in the laboratory. For transgenic varieties of these organisms, the possible environmental effects should be well known following evaluation of their performance in a «step-by-step» fashion. The performance of many large, long-lived organisms, on the other hand, cannot easily be simulated, because in order to obtain ecological realism, the scale of the experiment would itself demand uncontained conditions.

Monitoring programmes are an essential part of the study of transgenic organisms, among other things because a well-documented record of no unwanted effects will provide a solid foundation for future releases. Such safety records are however, highly dependent on how well the released organism and the release environment can be monitored. In contrast to ecological simulation, we expect monitoring to be more difficult for small, short-lived organisms than for larger, longer-lived ones.

5.2.2 Gene flow

Some key questions regarding the effects of gene flow from a genetically modified organism are: (1) does the organism have close relatives (i.e., natural or semi-natural populations of the same or closely related species) in or near the release environment, (2) how large is the natural gene flow among populations of the species, and (3) does the release environment put a selective premium on the introduced gene?

Close relatives

The use and possible release of genetically modified organisms are considered safer if the organism does not have close relatives in the wild. By close relatives, we mean everything from semi-natural or wild populations of the species undergoing genetic modification, to other species of the same genus and even other genera with which the genetically modified organism can hybridise (Ellstrand 1988, Raybould & Gray 1993). The potential for introgression of the transgene into wild populations will be highest for situations where the genetically modified organism coexists with conspecific populations that form a natural part of the surrounding environment.

Natural gene flow

The level of natural gene flow in a species can be used as a yardstick for predicting the spread of genes from genetically modified variants of the same species. Several aspects of the natural population structure are important; for example the degree of patchiness of the distribution area of the species, and

the connectivity of these patches in terms of the realised gene flow between them. In a highly subdivided population, a transgene can become locally abundant even if there is selection against it, but it will rarely spread far. In contrast, a transgene may spread far into a highly connected population but will rarely reach a high frequency in any location (Gliddon 1994). At any rate, it is difficult to predict gene flow from experimental studies of the dispersal of pollen and seeds. For assessing gene flow on the longer term, estimates based on studies of genetic differences between extant populations may be more appropriate (Waples 1991).

Selective advantage

Fixation of the transgene in natural populations is almost inevitable if the transgene poses a selective advantage to its carrier (see also subsection 5.4.2). Hence, one crucial piece of information is whether or not the genetically modified trait has a selective advantage in the wild. Theoretically, it is often assumed that when an organism is pushed away from the optimum phenotype by for example directional selection on a phenotypic trait, the organism's response is accompanied by a loss of general fitness because of trade-offs between the selected trait (e.g. growth rate) and other vital traits (e.g. survival and reproductive ability). For genetically modified traits, however, such trade-offs may not occur, because the modified trait can be achieved by incorporating genes from other species. In such situations, better performance in some traits may be achieved without altering the performance with respect to other vital traits. Many of the genetic modifications that are now tested in agriculture are being developed with the aim of producing better-performing organisms in an agricultural setting. These organisms must also be evaluated with respect to performance in the surrounding environment if we are to predict environmental effects with any confidence.

5.2.3 Altered use

Some key questions about the effects of altered use of the organism, following genetic modification, are: (1) does area expansion occur, and (2) are new control agents favoured?

Area expansion

When genetic modification of an organism leads to expansion of the area where it is used, a number of otherwise unlikely environmental effects can occur. First, the environmental threats posed by the genetically modified organism will occur over a larger geographical area, and secondly, the fitness of the genetically modified organism becomes less important as a limiting factor for its spread, because this spread will be mediated through human action. Thus,

altered use can increase the threats against special species, habitats and ecosystems.

Control agents

A number of transgenic plants which show resistance to a particular herbicide, are now produced. The use of these transgenic plants invites new usage of herbicides, which may be tougher - or more benign - to the general flora. This application does however not seem especially important in boreal forests today.

5.3 Risk assessment of transgenic Norway spruce

General considerations of invasion potential and gene flow to related populations make it clear that Norway spruce is a high-risk organism. A subjective grading of the potential ecological and genetic risks is presented for a selected set of attributes of Norway spruce and the boreal (release) environment in **Figure 5.1**. Among these attributes, which were picked from Tiedje et al. (1989), the most important ones for grading transgenic spruce at the high-risk end of the spectrum are (1) the essentially wild (self-propagating) characteristics of Norway spruce, (2) the broad geographic range it occupies, (3) the high level of gene flow among populations/neighbourhoods and the proximity of cultured (transgenic) stands to wild populations, (4) the important role that Norway spruce plays in the structure and function of boreal ecosystems, (5) the virtual impossibility of simulating realistic ecological conditions in the laboratory or in field test orchards, and (6) the typically uncontrolled access for the public to test sites.

If transgenic spruce were to be based on only pre-reproductive (immature) stages outside of (controlled) greenhouses, the environmental risks associated with reproduction and gene flow would largely disappear (**Figure 5.1**). But several aspects of the ecological role played by spruce would still be valid reasons for concern, and the problem of controlling public access to test or growing plantations would not guarantee fully against unwanted reproduction and spread of transgenic spruce.

We noted above that valuable insights may be provided by reviewing the release history of non-transgenic varieties of the species. In addition, ecological and genetical knowledge of the organisms may be combined with the use of mathematical models to look into the future at realistic environmental scales (Williamson 1989). Among other things, the use of mathematical models may help us describe and

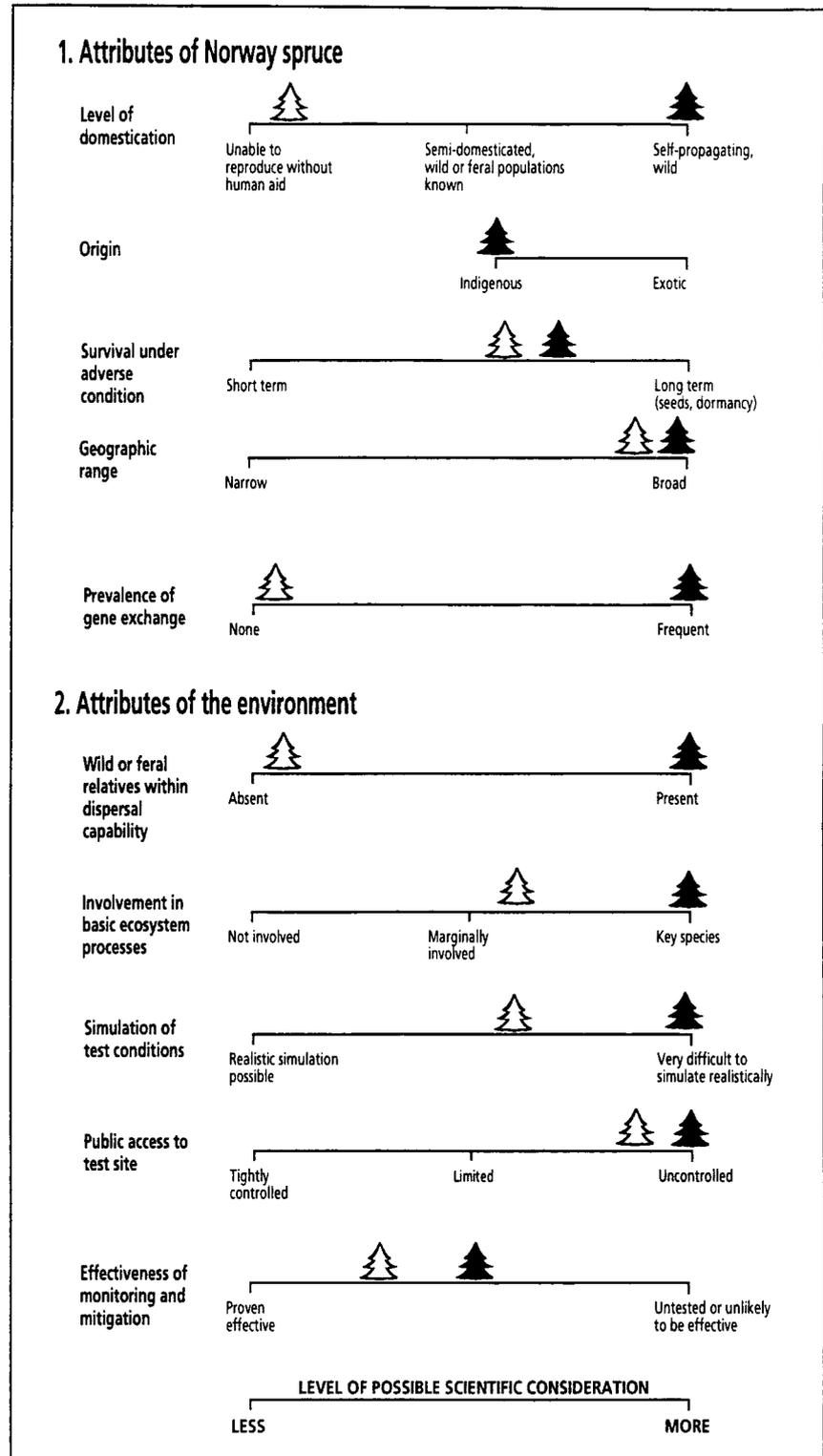
understand patterns of invasion and introgression, identify critical factors limiting the spread or establishment of the transgenic organism, and perform sensitivity analysis with respect to how variation in parameter values influences the likelihood of an environmental effect.

5.4 Mathematical models

This section reviews some models in the literature that can provide insight into how some evolutionary forces such as migration, selection and genetic drift, might change the frequency of a transgene when introduced into a natural population. A complete understanding of the simultaneous effect of all evolutionary forces that act on transgenes is difficult. In the subsections below we only give examples of some simplified models that we believe incorporate the most important factors. We will in general consider a diploid species with two alleles, *A*, representing the local gene, and *a*, representing the transgene with relative fitnesses $w_{AA} = 1 + 2s$, $w_{Aa} = 1 + s$ and $w_{aa} = 1$. We will however first emphasise some of the conclusions.

If a transgene gives only a slight selective advantage, it will almost inevitably increase in frequency in a natural population once only a few copies have escaped from a transgenic population (subsection 5.4.2), unless the effective size of the invaded population is very small. For plant species such as spruce with relatively limited dispersal, we should however expect the actual rate of increase, or, more precisely, the speed of the wave front of the transgene as it moves through neighbouring natural populations to be very slow (subsection 5.4.3), and a small increase in the frequency of the transgene in neighbouring populations may therefore not be statistically detectable during short term field trial experiments. Furthermore, during the first generations, the frequency of the transgene in the neighbouring population will, for the most part, be determined by the one-way migration process. The effect of selection will only become apparent much later when the wave front is "on its own". This suggests that short term monitoring programs that last for, say 10 generations, are insufficient to gain information about critical parameters such as the selection coefficient *s* acting on the transgene. It must be emphasised though, that properly designed comparative experiments lasting only one life cycle may still provide precise estimates of *s*. It should also be possible to synthesise physiological knowledge into estimates of *s*.

Figure 5.1. Subjective evaluation of the level of scientific considerations demanded by transgenic Norway spruce on a scale from less (low risk) to more (high risk). Two general situations are covered; one which involves reproductive stages (filled silhouettes) and one with obligate harvesting of immature trees (open silhouettes). Modified from Tiedje et al. (1989).



5.4.1 A simple deterministic model with continual immigration, and selective disadvantage

In the simplest case, assume that the invaded population can be treated as a panmictic unit, and that the effective size of the population is large so that genetic drift can be ignored. The change in the frequency p of the local gene due to selection and migration is then

$$\frac{dp}{dt} = sp(1-p) - mp,$$

where s is the selection coefficient, and m is the immigration rate (Crow & Kimura 1970). Setting $dp/dt = 0$ in and solving for p we find that the gene-frequency at equilibrium, when migration is exactly balanced by selection, is $p^* = 1 - m/s$. If the local gene is to persist, we must have $p^* > 0$ which implies that $s > m$ as shown by Haldane (1930). In other words, if the immigration rate is large compared to the selection coefficient the local gene will be lost. Depending on the gene's role in population regulation, a possible consequence of this fixation might be a reduced viability of the invaded population.

The above model is too simplistic for several reasons, and some important extensions will be discussed in the next three sections.

5.4.2 Selective advantage and genetic drift without continual immigration

Assume that some copies of the transgene escapes, maybe accidentally, from an enclosed transgenic population, and that the frequency of the transgene in the neighbouring natural population, after this release is p . Assume further that this release occurs only once. Of primary interest is the ultimate probability of fixation of the transgene. This probability depends strongly on the selection coefficient. (The actual time to fixation may however be very long, as we shall see in the next subsection). We will start by considering the case of neutral genes.

With no selection ($s=0$) the frequency of the transgene will fluctuate for many generations until it eventually becomes fixed or is lost by genetic drift. The probability that the gene becomes fixed will be equal to the initial gene-frequency p , and the probability that it is lost will be equal to $1-p$ (Crow & Kimura 1970, p. 426). For example, if $p = 0.5$, the probability that the gene is lost equals the probability that it becomes fixed.

However, with only a slight selective advantage, the ultimate fixation probability tends to one for all but small values of p . With a selection coefficient equal to s and effective population size N_e the ultimate probability of fixation of the transgene u is approximately

$$u = \frac{1 - e^{-4N_e s p}}{1 - e^{-4N_e s}}$$

if the initial gene-frequency is equal to p (Crow & Kimura, p. 425). From the above equation we see that the fixation probability depends on the product between N_e and s . As seen from **Figure 5.2** fixation is almost inevitable (u is close to 1) unless the initial gene-frequency p is very small, or unless the effective size of the invaded population is very small.

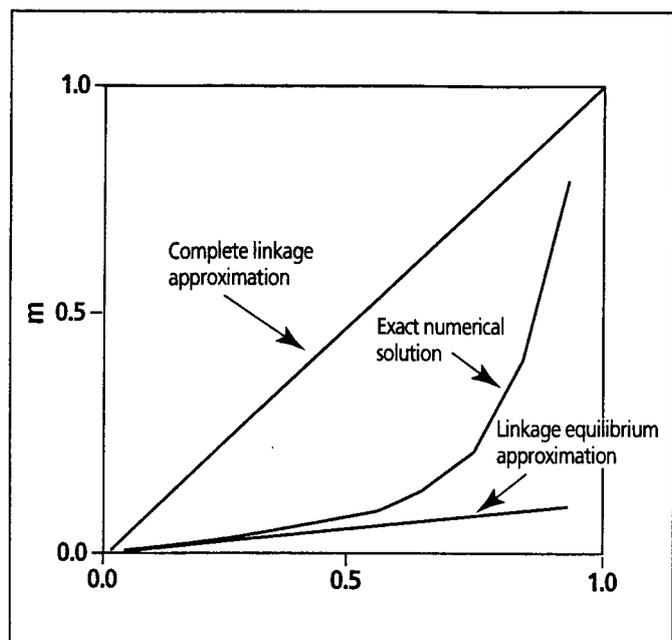


Figure 5.2. The probability of fixation of an advantageous transgene in a population of effective size N_e as a function of the initial frequency p . Note that fixation is almost inevitable unless the selection coefficient and the effective size of the population is small ($N_e s$ small).

5.4.3 Geographic structure

Of particular importance, in plant populations such as a spruce forest, is the relatively limited amount of gene flow in such a population. The geographic structure of the populations must be incorporated into the model, unless the populations under consideration are very small. We will consider a population model (similar to Slatkin 1973) with discrete generations and ignore age structure, genetic drift and mutations. For simplicity, we will assume that the population is distributed along a

single dimension x . The model may still describe the genefrequency dynamics in two-dimensional populations, if there is little genetic variation along the second dimension. The model may for example describe the genefrequency dynamics across the border between a transgenic and a natural forest. In this case the variable x would be the distance from this border.

The state of the total population at time t can be characterised by the genefrequencies $p_t(x)$ at all possible locations x . We will assume that dispersal of genes from one generation to the next follows a probability distribution $f(x_m)$, where x_m is the relative dispersal distance of genes from one generation to the next. (The dispersal distribution f can take any form, but is often strongly leptokurtic, that is, much of the probability is concentrated in the tails and around origo.) With these assumptions, given $p_t(x)$, we can express the state of the total population in generation $t+1$ as

$$p_{t+1}(x) = \int_{x_m=-\infty}^{+\infty} p_t(x - x_m) f(x_m) dx_m + sp_t(x) [1 - p_t(x)].$$

The first term on the right hand side represents migration and is simply an average of the gene frequencies close to x , weighted by the probability of migration over different distances x_m . The second term on the right hand side represents the increase in the genefrequency locally due to selection. To a good approximation, the dynamics of the system can be described by the partial differential equation

$$\frac{\partial p}{\partial t} = \frac{1}{2} \sigma^2 \frac{\partial^2 p}{\partial x^2} + sp(1-p),$$

where $\sigma^2 = \int x^2 f(x) dx$ is the variance of the dispersal distribution f (for details see Slatkin 1973). The genefrequencies now change continuously in time in this approximation of the model. **Table 1** lists some estimates of σ^2 (and some other dispersal measures) for Norway spruce and some other tree species.

If the selection coefficient is positive, that is, if the transgene has a selective advantage, it will soon become fixed locally, and at the same time disperse through other parts of the population. After a few generations, a steady state wave front will be formed (**Figure 5.3a**). It can be shown that the speed of this wave front only depends on s and σ^2 , and that the wave speed will equal $v = 5\sigma\sqrt{s} / (2\sqrt{3})$ (Fisher 1937, Ablowitz 1979). Other properties of $f(x)$ such as kurtosis turns out to be of little importance as long as the selection coefficient s is small, that is, different dispersal distributions leads to the same wave speed provided that their variance is the same (J. Tufto, unpublished simulations).

Table 1 Estimates of different dispersal measures of Norway spruce (*Picea abies*) and some other coniferous tree species.

Species	Dispersal (m/gen)	Source
<i>Picea abies</i>	30.6*	Wright (1952)
	91.4**	Langner (1953)
	91.4**	Strand (1957)
<i>Pinus cembra</i>	16.8*	Wright (1952)

* The dispersal standard deviation σ estimated assuming an exponential dispersal distribution.

** Distance within which 91 % of the pollen is deposited on the ground (according to Di-Giovanni 1991). Not comparable with σ .

As an example, assume that a genetic modification of Norway spruce produces individuals with a selective advantage as large as, say, $s = 0.1$. If we then use the estimate of the dispersal standard deviation $\sigma = 38.4 \text{ m/gen}$ from **Table 1**, the wavefront will not move faster than 0.70m/year or 700 m/1000 years, if we assume a generation length of 25 years. These calculations are of course only rough approximations but may still give us an idea of the relevant temporal and geographical scale of the problem. The estimate is however surprisingly small compared to the invasion of spruce through Finland and Sweden into Norway after the last glacial period. The speed of the advance through northern Sweden has been estimated to 900 m/year (Moe 1970, also see subsection 2.1.4). Through southern parts of Norway from the birth of Christ till today, Norway spruce advanced at an average speed of 200 m/year (Hafsten 1992a, figure 12). It must however be emphasized that the invasion of Norway spruce is a different process occurring on the interspecific level, while the wave speed of 0.70m/year calculated above refers to the speed by which new genes may spread into an already existing population. Part of the discrepancy may also be due to the fact that the dispersal variance σ^2 depends on the density of the vegetation, and local wind conditions (e.g. Di-Giovanni 1991), and the estimates in **Table 1** may therefore not be representative. Furthermore, a critical assumption of theoretical population genetics models such as that of Fisher (1937) and Ablowitz (1979) is that there is no directional bias in the movement pattern.

If there is selection against the transgene it will, to a certain extent, still become quite frequent far into the natural population. Assume that there is no natural regeneration within the transgenic part of the forest, so that the frequency of the transgene within the transgenic part of the forest remains equal to $p(x,t) = 1$, for $x < 1$. **Figure 5.3b** shows a numerical solution of the change in the gene frequency change over time at different distances from the transgenic forest edge. The transgene is assumed to be initially absent from the natural part of the population.

It is interesting to note that the difference in the frequency of the transgene between **figure 5.3a** and **5.3b** in the first 10 generations is surprisingly small. This is because the one-way gene flow from the transgenic part of the forest dominates the selection process which only becomes apparent in later generations.

5.4.4 Multiple gene copies

Most techniques such as the use of *Agrobacterium* result in individuals with several gene copies present. There is however a tendency of several gene copies to interact and inhibit each other when present in the same individual (Assaad 1993, Hobbs 1990), and transformed individuals are therefore crossed and reselected until only a single gene copy is present before considered commercially interesting. Due to the rapid development of gene technology, we can however not exclude the possibility that some of the difficulties that make single copy traits desirable, will be solved. It is therefore important to understand how multiple copies of the transgene might change the invasiveness of the transgenes.

Assume that identical copies of transgenes are present at n loci in all individuals in the genetically engineered part of the population, and assume that the immigration rate into the natural population is m . We shall also assume that there is no interaction between loci (no epistasis) so that the fitness of crossed individuals where i copies of the transgene are present can be written $w_i = 1 - si/n$. The fitness of a transgenic individual entering the natural population is then $w_{2n} = 1 - 2s$. However, due to recombination with local genes the population will move towards linkage equilibrium (random association between genes at different loci), while linkage disequilibrium at the same time will be created by the immigration and selection process. An interesting question is now how strong the selection has to be if the local genes are to persist. If we ignored recombination, so that invading genes behave as "one single gene", the condition for persistence would be, as before (see section 5.4.1), that $s > m$. On the

other hand, if invading genes recombined with local genes immediately after they entered the population, we could ignore linkage disequilibrium, and the change in the gene frequencies would then only depend on the effect of each allele on fitness which is s/n . The condition for persistence of the local gene would then be that $s/n > m$. The exact solution of the problem can be found numerically (using methods in Barton 1992), and the solution turns out to be close to the linkage equilibrium solution as long as selection is not very strong (see **Figure 5.4**).

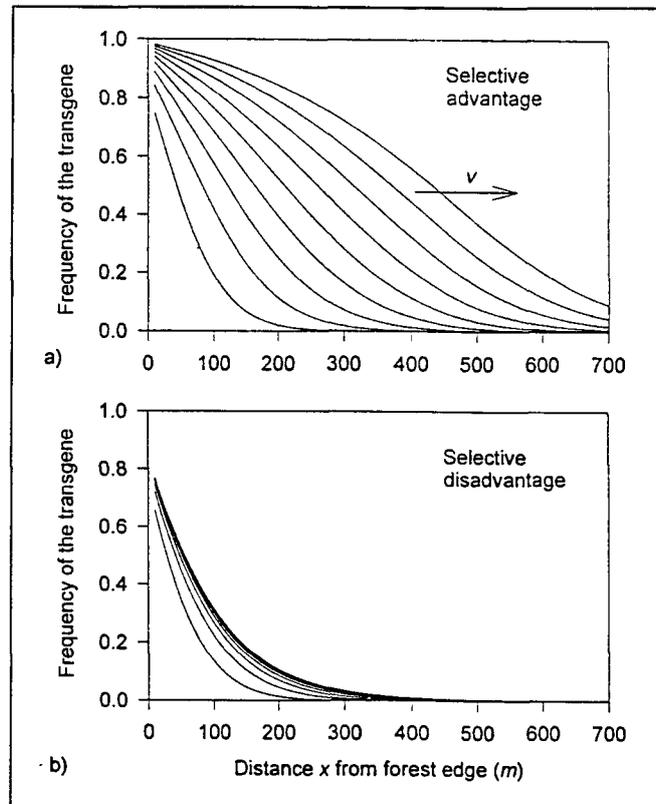


Figure 5.3. Simulations of the spread of a transgene through a geographically structured population. The lines represent the gene frequencies every 5th generation at different distances x from the border between the transgenic and natural part of the forest. A dispersal standard deviation of $\sigma = 38.4\text{m/gen}$ was used and the selection coefficient was set to $s = \pm 0.1$. a) If the transgene gives a selective advantage a steady state wave front will be formed after a few generations. b) If the transgene reduces fitness, a stable gene frequency cline will be formed after approximately 40 generations. Note that the transgene will become quite frequent much further into the natural forest than the standard deviation of the dispersal during a single generation might suggest. Also note the small difference between a) and b) during the first 10 generations.

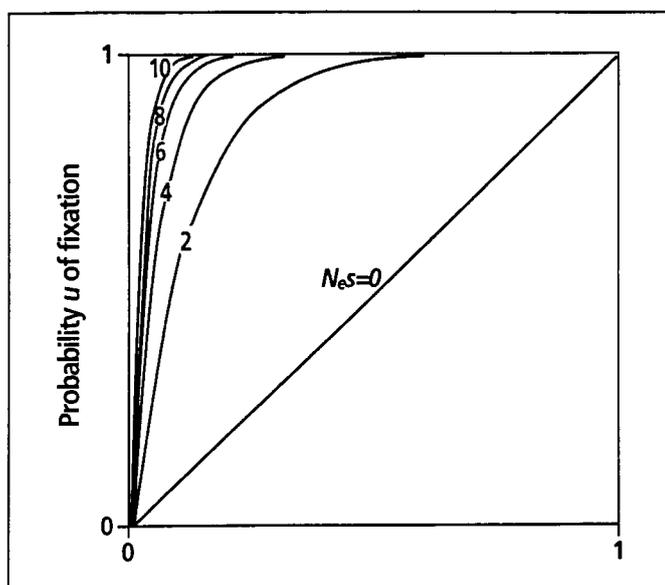


Figure 5.4. The exact threshold immigration rate that leads to fixation of the transgene as a function of the selection coefficient in a multilocus systems with 10 loci, compared with solutions assuming no recombination (complete linkage), and linkage equilibrium. As long as selection against the transgene is weak, the approximate condition for fixation is that $m > s/n$.

This means that, in a multilocus system, very little immigration can have a large effect on the genetic composition of the natural population, even if the total selection against invading alleles is quite strong, as also emphasised by Barton (1992) and Phillips (1995). Fixation of a large number of slightly disadvantageous alleles may thus lead to a large decrease in the viability of the invaded population.

6 Evaluation of specific GM-spruce cases

A few relevant traits that in the future may be manipulated by genetic engineering in Norway spruce are here shortly reviewed. The following specific comments on ecological risks are meant as an addition to the general points discussed in chapter 5.

6.1 Enhanced resistance to pathogens

The root rot fungus (*Heterobasidion annosum*) is the most noxious fungus in our forests. A rough estimate suggests that 50 mill. NOK is lost annually due to the reduced quality of the timber, and premature death of trees (H. Solheim, pers. comm.). If genetic engineering in the future can improve the resistance to this disease, it would be of great economical importance. Let us assume that this trait will be composed by the action of several (natural and/or alien) genes (general resistance, reducing the risk of counter-evolution of the fungus), and that the trait is faithfully transmitted through subsequent generations in a predictable manner. Under these assumptions we will try to evaluate the ecological risks involved in the releases of fertile transgenic plants of Norway spruce.

In areas with active podzolisation, that is, in most spruce forest areas, fungi are the most active component as to the decay of wood and decomposition of organic matter. If the actual «antifungus» gene is rather broad or general in its effect on fungi, this might influence decomposing fungi in some way, leading to a faster or slower decomposition rate (see **Figure 6.1**). Both paths of change will have consequences for biodiversity. A possible slower mineralisation process might cause a slower growth of spruce followed by formation of bogs and heaths.

The mycorrhiza fungi are regarded as very important for Norway spruce on dry habitats, on habitats where soil moisture is variable, or in general in habitats with marginal growing conditions (E. Sivertsen, pers. comm.). In optimal growing conditions for Norway spruce, mycorrhiza is not well developed, whereas mycorrhiza is important for Norway spruce on marginal habitats, like in dry habitats where mycorrhiza facilitates the water uptake. If an «antifungus» gene is introduced to reduce damage from the root rot fungus (*Heterobasidion annosum*), this may also change the response on the mycorrhiza fungi on Norway spruce. Hundreds of mycorrhiza species are described on Norway spruce (Sivertsen pers. comm.). If the close connection (symbiosis) between root and fungus is broken due to a general response

normal development. An international project at NISK are aiming at a greater understanding of the defence mechanism in these processes, and to use this knowledge to identify individuals showing high resistance (Christiansen 1995). Nevertheless, let us assume that transgenic spruce can be produced which synthesise toxic and inhibitory compounds to both the bark beetle and the fungus, and that the inserted genes are only activated during attack.

The large pine weevil (*Hylobius abietis*) represents a serious economical problem in forestry. They feed on small seedlings of Norway spruce and strip the bark off the stem in a border zone between stem and roots and thus kill the plants. Intensive research has been going on to find if mechanical (e.g. wax) or chemical protection against the attack or biological control of the species is feasible (Tømmerås 1993). However, if we could produce transgenic plants which synthesise *B.t.*-toxins or proteinase inhibitors during the first four years (using age specific or wound specific promoters) from seeds, and these internal compounds conferred enhanced resistance, such transgenes would be very valuable from an economical point of view.

The ecological risks involved in the release of such transgenes are on at least two levels (Hengeveld 1995). Firstly, the use of *B.t.* carries a possibility for resistance to develop both for the target insect(s) and non-target organisms. This is specifically serious when the insects are presented for the toxin continuously. Secondly, in a forest ecosystem with hundreds of species involved, the effects on the ecosystem of changing species abundances are not predictable.

The change in selection pressure on other organisms following the use of GM- spruce will produce unpredictable changes in the ecosystem. Due to less genetic variation in GM-spruce, adaptations to genetic monoculture will occur. This creates effective phytophagous insects and other potential pests and decreases populations in the complex of predators and parasitoids (Tømmerås 1993).

6.3 Enhanced frost tolerance during active growth

The frost hardiness in autumn, winter and early spring is quite high in Norway spruce, and natural variation in timing of cold acclimation and deacclimation is great. However, during active growth (May-June), Norway spruce is very sensitive to frost (Sokolov et al. 1977). In June, when active stem elongation is taking place frost damage is observed at -2 ° to -3 °C. The stem and branch quality are reduced on sites where summer frosts frequently occur, and the natural variation in frost tolerance during active growth is very small. If it is possible to produce transgenic spruce with improved

frost tolerance during active growth by means of high production of extracellular plant antifreeze proteins, such plants could be very valuable.

Norway spruce with increased frost resistance, will probably have local consequences on biodiversity in frost pockets and other places with stagnating air, due to increased vitality and better growth of Norway spruce in such habitats (see **figure 6.2**). Increased frost resistance will probably not lead to a generally higher vitality of Norway spruce at its alpine timberline, but increase the competition ability in frost pockets. Special frost pocket habitats are potentially threatened by invasion.

6.4 Increased drought resistance

Increased drought resistance will make *P. abies* more drought tolerant, that is, it can become more competitive and invasive in habitats which are naturally dominated by Scots pine (*Pinus sylvestris*) and xerophilous vegetation, or where we find a co-dominance of Norway spruce and Scots pine to-day. In the association *Vaccinio-Pinetum boreale*, which is typically developed (Kielland-Lund 1994) in the south boreal zone, with extensions into the middle and north boreal zone, there exists such a co-dominance between Norway spruce and Scots pine. However, Scots pine is the most vital on the alluvial sand plains of SE Norway. A gene modified and more drought resistant Norway spruce can change this picture in favour of Norway spruce (see **figure 6.3**). Because of the inherent shade-tolerance of Norway spruce, a new and more dark and damp micro-climate will follow and may change the habitat towards a pure spruce forest with the local extinction of plant species like *Pyrola chlorantha* and *Lycopodium complanatum*. The edaphic conditions will probably change towards a thicker raw humus layer.

A more drought resistant *P. abies* could also to some extent invade the continental association *Cladonio-Pinetum boreale* in the south boreal and middle boreal zone in SE Norway. However, due to the low nutritional status of this habitat, Norway spruce will probably not outcompete Scots pine in *Cladonio-Pinetum boreale*.

The greatest changes in biodiversity may be foreseen in the lime-rich thermophilous habitat with a current co-dominance of pine and spruce in the boreone-moral zone. A more drought resistant spruce will dominate this habitat and lead to unfavourable light conditions for e.g. some rare heliophilous orchids. Potential associated invertebrates might also come under threat.

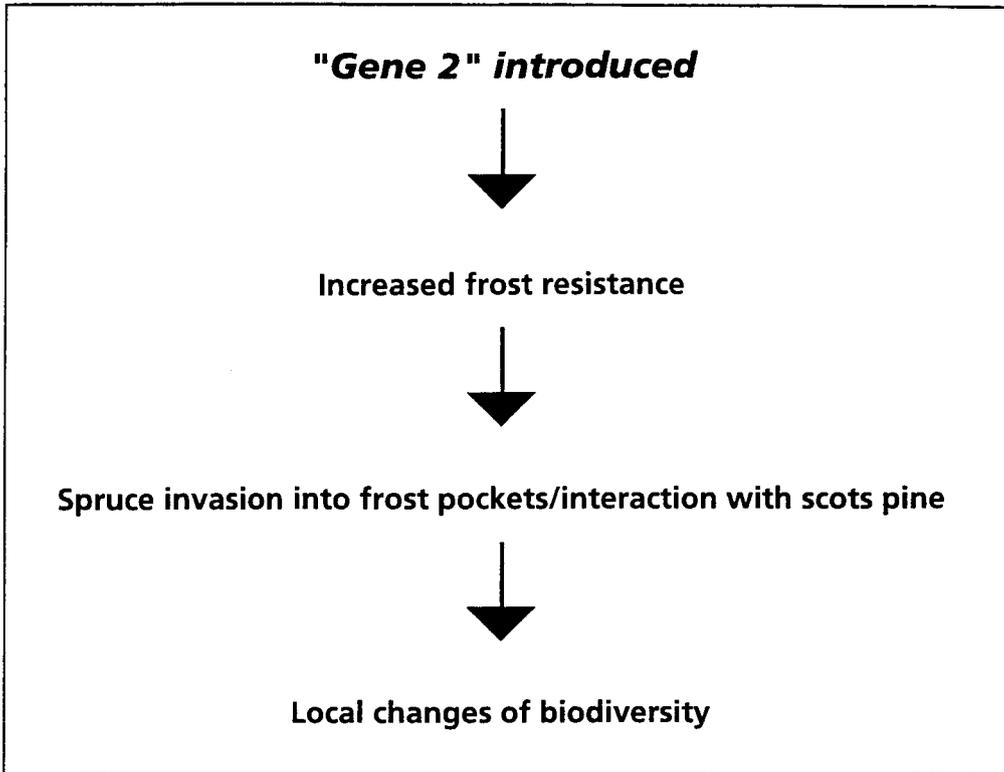


Figure 6.2. Some possible ecological consequences of increased frost resistance in Norway spruce.

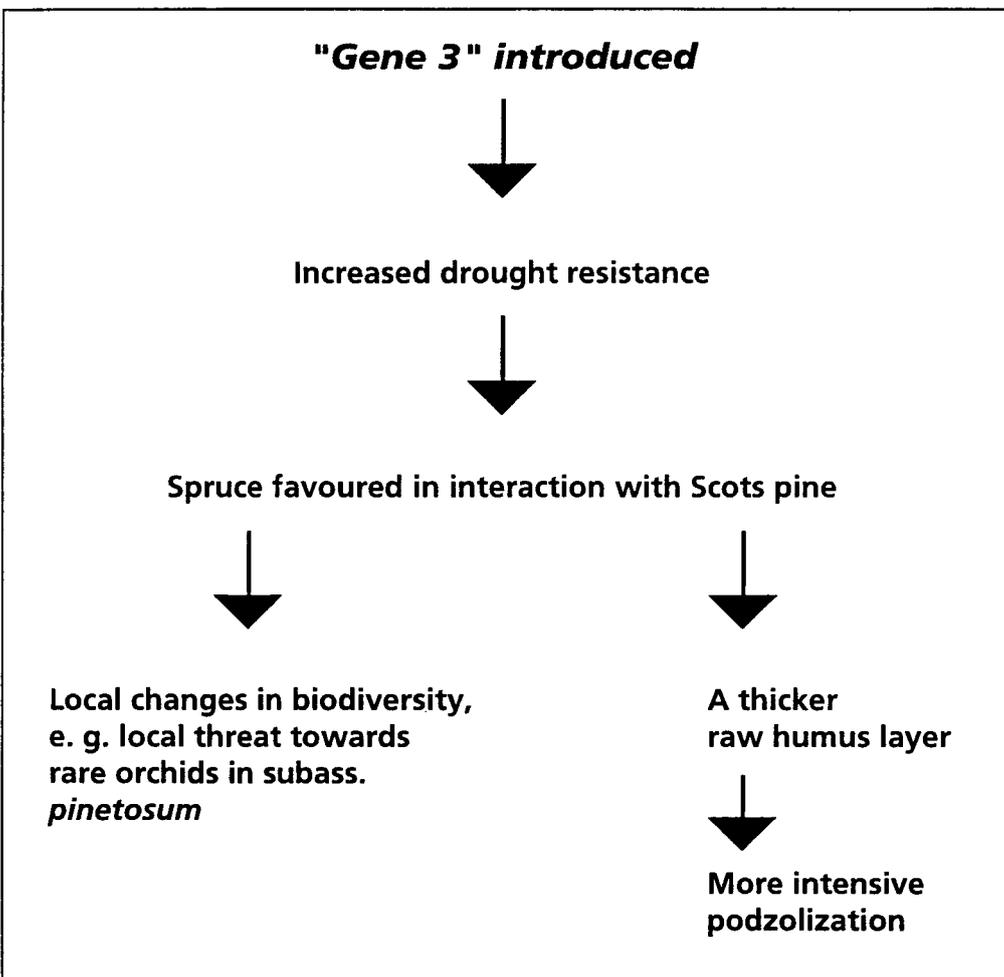


Figure 6.3. Some possible ecological consequences of increased drought resistance in Norway spruce.

In **figure 6.4** we have visualised a comparison of the level of scientific considerations needed before release of three different transgenic Norway spruce. The genetic modifications are fungal disease resistance, insect resistance and frost tolerance. By the level of scientific considerations, Tiedje et al. (1989) mean perceived risk to the environment, based on existing knowledge. As illustrated in the figure, the three genetic modifications differ in perceived risks for the various attributes evaluated. The ranking of risk associated with the three genetic modifications is uncertain, and additional uncertainty about weighting of attributes makes a summation of total risk impossible at current level of knowledge. For example, even for a trait such as frost tolerance, which has been studied by provenance transfer (chapter 3), our knowledge about transgenic, frost-tolerant spruce is far from complete. One aspect of uncertainty relates to the fitness consequences when non-native provenances interbreed with native ones; another uncertainty relates to how far lessons from provenance transfer can be used to study frost tolerance achieved by genetic modification. There is no way around this problem except doing the hard work related to studying risks in controlled (and confined) experiments, paying attention to a long checklist of attributes for each genetic modification to be evaluated. This checklist must include the general attributes suggested by Tiedje et al. (1989), as well as specific attributes based on knowledge about the focal organism and release environment, such as those emanating from chapters 2-5 in this report.

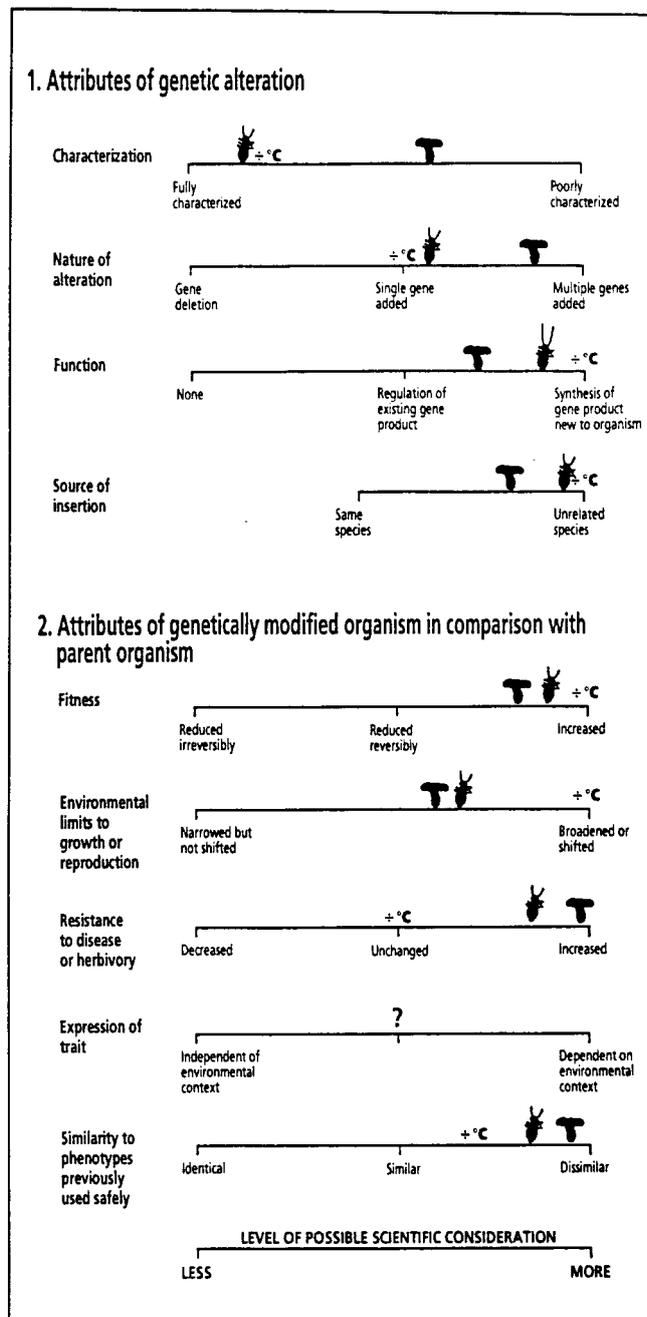


Figure 6.4. Evaluation of the level of scientific consideration demanded by transgenic Norway spruce expressing increased fungal disease resistance (T), insect resistance (fly) or frost tolerance (+°C).

7 Summary remarks

The intended effects of releases of transgenic organisms are usually specific with regard to species and environment, and limited in time. Evaluations of environmental effects must, however, also include other species and environments, because both the transgenic organisms and their genes can spread after deliberate or accidental release. Moreover, genetically modified organisms are exposed to natural selection and may evolve better adaptations to the environment over time. It will therefore always be a formidable task to provide documentation on the environmental effects of a release, and almost impossible to predict them.

Stability, gene expression and horizontal gene transfer are topics where there generally are discussions about the present status. When releasing a genetically engineered organism this general problem has to be addressed.

Many releases of GMOs are quite trivial relative to a ecosystem key species such as Norway spruce. Species can be fully domesticated, fully dependent on humans or easily controlled by human intervention. Spruce biology, on the other hand, shows that it is a key species in major ecosystems containing a mosaic of natural and managed forests. Approximately half of the species diversity in Norway is connected to forests, half of the Red list species as well. Hundreds of mycorrhiza fungi species are described on Norway spruce. These fungi are in general very important for spruce growth and especially crucial on dry habitats.

There are strong economic incentives to produce GM-spruce e.g. more resistant to frost, herbicides, fungi or pest insects. Some positive and negative effects of provenance transfer on economic traits have been shown. However, controlled provenance transfer are still too recent (in terms of spruce generation time) to study fitness effects of interbreeds between transferred provenances and local spruce.

There are uncertainties concerning spread and fitness of GM-spruce. There is furthermore impossible to call back unwanted effects after several spruce generations. A possible trait of transgene inactivation is highly premature, and still not considered as reliable. A potential use of only juveniles is difficult to regard as safe due to problems in controlling access.

Evaluation of specific GM-spruce must also include the conservation of biodiversity dependent of the species Norway spruce. There are differences in the risk assessment between various potential types of genetically engineered Norway spruce. For some GM-types the main risk is invasion to new areas (e.g.

frost tolerance) while other types affect the forest where spruce already is the dominating species (e.g. enhanced pathogen resistance) and finally can both today's Norway spruce areas and new potential invasive areas be affected (e.g. enhanced drought resistance).

A type of GM-spruce able to resist or change the decomposition of wood (e.g. insect resistance) are considered as specifically questionable due to threat to natural ecosystem function, habitats and species. The impact of using GM-spruce with enhanced fungi resistance is also uncertain, and can seriously affect, for the ecosystem necessary, mycorrhiza system in an unpredictable way.

By releasing GM- spruce in an area where inserted genes have the possibility to spread over the natural population, spruce in its main natural (native) range can have great impact of most of the genetic resources present there. The uncertainties about probability for potential irreversible threat independent of the intended benefit from the genetic modification, ought lead to no release of genetically modified Norway spruce. Both lack of knowledge and the stochasticity in biological systems are additional strong arguments for use of the «precautionary principle». So far there are no evaluations available giving answers to all the important questions about release of genetically modified spruce in the main area of natural occurrence range.

8 Literature

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