EUROPEAN FOREST GENETIC RESOURCES PROGRAMME (EUFORGEN)

Technical guidelines for genetic conservation of Norway spruce (*Picea abies* (L.) Karst.)

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IPGRI

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The European Forest Genetic Resources Programme (EUFORGEN) is a collaborative programme among European countries aimed at ensuring the effective conservation and the sustainable utilization of forest genetic resources in Europe. It was established to implement Resolution 2 of the Strasbourg Ministerial Conference on the Protection of Forests in Europe. EUFORGEN is financed by participating countries and is coordinated by IPGRI, in collaboration with the Forestry Department of FAO. It facilitates the dissemination of information and various collaborative initiatives. The Programme operates through networks in which forest geneticists and other forestry specialists work together to analyze needs, exchange experiences and develop conservation objectives and methods for selected species. The networks also contribute to the development of appropriate conservation strategies for the ecosystems to which these species belong. Network members and other scientists and forest managers from participating countries carry out an agreed workplan with their own resources as inputs in kind to the Programme. EUFORGEN is overseen by a Steering Committee composed of National Coordinators nominated by the participating countries.

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Introduction

Norway spruce (*Picea abies* (L.) (Karst.) is a principal species of different types of complex forest ecosystems in the boreal and temperate zones of Europe. Its distribution area and forestry importance increased after the extensive planting which began more than 200 years ago and concentrated on lower altitudes of the temperate and to some extent also boreal Europe.

Norway spruce is a traditional object of forest genetic and breeding research. The well-known provenance experiment IUFRO 1964/68 has provided basic information about geographic patterns of variation for adaptive traits (Krutzsch 1974, 1992). Advanced breeding techniques, management of seed orchards and vegetative propagation methods were developed on the model of this species. Phenology and physiological traits were described. A number of studies were carried out with biochemical and molecular markers, focusing on the mating system and the levels and structure of genetic variation.

Populations of Norway spruce face severe threats, especially in some regions of central Europe. The overall dieback of conifer forests, caused by industrial air pollution and global warming, undoubtedly affects the species' genetic resources and requires the intensification of joint efforts for genetic conservation. Despite the high levels of intrapopulation genetic variation and known high adaptability of Norway spruce, rapid changes of the distribution area margins due to high mortality are predicted. Another important concern to conservation of genetic resources is the past transfer of forest reproductive material and subsequently the unknown, non-local origin of spruce in vast areas of Europe.

Norway spruce has been frequently identified as a priority species in national gene conservation strategies (Koski 1993). The urgent need to conserve its genetic variation has been recognized in European countries since the 1980s and funds and expertise were allocated at different levels. Many efforts have been devoted to the conservation of genetic material prior to or even without an explicit elaboration of national gene conservation programmes, mainly within tree breeding and nature protection. The interest to strengthen collaboration among the specific national activities and programmes was affirmed by signatory countries of the Strasbourg Ministerial Resolution S2 (Conservation of Forest Genetic Resources) in 1993 when Norway spruce was selected as one of the four pilot species for collaborative gene conservation networks. As its implementing mechanism, the EUFORGEN *Picea abies* Network later benefited greatly from the work of the IUFRO working party 2.02.11 (Norway spruce breeding and genetic resources) which has been promoting research and activities at the scientific and technical levels for decades.

The comparably high intensity of measures for the genetic conservation of Norway spruce is determined by the enduring high economic importance of the species for timber production, the actual threats to its genetic variability and the sound biological and genetic knowledge acquired by the scientific community. While the results of experimental work on the species' ecological requirements, physiology, morphology, reproductive biology, population dynamics and genetic variation have been obtained from many countries and cover significant parts of the distribution area, information on more practical experiences with the conservation of genetic resources is still missing. Also, the scientific knowledge is often inaccessible to local forest officers and national or regional authorities responsible for the genetic conservation of forests. The shortage of resources available for research, and for making research results available, may furthermore restrict access to knowledge, especially in countries with economies in transition. Gene conservation programmes, in order to be undertaken efficiently and safely, require a substantial knowledge base. Even though the level and amount of information available on Norway spruce is high compared with other conifers and broadleaved trees, not to mention Mediterranean or tropical species, much more scientific and practical knowledge is still needed for well-informed decisions to be taken.

Waiting for all scientific uncertainties to be solved at a time would, however, delay taking action for the conservation of precious, and in many cases severely threatened, genetic resources. These Guidelines aim to contribute information and provide guidance for the management of Norway spruce gene conservation units. This work results from collaborative activities of European countries in the EUFORGEN *Picea abies* Network. The focus is on synthetic but practically oriented advice and its easy, cost-effective implementation in the field almost everywhere in Europe. Wherever suitable, the Guidelines attempt to discuss several options, while the implementation of each choice will always have to adapt to local conditions and practices.

The Guidelines are divided into four chapters: *in situ* conservation, *ex situ* conservation of genetic resources in populations, in clone collections/seed orchards and in genebanks. Timely and comprehensive actions, based on best available local knowledge and on generalized guidelines, should contribute to the successful conservation and use of forest genetic resources. The key characteristic of *in situ* conservation is its dynamic nature allowing for continued evolution of a gene conservation stand. This approach will generally be preferred for Norway spruce and other long-lived, outbreeding and undomesticated trees with common occurrence and widespread distribution. It will be easier to take into account the considerations about *in situ* conserved genetic resources but still closely following the forestry practice common in the respective area. Even if *in situ* conservation is aimed at the particular species Norway spruce, genetic resources of associated plant species will be preserved as well. Some circumstances will make *ex situ* measures necessary. These should be encouraged in order to complement or 'back up' the *in situ* gene reserves. Genetic resources held *ex situ* often represent characterized variation which gives additional options for genetic management and use. The basic link between conservation, tree breeding and managed use of forests is widely recognized. It will obviously be very important not only to find a balance between the *in situ* and *ex situ* approaches, but also to integrate the principles of genetic conservation into routine silviculture and forest management, as well as nature protection and tree breeding.

These Technical Guidelines are concerned with the practical management aspects of the conservation of genetic resources in Norway spruce. Preceding the actual conservation, a process of setting priorities for species and then for populations within a species will be needed according to the preferences and possibilities at a national or regional level. Decisions on the choice of which resources and how they should be conserved will ideally be based on all available information including ecogeographic and forestry-related information, adaptive traits and genetic inventories. Most importantly, before starting action in the field, objectives for gene conservation will have been clearly formulated. The Guidelines emphasize the preservation of a broad genetic variation and hence evolutionary adaptability of populations to a changing environment over generations as the main goal of genetic conservation. The preservation of broadest genetic variation is also crucial for its effective use for human needs in the future.

Unlike in the case of agricultural crop plants, high genetic variation not altered or reduced by domestication can be assumed for natural as well as planted and managed Norway spruce forests. The high genetic variation at the level of individual trees, within populations and among populations, enables adaptation to environmental changes faced during the very long life cycles of forest stands. The need to ensure and enhance this potential for future evolution is the driving force for genetic conservation. From a more practical point of view, the conservation of forest genetic resources must take into consideration not only timber and other forest products, but also the whole range of resource values of a forest ecosystem including the ecological and social benefits.

Because of the continuing alarming losses of Norway spruce stands and their genetic diversity in Europe, the importance of international collaboration is becoming increasingly important. Although priority is given to the *in situ* conservation of genetic resources in autochthonous and well-adapted forests, genetic material conserved locally may become indispensable for the preservation or re-establishment of spruce forests anywhere else at any time in the future. The interdependence of countries thus concerns exchange of reproductive material, and exchange of experience. The latter is particularly important with regard to exchange of information about initial steps, for monitoring progress and for providing mutual support. The second aim of these Guidelines is therefore to contribute to raising awareness about the often neglected task of conserving the genetic resources of our forests.

Following the four chapters of these Guidelines, the Bibliography offers a selection of references with relevance to genetic resources management in Norway spruce. Besides the literature cited in the text, it includes some important published results of research carried out with an increasing intensity during the past three decades, as well as recent review articles and proceedings of specific meetings. The selected bibliography provides suggestions for further reading, and is not to be seen as an exhaustive bibliographical source. A brief glossary of terms used in the Guidelines is also provided. The definitions attempt to facilitate the reading of the text by describing the essence of terms which are widely used but often have an ambiguous meaning or lack overall acceptance. Various sources were used to compile the list, with particular reference to the OECD Scheme. The terms were modified where appropriate to meet the needs of the *Picea abies* Network.

These Guidelines will be developed further. The authors, the EUFORGEN *Picea abies* Network and IPGRI very much welcome receiving comments and suggestions for improvement of the current version.

The authors gratefully recognize the key role of the Follow-up Committee of the Strasbourg Resolution 2, chaired by Dr Michel Arbez, in the establishment of the gene conservation networks. We thank all members of the *Picea abies* Network for their input and the Steering Committee of EUFORGEN for fruitful and encouraging discussions.

1. *In situ* conservation of genetic resources

Aims and approach

In principle, gene conservation aims at maintaining the evolutionary genetic adaptability of populations and species over many generations. For most cultivated crop plants the original natural populations from which they developed have disappeared, and consequently an unknown amount of their genetic information has been lost. In contrast, many forest trees, including Norway spruce, typically still grow in large natural populations. *In situ* gene conservation then simply involves the saving of appropriate populations over generations, in order to maintain the adaptively or randomly developed genetic structures within the species. The purpose is not to fix the currently existing gene or genotype frequencies at any expense, but rather to ensure the existence of broadest genetic variation.

A successful *in situ* gene conservation practice must fulfil certain fundamental prerequisites:

1. A network of gene conservation stands is to be created with sufficient coverage of the spatial genetic variation of the species.
2. The number of individual genotypes per population must be large enough to include most of the genepool existing in the respective population (the conservation of common, essential genes is crucial).
3. The system of regeneration must maintain the population and the regeneration stock should predominantly originate from matings within the respective population.

The concrete requirements and management guidelines will be derived from these premises.

The elementary advantage of *in situ* conservation is that the tree population already exists. Thus, the establishment of a gene reserve needs neither expensive measures nor years to wait. The continuity of natural populations may seem to be assured, but in fact the inherent dynamics of forests today no longer functions as in the past. Human impact on forests has, to a large extent, disturbed the natural conditions of reproductive processes.

Wind pollination is a random process that 'wastes' huge amounts of pollen and is effective in large stands of relatively high density characteristic for Norway spruce in the boreal zone. Even though pollen grains of Norway spruce are bigger than those of many other wind-pollinated trees, they are carried by wind over long distances. On the other hand, adequate pollination of female strobili requires a dense pollen cloud, which is formed only in stands of sufficient size. In small stands the total pollen pool is limited and the proportion of self-pollination is likely to be higher. Consequently, the amount of viable seeds is relatively small and the overall vigour of the offspring is reduced. In regions where the species is common, the pollination of small stands, even if isolated, originates predominantly from outside sources. In the case of autochthonous origin of populations, background pollination does not cause any harm in terms of genetic information. Stands of unknown or undesirable origin, however, often exist in the surroundings and geneflow from those is to be avoided.

According to the current knowledge of reproductive biology and demography of populations and taking into consideration the basic gene conservation objectives, it is recommended that the ideal area of gene reserve forest composed of one or more gene conservation stands (i.e. an *in situ* gene conservation unit) should be at least 100 ha. This area is not too large, if compared with most nature protection areas. However, sometimes it is not easy or even possible to find such large areas. The size of each gene reserve forest will depend on the local situation and on regional peculiarities. It should be emphasized that if a number of individual gene conservation stands constitutes the relatively compact gene reserve forest (with total size corresponding to 100 ha or more), they should preferably cover all the divergent sites within that unit. Such a situation will in most cases be applicable to the conditions in central Europe. The creation of a 'buffer zone' may be considered, depending on the situation and local conditions of the gene reserve forest.

Gene conservation *in situ* of Norway spruce populations may take place in both unmanaged forests and managed forests. The unmanaged category concerns different types of nature protection areas, such as nature parks, national parks and nature reserves. Besides their conventional functions, ecosystem and landscape protection as well as habitat preservation for endangered fauna and flora, protected areas significantly contribute to maintaining the genetic variation of forest tree species. However, protected areas alone do not fulfill all actual needs and specific requirements of the conservation of forest genetic resources. They have two main limitations: (1) their distribution and coverage are seldom adequate because they have been selected for other purposes and often include fairly extreme environments rather than being representative of the existing different forest types; and (2) the access to reproductive material is in many cases limited by law or otherwise restricted, thus the continued regeneration of populations may not be secured. The practice varies from country to country and the strength of restrictions varies among the different categories of protected areas. Still, the general approach is passive and stationary from the point of view of human interference. This aspect of *in situ* gene conservation shall not be described in more detail here.

Actively managed *in situ* gene reserves can avoid the constraints to conservation of genetic resources associated with nature protection areas. The most crucial point is ascertained continuous existence of populations over long periods of time. Firstly, the proposed gene conservation unit is to be in permanent ownership and saved from changes in land use, such as the building of highways and houses. Maintenance with adequate silvicultural measures, protection against fire and other damage factors must be available. From the economic point of view, the preconditions are not as stringent as for conventional nature reserves. Timber harvesting is allowed, which will not eliminate income. The required area of 100 ha is fairly modest in relation to the size of most protected areas. The long-term commitment to such specialized land use of forest normally restricts the efforts to state forest and possibly also private companies of sufficiently large size. Ownership does not pose such a problem in countries where forests are mainly owned by the state or large private companies.

Requirements of gene reserve forests

1 All *in situ* gene conservation stands shall be autochthonous (of local origin). Stands of foreign or unknown origin should not be included. Plantings established with material of undesirable origins may even be removed in order to ensure that only trees carrying genotypes of local origin remain. As an exception to the rule, it may be acceptable or even desirable for certain purposes to conserve genetic variation of well adapted 'landraces'. This specifically concerns regions or countries outside the natural distribution area, where Norway spruce is not autochthonous or where the local populations have been completely destroyed. Areas exposed to heavy selective cutting and other kinds of actions reducing natural genetic variation are not acceptable as gene reserve forest. It is important to realize that the whole area does not need to be composed of pure Norway spruce. Gene reserve forest may consist of a mixture of species in various proportions.

2 The target area of a gene reserve forest shall be at least 100 ha. The shortest diameter shall be 400 m or more. The whole area need not

be a compact, even-aged mature forest, but the populations should preferably consist of individuals of various ages or be composed of different age classes, even open areas, and include components of other (non-hybridizing) species. Areas smaller than the mentioned 100 ha can be accepted for gene reserve forest in regions where Norway spruce forests are not common or widely distributed, with particular reference to regions or countries outside the natural distribution area. Such areas should, however, have the potential for regeneration and possible enlargement. The minimum size of a gene reserve must be decided on a case-by-case basis according to the given situation. The target area of a gene reserve allowing enlargement by means of artificial regeneration (replanting) should be at least 10 ha. Such cases should be seen as exceptional, and should be applied in specific cases where the population(s) in question have been greatly reduced and survive as important remnants.

Registering of gene reserves

After a suitable area has been identified following the agreed criteria as a gene reserve forest and agreement on the reservation of the area for this special purpose has been achieved, the forest needs to be registered. Information on the location and characteristics of stands, ownership of the gene reserve forest, etc. (see Descriptors in the Report of the second Network meeting) is to be delivered to the responsible national authority. Prior to registering the area, the owner/manager of the gene reserve forest should formally agree to follow a set of guidelines regarding mainly the silvicultural treatment and management as part of a forest management plan (also to be held by a national registering authority). The possibilities of compensations to forest owners for the economic losses resulting from the registration and subsequent management of a gene reserve forest should be investigated, according to the practice in individual countries. The area needs to be clearly defined and depicted on a map. Permanent marking in the field is not always necessary and, as a rule, the area can remain unfenced and freely accessible. Fencing may be necessary in cases when regeneration must be protected. It is recommended to put an appropriate sign board at the ’entrance’ of the gene reserve forest to raise awareness of visitors.

Management of gene reserves

The main objective of silvicultural management is to ensure the continuation of a population or populations that represent the gene reserve forest. Thus, firstly, management should aim at the protection of stands against all types of damage that might threaten their existence. Secondly, management should aim to prepare the conditions and ensure the potential of a stand for regeneration, with particular emphasis on the reproduction of genetic variation.

Obviously, trees contribute directly to the genetic evolution of a population only as long as they are alive, even if the life cycle of trees is extremely long compared with other organisms. A very old, even-aged stand is indeed very vulnerable to harmful abiotic and biotic factors. Therefore, occurrence and regeneration of more than one age class is a necessary prerequisite to secure continuity at the stand level. An uneven-aged stand structure of Norway spruce is more likely to develop naturally in certain ecological situations, e.g. in mountain forests (small-scale mosaic of sites), but it is not easy to maintain everywhere. A pattern of stands with even-aged trees, i.e. with age class distribution, is a result of common practice and can be observed in many regions.

Stands approved for seed collection (seed stands) are often proposed for gene conservation and in many cases are considered priority for that purpose. Seed collection stands commonly represent mature or overmature, even-aged stands of tall trees with superior phenotypic quality. *Ex situ* conservation, involving long-term storage of viable seeds in genebanks, is an important back-up conservation measure undertaken in the seed stands (see Chapter 4).

Old stands are vulnerable to both abiotic and biotic harmful agents. Pollutants, pests and diseases adversely affect older trees to a greater extent than for younger, more vigorous trees. Even though gene conservation stands are intended to reach maximum rotation age, overmature stands in particular need to be regenerated periodically. For many species, including Norway spruce, this is not a straightforward process. Large openings in the stand cause wind throws and consequently promote too vigorous growth of ground vegetation. A careful and repeated opening up of the stand with strips, preferably not straight ones, or small patch clearings is probably the best way to appropriately encourage the process of regeneration. Regeneration can also be stimulated by means of site preparation and weed control, followed by sowing or planting. In order to secure the reproduction of genetic information contributed by as many individuals as possible, regeneration cycles with longer duration than in the normal forestry practice should be aimed at.

Felling and removal of large trees is difficult and may cause damage to the remaining trees. However, simply leaving the Norway spruce forest unmanaged will eventually lead to a dead end. The ancient 'nature method' whereby spruce forest is completely burnt by fire, a frequent factor in some environmental situations and regions, turns into broadleaved forest by natural regeneration and after 50 years is recolonized by Norway spruce, would not be realistic today and may not even be compatible with the prerequisites of gene conservation because of the extent of forest fragmentation. If the gene reserve forest is as large as 100 ha, a portion of it, in most cases the core area, or several of the included populations, may well be left without intervention.

Thinning in gene reserve forest is not only permissible but a necessary measure ensure the stability and regeneration of stands. It is sometimes argued that thinning is a form of unnatural, directed selection which can modify the genetic composition of a population. This, however, will mainly depend on the criteria used to select trees for thinning. In any case, it would hold true for the current tree generation. The next generation will, with or without intervention, have a different genetic composition from the parent stand.

It is impossible to give detailed thinning guidelines covering all probable circumstances. The main principle is to thin in a timely manner avoid too high density and its negative consequences. Most frequently, gene reserves will predominantly consist of old growth or mature stands with even-aged structure. Thinning should then be mainly from below, removing suppressed, injured and deteriorated trees, thus simulating natural selection processes in the forest. If the stand is originally very dense, the operation must be carried out with caution and requires careful planning. Besides the possible damage caused by falling large trees to the remaining ones, heavy machinery may severely damage bark and roots. Excessive thinning, leading to wide spacing of trees, may expose them to sunburn and wind throw. Steep slopes give rise to special problems. Small openings or narrow, not straight, strips may be suitable and allow gradual regeneration. In every case, the local professional foresters must choose the most appropriate mode of thinning.

Gene conservation stands at earlier stages of ontogenetic development are easier to manage. Moderate thinning enhances the growth of dominant trees, supports stability of the stand and maintains it in a healthier condition. With the recommended method of thinning from below the probable losers are removed in advance, but in case some particular forms, such as *pendula* or *aurea* are present, then these should be retained at the expense of 'normal' trees.

In some cases stands with uneven-aged structure ('Plenterwald') will be utilized as gene reserve forests. Then, of course, thinning is mainly from above. Forests treated with strong selective cutting over generations may suffer from loss of genetic variation. Therefore, selection forests of Norway spruce are in general terms less likely to be suitable as gene reserves, if there are other options.

Commercial harvest of timber is the most obvious phase of treatment which distinguishes gene reserve forests from nature protection areas. Harvesting is an essential part of the active approach aimed at the maintenance of stand stability and regeneration potential. Selling of merchantable timber may mitigate at least part of the economical losses associated with gene conservation *in situ*. On the other hand, the purpose of commercial cuts in gene reserve forests cannot be to maximize net income. Extreme caution must be taken during harvesting operations, with an absolute minimum of damage to remaining trees. Clear-cutting is acceptable, but the openings must be small, with their size and position depending strongly on the local conditions (see next section on Regeneration). In particular on wind-exposed sites and loose soils, the stability of the remaining stand must be taken into account. As a rule, discrete openings or corridors are preferable to selection cuts, i.e. the removing of individual selected trees out of the stand. Selection cutting systems are less cost-efficient and probably deleterious from a genetic standpoint.

In younger, rather even-aged stands the first thinning may be delayed until trees have reached dimensions of merchantable fibre wood. Normally the first thinning is from below and consequently the economic returns are not great. Despite that, any selection and cutting of the tallest trees with higher timber value should not be permitted at this stage.

Regeneration

Regeneration is the key aspect of *in situ* (but also *ex situ*) gene conservation. Natural regeneration is immediately associated with *in situ* conservation, but one cannot always rely on the success of this method alone. The entirely natural process, which may in some cases include forest fires or windbreaks and the alternation of cycles with broadleaved species, is seldom applicable for practical reasons. Generally long (longer than in normal forestry practice) regeneration periods should be aimed at in gene reserve forest. The longer the regeneration period the larger the proportion of trees involved in seeding, the higher the probability of genetic variation of the population being sufficiently represented in the next generation. It is obvious that natural regeneration should usually be enhanced and controlled in one way or another. Artificial regeneration is thus permissible in gene reserve forests, to complement, and exceptionally to replace, the preferable natural method.

An ideal gene reserve forest is a mosaic of stands, having differentiated stand composition, structure and age classes. This means that the whole area (of 100 ha or more) would never be purposefully regenerated in one shift. A plan needs to be made for regeneration of the gene reserve, which includes both spatial and temporal elements. If the entire area of the gene reserve forest consists of mature or overmature stands, then establishment of new generations ought to be started without delay. If the area includes sapling and pole stage stands, the regeneration may still be delayed but planning should take into consideration the need for prolonged regeneration periods.

The actual process of regeneration must be fitted to local circumstances. Universal and detailed guidelines cannot be given. One principle is to keep the size of regeneration areas rather small, up to a maximum of 5 ha in the boreal zone, for example. On the other hand, several separate openings may exist in the same gene conservation stand. In mountain forests, the small-scale mosaic structure allows regeneration of overlapping generations on the same site.

Regeneration from natural seeding is, of course, most desirable from the genetic conservation aspect, as well as economical. As a rule, site preparation needs to be undertaken prior to seeding and weed control is often necessary during the seedling stage. In situations where natural seeding is insufficient or does not adequately cover larger openings, direct sowing into properly prepared sites can help ensure the success of regeneration of the gene reserve forests. In many cases the more rapid and successful regeneration method will be planting, especially on fertile, nutrient rich soils. Planting is a fully acceptable method of artificial regeneration in gene reserve forests.

Most importantly, in the case of both direct seeding and planting, the material for artificial regeneration must originate from the same gene conservation stand. The conditions should as closely as possible follow the conditions of natural regeneration (insolation, wind, soil temperature), in order to avoid any unnatural selection pressure.

Seeds are to be collected from at least 100 trees per population (gene conservation stand), preferably from the more central parts of the forest rather than forest margins. Bulk seed will be a representative mixture of all collected lots. It is recommended that this kind of bulk seed be collected at the first occasion of abundant seed crop year and kept in storage for future use. To be absolutely certain that enough seeds will be available to regenerate parts of the gene reserve forest, in the worst scenario when the entire gene reserve is destroyed, it is recommended that at least 5 kg of seeds be stored per population of the gene reserve forest in a seed bank (Chapter 4).

Utilization

The main purpose of *in situ* gene reserves is to maintain natural genetic variation over long periods of time. It is difficult to envisage the potential utilization of gene reserves carrying valuable genetic information and variation, but future generations will not thank us if we do not take care to conserve the valuable *in situ* genetic resources now. Examples can be taken from many cultivated crops used in agriculture, where the costly efforts to supply more genetic variation for breeding of domesticated cultivars from their scarce wild relatives have to be undertaken. Such an approach can certainly not become applicable for Norway spruce and other long-lived forest tree species.

Gene reserve forests have the advantage that they can be utilized in several ways, instead of standing as protected relicts. Economically important is their continued, although restricted, availability and use for timber production. Besides that conventional utilization, several more genetically relevant functions can be mentioned:

1. **Seed source.** Commercial seeds can be collected from standing trees and also from felled trees as part of management operations. The seeds may often be regarded as a highly valued or useful provenance for specific use in another area.
2. **Reference populations.** Local natural populations provide the most suitable reference material for use in provenance trials and progeny tests. *In situ* gene conservation stands are intended to remain as examples of natural populations representative of the surrounding forest area or region. Seeds collected from the internal parts of the forest represent the best available natural genepool even if cultivated stands of foreign origin or genetically improved stock become dominant or perform better in the region.
3. **Research.** Genetic structures of natural populations, mating system, evolutionary processes and co-adaptation of trees and their parasites/symbionts are examples of research subjects that will have increasing relevance in the future.
4. **Complementary breeding populations.** It can be assumed that, in future, new kinds of selection procedures will be developed. DNA methods may produce a new tool to select desirable genotypes

directly, or so far concealed traits may turn out to be valuable for certain purposes and consequently complementary selections may become useful.

In conclusion it is remarked that *in situ* gene conservation in managed forests is usually quite close to normal silvicultural practice. Conservation of common, essential genes is the crucial issue. This is certainly possible within the capacity of trained professional foresters locally. Awareness and knowledge of the objectives, systems and wide experiences with gene conservation will motivate the forestry profession and the public more generally, to make greater efforts for achieving progress in this important area.

2. *Ex situ* conservation of genetic resources in populations

Objectives

Ex situ conservation of genetic resources entails removal of individuals or reproductive material from their original environment. With forest trees, the term covers three different types of methods: (1) plantations that are either established to serve the purpose of gene conservation from the beginning or converted for that purpose although originally intended for other use; (2) collections of trees including clone collections, seed orchards and arboreta; and (3) genebanks storing seed lots, pollen or tissue. This chapter concentrates on the first type of *ex situ* gene conservation methods.

Planted *ex situ* gene conservation stands or populations of Norway spruce can be maintained complementary to *in situ* conservation activities (see Chapter 1), but are particularly important when *in situ* methods cannot be applied or are less suitable or less efficient. They become very useful when it is desirable to develop tree populations for increased timber production or to serve human needs for other products and benefits, but at the same time adaptation close to the natural conditions in the forest must be secured. This type of gene conservation provides for fast adaptation of a population to its environment, by combining natural processes with human management. Conservation of genetic resources in designated *ex situ* populations is, therefore, a dynamic approach.

Ex situ conservation methods can also be static in that they maintain a fixed genetic structure without any influence of evolutionary forces. This is most often the case with collections of clones (see Chapter 3) and seed and genebanks (see Chapter 4).

No gene conservation unit should be established without a clear objective in mind (Eriksson *et al.* 1993; see Introduction). All activities such as collecting, establishment of a plantation, its management and regeneration should follow the main objective, taking natural conditions and available institutional/financial resources into consideration. In general terms, the main aims for *ex situ* gene conservation populations will thus be to maintain and enhance the future adaptability of the populations combined with their improved value for human use.

Types of *ex situ* gene conservation populations

Norway spruce populations outside the natural distribution area

Many commercially important species are introduced to new environments. Some of the introductions develop distinctive properties and prove to be very successful in terms of adaptation associated with high survival rates, good growth and overall performance. Often the origin of the introduced material is unknown. Adaptation to the new environment may be determined by natural forces during the establishment and especially during the phase when competitive selection occurs in the stand. The genetic variation in such promising stands may be conserved and utilized by managing them as *in situ* gene reserve forest. The stand may also be sampled and a new population artificially established in truly *ex situ* conditions. In several European countries or regions, Norway spruce represents one of the most important exotic forest tree species. The genetic structures well adapted to environmental conditions outside the species' natural distribution area ('landraces') are thus of concern to both *in situ* and *ex situ* gene conservation activities.

Endangered populations

Within the distribution area of Norway spruce, the further existence of many populations designated for gene conservation is threatened by unfavourable climatic conditions and by increasing industrial air pollution. This is the case particularly in some areas of central Europe. Protection of such populations in their natural habitat will mostly be difficult, if not impossible. To avoid risks and to eliminate detrimental effects on genetic variation, a solution for the conservation of genetic resources will be to establish new populations at another location. This can be done by planting seedlings, if seeds are available, or by grafting.

Another situation arises when small-sized, autochthonous and valuable populations are surrounded by artificially established forests of non-local origin. The adaptive genetic variability of the local stand may not be conserved in the offspring resulting from mating events in the course of natural regeneration. It may be necessary to artificially establish a new population from sampled material and in appropriate conditions outside of the range of contaminating pollen sources.

Conservation of known genetic variation

Forest geneticists have established trials at multiple sites testing provenances, families or clones. Provenance trials have often been planted both within and outside the natural distribution area of the species and involve many countries. These trials are planted according to specific experimental designs with replications and the origin or family identity of each individual is known. The genetic variation of individuals belonging to the experimental populations has been characterized. Their value as genetic resources may depend on the number of populations included in a trial, the number of individuals per population and on the geographic coverage of the distribution area. At least for the first generation and as long as individual identity is kept on record, some of the research trials should be maintained as gene conservation units. Depending on the management and regeneration methods, the trials might continue to serve as genetic resources, but the occurrence of provenance hybrids in future generations must be taken into consideration. Due to the effects of natural and artificial selection they may develop into local 'landraces', with known genetic structures. This characterized genetic variability can be then be utilized for specific purposes.

Breeding and genetic resources conservation

Breeding tests are artificial populations established on the basis of specific selection criteria. Their main purpose is to produce afforestation material for generating future forest stands with specific adaptive properties and qualities beneficial for human use. Breeding populations are considered to have the largest possibilities for direct influence on the population genetic diversity through their initial composition, management and regeneration. The most targeted management of genetic diversity occurs in tree breeding programmes structured into multiple populations under different selection conditions.

A system of multiple populations was proposed by Namkoong (1976, 1984, 1986) to integrate tree breeding and gene conservation. According to this system, a large gene resource population is split into several smaller populations. The gene resource populations should be sampled to capture maximum genetic variability among populations, still keeping a large genetic variation within the populations. In the most elaborate form, the populations should be established over a broad array of sites, thus sampling a wide range of environmental conditions. In managed populations artificial selection is carried out to improve the adaptedness of each population and to increase genetic variability among them. The populations can also be left unmanaged. They can be established directly from natural populations and be actively selected and then either managed or unmanaged. Each population can be kept relatively small.

A set of multiple populations for joint breeding and gene conservation purposes may initially capture a large portion of the existing genetic adaptedness in the species. Natural and artificial selection will allow for a dynamic development both to changed environmental conditions and to human needs. A set of populations initially established in different ways and managed differently may thus be beneficial to match different breeding objectives.

Existing examples of the multiple population approach

A multiple gene conservation strategy has so far been adopted in forestry practice for *Quercus suber* in Portugal (Varela and Eriksson 1995). The plan involves both small, managed natural populations and large, managed and unmanaged natural populations covering most important environmental and management types. This approach will ensure that both the evolutionary and breeding objectives of gene conservation, as well as the conservation of accompanying species, are taken into account.

Another example concerns the proposed long-term breeding plans for Scots pine and Norway spruce in Sweden (Danell 1991, 1993). Based on large sets of initially selected and tested parents, a number (>20) of small nucleus populations will be selected and established. This selection will in particular be based on phenotypic traits related to the adaptation to climatic conditions. The populations will be established at locations that represent a wider range of variation in light and temperature conditions than the species covers today. Each population will be matched to the site conditions and will be selected and managed to achieve a high degree of adaptability. The set of populations will serve long-term conservation needs and can easily be utilized.

Changes in genetic structure

In dynamic conservation of genetic resources, changes in the genetic structure of populations occur during the adaptive process which is intended as part of the conservation strategy. However, adaptation to new site conditions may not be wanted in some cases when the original genetic structures should be kept, such as evacuation or rescue of genetic material. This purpose may generally not be possible to achieve with *ex situ* populations and it may be more appropriate to establish clonal collections in which seed can be produced through controlled crosses. Artificial regeneration by sowing or planting can then be made at the original sites, if required.

Sampling

The sampling of material to be conserved in *ex situ* populations depends on the conservation objective. Within a region of provenance, attention should be given to sampling of genetic variation both between and within stands. It is recommended that sampling 10-20 stands within a region of provenance would provide a very good and representative sample. Seeds should be collected preferably in a year with abundant seed crop and the seed-bearing trees should be well-distributed in the stand, at least 30-50 m apart. When a single stand (population) is the basic target unit of conservation, it is advisable to collect seeds or take scions for grafting from a sample of a minimum of 100 trees.

When tree breeding and gene conservation purposes are combined, the specific breeding strategy will determine the number of genotypes and the type of genetic variability needed in each population. In converted experimental populations, the original genetic composition is fixed. It can be changed by thinnings based on directed, genotypic selection to obtain a more directed diversity, depending on the specific objective of the gene conservation unit.

Establishment

The establishment of an *ex situ* gene conservation stand or population can be carried out by sowing, planting or by vegetative propagation, usually grafting. Direct sowing in the field will allow for adaptation to the new environment from the very beginning. The same can be achieved by planting at narrow spacing (Hattemer 1995). Both methods will require early thinnings. Nursery treatment and planting methods for the establishment of *ex situ* gene conservation populations should follow local standards. The size of plantations will again depend on the objective of the conservation unit. If the aim is to conserve genetic resources in a single region of provenance then 2 or 3 plantations of sizes 2 to 5 ha each are suggested.

A randomized design should be applied when populations are established by grafting. If possible, the identity of the grafted material should be kept on record. This is important if controlled crosses are to be made in the future. The design of combined breeding and gene conservation units will depend mainly on the specific breeding strategy.

Management

As it is important to secure the physical stability of the stand, a careful tending is advisable. This should be done by normal silvicultural practices used in the respective area. Thinnings should be made in any case, initially systematic and later selective (see Chapter 1). Thinnings should take into consideration the specific experimental design used in *ex situ* gene conservation populations with individual identity possibly kept and the overall objective of the gene conservation unit.

Regeneration

Gene conservation populations established in *ex situ* conditions can be regenerated both naturally and artificially. Natural regeneration should be used if applicable and if maintenance of specific useful diversity is not the main objective. The usual regeneration of an *ex situ* population will be by artificial means. The principles for selecting and harvesting trees should be similar to the first generation. Equal amounts of plants should be used from each parent tree in order to keep a high effective population size. In case of a grafted plantation, as much as possible of the originally selected diversity should be kept and stimulation of flowering may be necessary.

General remarks

The *ex situ* gene conservation populations can belong to a variety of types: progeny test plantations from seed lots originating from one or several stands; grafted populations for evacuation purposes; breeding populations in a multiple population system; converted field experiments or breeding tests. The latter type may be thinned provenance, family or clonal trials and also abandoned seedling seed or clonal orchards. The potential risks associated with the occurrence of provenance hybrids in future generations of trials should be carefully considered.

Ex situ conservation of genetic resources may be carried out at different levels of complexity. In its simplest form it means the establishment of a forest plantation based on the target genetic resource population which was selected and designated for this purpose. The plantation can be managed as a traditional forest plantation to develop into a mature stand. It can be regenerated naturally or by reforestation. If regular interventions are needed to meet specific objectives, then the management becomes more complex and expensive. Such types of gene conservation populations will therefore be preferred as an alternative *ex situ* conservation method for species with high economic value, and Norway spruce represents a good example.

3. *Ex situ* conservation of genetic resources in seed orchards and clone collections

Objectives

Genetic resources of Norway spruce can be conserved *ex situ* by the establishment of seed orchards and clone collections. These types of gene conservation units will be applied whenever the *in situ* and the other *ex situ* conservation methods described in these Guidelines are not successful or are very difficult to implement.

Seed orchards may be based on the material from vegetatively propagated trees (clonal seed orchards) or from generatively propagated trees (seedling seed orchards). Seed orchards established specifically for, or fulfilling, gene conservation purposes produce seeds of valuable autochthonous or non-autochthonous populations as well as single trees worthwhile to conserve owing to (i) their endangered status (including heavily air-polluted areas or areas affected by extensive human activities), (ii) insufficient fructification in their natural environment, (iii) unwelcome pollination in their natural environment or (iv) the necessity to produce seeds of good quality over longer periods of time than the lifespan of the basic material. If the number of clones from a certain population is not sufficient for the establishment of a gene conservation seed orchard, or only rare genotypes or single trees should be conserved, then the genetic information of these individuals can be conserved in clone collections for a longer time than the lifespan of the basic material. Additionally, the establishment of gene conservation seed orchards or clone collections is the necessary regeneration measure to complement the storage of pollen in genebanks.

Changes in genetic structure

The possible genetic implications of seed production in seed orchards were described elsewhere. According to Hattemer *et al.* (1982), there is a strong relationship between the number of clones or single tree progenies included and the probability of allele losses. The genetic structure of progenies in a seed orchard may also be influenced by the respective reproductive phenology and by the annually changing capacity of individual female and male clones to produce flowers.

It is recommended to include the highest possible number of clones in a gene conservation seed orchard. Losses of genetic information caused later by insufficient pollination or self-pollination can further be minimized by suitable seed orchard design as well as careful consideration of the flowering intensity of the clones and the distribution of flowering clones before seed collection. If the infrastructure is available, changes of genetic structure between the original stands, the clones used and the seeds produced in a seed orchard should be evaluated by genetic studies at marker gene loci, in order to assess the genetic implications of the conservation practices on the material conserved or produced.

Genetic comparisons of seed orchards with basic material

Studies comparing the genetic structure of Norway spruce seed orchards with the genetic structure of the natural stands where the clones or single trees were sampled are quite rare. In a study carried out at isoenzyme gene loci (Bergmann and Ruetz 1991), no substantial differences were found with regard to gene frequencies and genetic diversity between a seed orchard and the originally sampled Norway spruce populations. The differences were greater for heterozygosity. Unfortunately, there are no comparisons of the genetic structure of the seed orchard progenies. Very few differences for genetic diversity were observed between seed lots from several seed orchards and natural stands of Scots pine (*Pinus sylvestris*), but the embryos descended from the seed orchards had a lower degree of heterozygosity than embryos from the natural populations (Szmidt and Muona 1985).

Legal regulations

The production and movement of Norway spruce reproductive material is under the rule of national legal regulations for forest reproductive material in most European countries. Therefore, the establishment of gene conservation seed orchards should be given a particular emphasis and should always be carried out according to the respective valid regulations.

Collecting and propagation

In order to avoid losses of genetic information in a seed orchard or clone collection, the number of clones or single tree progenies should be as high as possible and should in any case exceed 50. If the size of a valuable relic population that needs to be conserved is not large enough to achieve the minimum sufficient number of clones, then the establishment of a clone collection should have priority. If there are different autochthonous relic populations in one provenance region, clones from these populations may preferably be collected and put together in one gene conservation seed orchard, in order to achieve the minimum sufficient number of clones.

If the designated population has a sufficient size, the selection of trees to be sampled should be done randomly, e.g. using a raster screen. In order to avoid the selection of trees related to each other, a minimum distance of 30-50 m should be kept between the selected trees (see Chapter 2).

For the establishment of clonal seed orchards, vegetative material collected from old trees is normally used. Taking into account local climatic peculiarities, the scions should be collected by tree climbers during winter (from December to March) in those parts of the crown capable of flowering. The number of scions per clone depends on the seed orchard design and the number of orchards to be established. During tree climbing, damage of the trees should be avoided by the use of sophisticated rope-climbing techniques and careful climbing.

In most cases, grafting of Norway spruce is uncomplicated. It can be carried out in a greenhouse where the scions will be grafted on 3-year-old root stock potted in the previous year. Collecting and grafting should then be organized simultaneously, so that storage of the scions is not necessary. Grafting can also be done on seedlings in the field. In this case, the scions should be stored covered with plastic film in cold storage at 0°C, until the root stock has been prepared in the right condition for grafting. If no cold storage facilities are available, the scions may be stored on ice in a cellar. If scions are collected in winter at very low temperatures, they can be stored at temperatures as low as –10 to –20°C. The best success will be achieved if the scions are grafted on root stock just before flushing. The most common grafting technique is veneer grafting if normally developed scions are used. In the case of underdeveloped scions, the 'incrustation in head' technique may be used.

If very old Norway spruce trees should be conserved by the described techniques, the vegetative propagation by grafting may be difficult. In this case, the establishment of seedling seed orchards can be a suitable alternative if the number of single trees is sufficient. For the establishment of seedling seed orchards, the progenies of the single trees are to be collected, handled, stored and raised strictly separately.

Establishment, management and tending

The location of each seed orchard should be at least 400 m away from the next Norway spruce trees or stands to reduce unwelcome pollination from outside sources. Additionally, the possibility of pollen contamination can be minimized by planting of border trees or increasing the seed orchard size. The best isolation is assured if the seed orchard is located within a region covered only with broadleaved species. To ensure sufficient flowering and seed production, the site should be exposed south to southwest on medium to moderate wet soils. The management of the seed orchard may be facilitated if the site is accessible by machines.

The aim of the clonal or seedling seed orchard design should be to maximize the outcrossing rate and to minimize inbreeding. Therefore, each clone should have every other clone as a neighbour. At the same time, the distance between individuals of the same clone should be maximized. Different methods for the mixture of clones in seed orchards have been described by Giertych (1975), Nester (1994), Schmitz (1995) and others. Finally, duly and early consideration of future schematic thinnings will help to avoid any possible disruption in the distribution scheme.

Each clone or single tree progeny should be represented by an equal number of individuals. Graftings or seedlings which reached the age of 4 to 5 years and height 0.4 to 0.5 m might be planted in a rectangular pattern of 4 x 6 m. If necessary, the initial space of 24 m^2 per individual can then be extended to 48 m^2 by removal of each second tree within a row, thus keeping the original distribution scheme.

In regions with high pressure of game on forest vegetation, the seed orchard area should be protected and fenced. During the first years, normal weeding including replanting is recommended. After the establishment of the plants' growth, weeding is required only once a year. Owing to the value of the material conserved, special attention should be paid to fertilization and plant protection. Before the canopy is closed, a schematic thinning should be carried out to ensure the flowering of lower branches.

Seed collection in gene conservation seed orchards

Sufficient flowering and seed production in a seed orchard can be expected 15 to 20 years after the establishment, at the earliest. To prevent unnecessary losses of genetic information, the flowering and seed production in gene conservation seed orchards should carefully be observed prior to the seed collection. Seed collection should only be conducted after a regularly distributed male as well as female flowering has been observed at a minimum of 70% of the clones or single tree progenies. The smaller the number of clones or single tree progenies, the more careful the question of regular flowering and seed production has to be considered.

To ensure the broadest possible genetic diversity, all individuals of a clone or single tree progeny bearing sufficient seed crop should be harvested. Collecting seeds only from individuals with abundant seed crop or carrying big cones should be avoided. Within the seed lot, each clone or each single tree progeny has to be represented with the same amount of seeds.

4. *Ex situ* conservation of genetic resources in genebanks

Approach

As a complementary method to both *in situ* and *ex situ* gene conservation with population being the basic unit to allow for dynamic changes of genetic structures (Chapters 1 and 2), genebanks represent the case of static conservation. Irrespective of whether the materials for *ex situ* gene conservation are seeds, pollen, tissues or clonally propagated material, they conserve genes in a static way and except for the changes due to genotype-dependent differences in mortality (loss of viability), there are no other causes for changes in the genetic structures during storage.

Objectives

The principal objective of *ex situ* conservation in genebanks is to ensure availability of genetic material at different localities and periods of time. Originally, the first genebanks developed from seed banks supplying forest reproductive material for plantations, mainly in the periods with insufficient seed crops. However, this purpose is not identical with gene conservation, it covers only approved stands for seed collection (seed stands), and very often contains a small number of parent trees which may correspond to specific aims of tree breeding.

The conservation activities in genebanks should follow similar principles as *in situ* or *ex situ* gene conservation units and offer the insurance for mainly *in situ* gene conservation approach in Norway spruce. In case of need, genebanks should also serve as the source of forest reproductive material with genetic properties of high quality. Other objectives of *ex situ* conservation approach using genebanks include the establishment (regeneration) of gene conservation units and the provision of genetic material for breeding and evaluation programmes in later periods. The advantage of *ex situ* genebanks is the fast starting point. To initiate gene conservation activities in genebanks is a relatively uncomplicated procedure assuming that existing facilities can be utilized, such as seed-processing and seed-storage centres established traditionally for conifers in many countries.

Requirements

Genebanks can conserve any type of forest reproductive material (seeds, pollen, tissues, vegetative parts of plants). The different types of material represent different options regarding storage technologies, time period and required number of stored samples.

A significant aspect is the hierarchical organization of a genebank. Genebanks can store samples mixed at the population (stand) level, i.e. bulk samples, or samples stored at the individual level. In both cases, the number of individuals included should be defined according to the main principles of a gene conservation strategy. It should be noted that larger seed lots and sampling modes which enable keeping parts of a seed lot separate will be required in the case of genetically heterogeneous populations. The amounts and organization of the stored samples should reflect stated conservation objectives, which will help determine whether samples covering the most representative genetic variation should be included, or whether sampling should be aimed at the capture of rare alleles. Sampling of populations should ideally be based on knowledge of overall levels and patterns of genetic variation and the mating system. Whenever possible, the samples included and stored in a genebank for conservation purposes should possess a hierarchical structure, e.g. trees – stands – regions of provenance.

Collecting

Collecting of samples which are to be stored in the genebank should be well documented, following commonly agreed guidelines and covering all important aspects, e.g. location, geographical and ecological data (see Descriptors in the Report of the second Network meeting). The selection of individual trees should be kept on record. Their position should preferably be marked on a map. Notes on the flowering intensity and seed crop should also accompany each sample included in the genebank. For taking a representative sample, the seed-bearing trees should be well distributed in the stand, adjacent or related trees should be avoided and the minimum distance between two sampled individuals (30-50 m; see Chapter 2) should be mentioned in the collecting protocol. If genetic inventory of a gene conservation stand has been carried out, then material from the same trees should be collected which were already characterized in the analyses.

The minimum number of trees sampled per gene conservation stand should not be less than 100 individuals (see also Chapters 1 and 2). Representative bulk seed collected from at least 100 trees can provide insurance against sudden loss of the forest population, and offer a source of suitable reproductive material where gradual artificial regeneration of the stand will be needed. Most representative material is to be collected in a mast year, when the highest number of trees carry cones. In the case of Norway spruce, abundant seed crops occur at irregular intervals. It is recommended to collect seeds during the first mast year after the designation of the gene reserve forest and regularly afterwards. Recent experimental results show that seeds collected in the same stand in different years will probably not have the same genetic properties. Additional sampling for gene conservation purposes should therefore be carried out also between the mast years, wherever possible.

Seed storage

For gene conservation purposes, storage of bulk seed lots with a defined number of sampled individuals will generally be sufficient. If the seeds (also pollen and tissue material) will be used for further breeding purposes or evaluation, storage of individual tree samples is preferred. Bulk samples can easily be created afterwards by mixing equal-weight proportions of seeds or equal numbers of full, viable seeds. The decision on storage of individual or bulk samples thus depends on their future use. The approach to storage of separate seed lots representing open-pollinated progenies of individual trees could aim to conserve the broadest genetic variation including rare alleles, and would be based on consideration of the effective pollen contributions in the mating system of Norway spruce.

Seeds always need to be kept and handled under the best possible conditions before storage, in order to maintain high levels of viability. The seeds should be held for a minimum period of time under temporary conditions that do not meet acceptable standards for conservation. Where Norway spruce seeds are stored at the preferred conditions, there is no known benefit in chemically treating them to control pests or diseases.

Seed-drying procedures in genebanks should be given high attention and dry seeds always handled with care. The objective of drying is to reduce the moisture content to a level which prolongs viability during storage. A variety of methods can be used, depending on the availability of technical equipment, number and size of samples to be dried, local climatic conditions and cost considerations. It is recommended for genebanks, however, to use a special drying chamber with dehumidified forced-air circulation and adapted to operate quickly with rather small quantities of seeds in the individual seed lots. Drying temperatures of about 30°C will usually be applied for Norway spruce seeds. The seed samples need to be dried without delay after being received. It should be noted that dry seeds are often brittle and can thus be damaged easily.

All seed lots should be as clean from dust, wing rests and foreign particles and free from pests and diseases as possible, but heavy cleaning procedures should be avoided for gene conservation purposes. Inappropriate equipment may damage the vulnerable seeds and the application of separators grading the seeds according to, e.g. size and specific gravity, might even eliminate certain genotypes during the genebank process. The use of fine seed extracting, dewinging and seed-cleaning are necessary steps of the technological process. In many cases it may also be acceptable to remove empty, damaged and filled non-productive seeds by special water-based technologies developed for routine use in Norway spruce.

A range of containers for storage is available which are moisture-proof and sealable. Choice of container will depend on its availability and ability to withstand the given storage conditions without leaks. The use of any type of sealed moisture-proof containers which are known to ensure the quality of stored material is acceptable. It should be noted that many plastics are not moisture-proof.

There are several options for the orthodox Norway spruce seeds concerning storage at an operational level in genebanks. Temperatures above zero (2-4°C) may be suitable for short-term storage of seed samples in hermetically closed bottles or tins; this method will maintain viability of seed samples for up to 15 years with 4-8% seed moisture content. The preferred method for long-term storage of seed samples in genebanks is the deep freezing at temperatures of –18°C with about 5% moisture content. It is more energy consuming and needs appropriate equipment, but is required for the conservation of rare or particularly valuable samples. The seed samples of Norway spruce for gene conservation purposes, maintained deep frozen for long-term storage, will not lose viability for a few decades. Some flexibility should be possible with regard to what should be considered acceptable, particularly in cases when refrigeration, to the extent required by the above preferred values, cannot be provided. Owing to the nature of the relation between seed longevity, storage temperature and correct seed moisture content, the same storage life can be achieved by different combinations of temperature and moisture. Experiences show that for medium-term storage, subfreezing temperatures (around –5°C) and relative moisture content up to 10% in the seed samples give acceptable results, if the standard long-term storage conditions cannot be met.

The viability and quality of the seed samples are influenced by the conditions of their storage, in addition to the initial quality of the seeds. Viability will usually be assessed by a germination test. The germination ability of stored seed samples has to be tested upon receipt of material and subsequently at regular intervals during storage. This interval will be determined according to the viability of the seeds at the start of storage, moisture content and storage conditions. It is recommended for Norway spruce that tests be carried out at least every 5 years of storage. Where the preferred conditions of long-term storage cannot be met, monitoring will need to be more frequent. Procedures for seed testing in genebanks will follow in detail the international rules described in ISTA (1996). Germination losses are considered acceptable up to 20-25% for gene conservation purposes; even though classical commercial seed storage facilities might frequently be aiming at lower rates. As far as possible, all sources of selection pressure should be removed and all possible care taken to minimize changes of the genetic structures in the seed lots during the process. It is essential that genebanks have, or have access to, sufficient laboratory equipment to enable viability monitoring tests to be carried out in a regulated, uniform and timely manner.

Regeneration of seed lots stored is the last, but most time-consuming and costly operation during the genebank process. It also means high risks which may easily jeopardize the integrity of genetic variation in the seed lots (populations) being conserved and should, therefore, be carefully planned. It should be undertaken when viability falls to 75-80% of the initial value. Under very unfavourable circumstances, only a small portion of each stored seed lot may be adapted to survive the environmental conditions prevailing at the time of regeneration. Even despite a high mortality rate, the establishment of a regenerated population from each population seed lot must be assured, and then maintained until the reproductive age of the trees is reached and sexual reproduction occurs.

Amounts

The amounts of seed material stored for gene conservation purposes vary according to the conservation objectives and depend on the practice applied in individual countries and regions. If, as an example, the suggested weight of 5 kg (see Chapter 1) corresponds to about 600 000 Norway spruce seeds (a maximum of about 500 000 viable seeds) and if 500-1000 individuals are considered a sufficient population size of the regenerated population in reproductive age, then the estimated size of representatively sampled seed lots given as 5 kg per stand (population) seems safe. It provides a sufficiently large basis for the different types of selection that occur during storage and regeneration, and is also applicable from the point of view of practical storage capacities. Numbers of seeds or plants per hectare used for the establishment of the regenerated population will follow local forestry requirements and practices.

Storage of pollen

With modern freeze-drying techniques, tree pollen can be stored at a very low moisture content and at subfreezing temperatures. For regeneration purposes, however, this technique requires complementary female structures to enable use of the pollen for seed production. Strategies for the use of pollen, including sampling, storage and regeneration, in the conservation of Norway spruce genetic resources still need to be defined and implemented. New developments and further testing of experimental material can be expected in this area in the near future.

Storage of tissues for *in vitro* propagation

Storage of tissue cultures also has the potential to provide a secure static *ex situ* conservation method. The technique involves micropropagation of meristems, embryos, or other types of tissues. It requires large investments in development, but if advanced cryogenic storage is used it will offer a secure conservation method. Conservation through tissue culture is still in the experimental stages for most forest tree species.

Deep freezing in liquid nitrogen and similar techniques of cryopreservation hold promise for storing vegetative plant material and embryos of plants whose seeds are difficult to store in a desiccated state. Undifferentiated tissues, somatic embryos and organized meristems are suitable subjects to be kept safely for long periods of time. Cryogenic storage, the preservation of biological material suspended above or in liquid nitrogen at temperatures from –150 to –196°C, has been used for many years as a means of keeping animal semen for breeding purposes. This technology is relatively new to tree seed storage and hence the optimum moisture content levels, treatment conditions and time limits for storage of orthodox seeds still have to be determined. Cryogenic storage in the case of species with small seeds may be very cost-effective and the technology promises fewer concerns from the genetic viewpoint than conventional seed storage. There are many reports which describe the regeneration of plants from a proportion of explants after a brief period at –196°C, but few describe successful storage for longer intervals. Some success has been reported with the freeze preservation of callus cells, for instance in the case of *Prunus cerasus, Rubus idaeus* or hybrid poplar, *Populus euramericana.* Callus cells of *Picea glauca* stored one year in liquid nitrogen gave rise to whole spruce plantlets. Although a considerable number of experiments have investigated cryopreservation techniques, methods are not yet sufficiently reliable to be used widely for storing the genetic material of major woody plants including Norway spruce. New advances in this field still remain to be made.

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Glossary of terms

adaptation	The process of change in structure and / or function that makes an organism or a population better suited to survive in an environment. Adaptation may be achieved by phenotypic tuning to prevailing environmental conditions, or through evolutionary changes of genetic structure at the population level.
adaptability	⇒ evolutionary adaptability
adaptedness	The state of being adapted that allows a population to survive, reproduce and permanently exist in certain conditions of the environment.
autochthonous population	⇒ population
basic material	Trees from which reproductive material is obtained.
breeding (forest tree b.)	The application of genetic principles and practices to the development of individual trees, varieties or populations more suited for the human needs.
character	A distinctive phenotypic expression exhibited by all individuals of a group, capable of being described and measured and determined by the individual's genotype and environment.
clone	Group of individuals (ramets) derived originally from a single ancestor individual (ortet) by vegetative propagation (e.g. cuttings, grafts, layers) and thus having identical genetic constitution.
collecting	The activity of gathering or acquiring genetic materials (seeds, pollen, parts of plants, etc.) for addition to a gene conservation unit.
community	A naturally occurring group of various plant

(forest c.)	organisms that inhabit a common (forest) environment, interact with each other and that generally are independent of other groups.
conservation stand	⇒ gene conservation stand or population
cryopreservation	The preservation or storage of seeds and tissues at very low temperatures, usually in liquid nitrogen.
distribution area	The geographical occurrence and arrangement of a species, or a population; usually refers to the natural extension of the area occupied by a species.
ecosystem	The ecological complex of, e.g. a forest community, including the non-living components of the environment and functioning together as a stable system in which exchange of nutrients follows a circular path.
ecotype	The product of genetic adaptation within a species, to a particular habitat or environment, as a result of natural selection (also local race).
effective population size	In broad sense, the number of individuals in a population successfully involved in reproduction in a given generation.
evolutionary adaptability	The potential or ability of a population to adapt to changes in the environmental conditions through changes of its genetic structure.
genebank	Facility where genetic resources are stored in the form of seeds, pollen or tissue culture.
gene conserv-ation stand or population *in situ*	Forest stand in which appropriate management is carried out to ensure the conservation of genetic resources of target species.
gene conserv-ation stand or population *ex situ*	Population established with the specific objective of genetic conservation using basic material collected by random sampling in the target gene conservation unit.

gene conservation unit — A common term for all units in which genetic resources are maintained, including gene reserves, *in situ* and *ex situ* gene conservation stands or populations, seed lots stored in genebanks, clone collections, seed orchards and arboreta.

geneflow — The exchange of genetic material between populations due to the dispersal of gametes (through pollen) and zygotes (through seeds).

gene frequency — The frequency of the occurrence of alternative forms of genes (alleles) in relation to the frequency of all the alleles at a particular locus in a given population.

genepool — The sum of all genetic information encoded in genes and their alternative forms (alleles) present in a population at a given time.

gene reserve — ⇒ (*in situ*) gene conservation stand or population

gene reserve forest — One or a continuous complex of ⇒ *in situ* gene conservation stands or populations.

gene(tic) conservation — All activities including, e.g. collecting, maintenance, storage, management, protection and regeneration, aimed at ensuring the continued existence, evolution and availability of genetic resources; *in situ* and *ex situ*:

in situ — Conservation of genetic resources ’on site’, in the natural and original population, on the site formerly occupied by that population, or on the site where genetic resources of a particular population developed their distinctive properties. Although usually applied to stands regenerated naturally, the *in situ* conservation may include artificial regeneration whenever planting or sowing is done without conscious selection and in the same area where the reproductive material was collected.

ex situ — Conservation of genetic resources that entails

	removal of individuals or reproductive material from its site of natural (original) occurrence, i.e. conservation 'off site'.
genetic diversity	The measure of genetic variation present in a population as a consequence of its evolution.
genetic resources	The biological material containing useful genetic information of actual or potential value.
genetic variability	The ability of a population to produce individuals carrying different genetic variants (alleles, genes or genotypes); the capability of a population to generate genetic variation.
genetic variation	The occurrence of genetic variants (alleles, genes or genotypes). Genetic variation is brought about by a change in genes, as distinct from differences due to environmental factors.
genotype	Genetic constitution of an individual tree possessing a particular set of alleles (i.e. different forms of genes which may occupy the same position on a chromosome).
grafting	The joining together of parts of plants in such a way that they will unite and continue their growth as one plant.
heterozygous	The condition of having unlike alleles at corresponding loci (as opposed to homozygous – having identical alleles). An individual organism may be heterozygous or homozygous for one locus, more than one or all loci.
hybridization	The formation of a diploid organism, mostly by sexual reproduction between individuals of unlike genetic constitution.
inbreeding	The mating system in which mating events occur between individuals that are more closely related than average pairs chosen from the population at random.
inheritance	The transmission of genetic information from parents to progeny.

in vitro	Biological processes made to occur in isolation from the organism ('in glass').
mast year(s)	Years at certain intervals in which some plant species produce large crops of seeds as part of their biological and ecological strategy.
mating system	The system whereby individuals of opposite sexual type are paired to produce progeny.
multiple population (system)	The arrangement when two or more populations of sufficient size, originating from a single large resource population, are established over a broad array of environmental conditions, managed or unmanaged, with the purpose of integrating tree breeding and gene conservation.
open pollination	Natural, or random pollination, i.e. when the transfer of pollen from an anther to a stigma is freely exposed to geneflow.
origin	For an autochthonous stand of trees the place in which the trees are growing; for a non-autochthonous stand the place from which the seeds or plants were originally introduced.
orthodox seeds	Seeds that can be dried to a low moisture content (of around 5%) and successfully stored at low temperature for long periods.
outbreeding	The mating system in which mating events occur successfully between individuals that are less closely related than average pairs chosen from the population at random. It is the most common mode of sexual reproduction of forest trees.
phenotype	The observable (structural and functional) characters of an individual resulting from interaction of the genotype with the environment.
population	A (Mendelian) population is defined as a unit present under certain (environmental) conditions, composed of biological organisms which are able to reproduce sexually and where

	every pair of individuals is enabled and allowed to have common ancestry over generations.
autochthonous p.	A population which has been continuously regenerated by natural regeneration. The stand may be regenerated artificially from reproductive material collected in the same population or autochthonous populations within the close proximity.
base p.	The population of trees from which selection of reproductive material is made for the next generation of breeding.
breeding p.	A subset of trees from a base population that is selected for their desirable characters to serve as parents for the next generation of breeding.
production p.	A population used strictly to produce seeds or vegetative material for afforestation or reforestation purposes.
progeny	Offspring; descendants of a particular mating or of a particular mate.
provenance	The place in which any stand of trees is growing. The stand may be autochthonous or non-autochthonous (see ⇒ origin).
provenance trial	A well-designed field experiment aimed at the comparison of growth of population samples from a distribution area of a species, established in two or more environments.
recalcitrant seeds	Seeds that cannot be dried and therefore lose their viability if stored at subzero temperatures without some damage from freezing, even under conditions that are normally conducive to seed longevity.
regeneration	The process of rejuvenation of a gene conservation unit (individual tree, accession stored in a genebank, live collection, stand or population). In case of a population, regeneration

	can be natural (regeneration stock originates from matings in the respective population) or artificial.
region of provenance	The geographic area with sufficiently uniform ecological conditions on which are found stands of a species showing similar phenotypic and probably genetic characters.
reproduction	The process of forming new individuals of a species by sexual or asexual ways.
reproductive age	The age at which the tree produces its first flowers and seed crop.
reproductive material (forest r. m.)	Seeds (cones, fruits and seeds) and vegetative parts of trees intended for the production of plants as well as plants raised by means of seeds or vegetative part; also includes natural regeneration.
sampling	The selection of the populations and trees within the populations from which seeds or other material is collected.
scion	Aerial part of a plant that is grafted onto the root-bearing part (rootstock) of another plant.
seed lot	A specified quantity of seeds stored in genebanks, physically identifiable (usually referring to a population sample), in respect of which analyses are made and the International Analysis Certificate of ISTA may be issued.
seed moisture content	The proportion of the total weight of a seed sample contributed by water.
seed orchard	A plantation composed of clones (clonal seed orchard) or progenies (seedling seed orchard) from superior selected trees which is isolated or managed to avoid or to reduce pollination from outside sources, established and managed mainly for the production of frequent, abundant and easily harvested crops of seed.
seed source	Trees within an area (stand or seed orchard)

	from which seed is collected.
seed (collection) stand	A stand of trees superior to the accepted mean for the prevailing ecological conditions when judged by a standard set of phenotypic criteria and which may be treated for the production of seeds.
seed (collection) zone	Zone defined for seed-collection purposes, occupied by trees with relatively uniform genetic composition as determined by progeny testing various seed sources. The encompassed area is based on geographic bounds, climate and growing conditions (e.g. range of altitude) and usually refers to a definite administrative unit.
selection	Any process, natural or artificial, which permits a change in the genetic structure of populations in succeeding generations.
self pollination	Transfer of pollen to the stigma of the same flower or a female reproductive organs (stigmae, strobili) of the same genotype (tree).
sexual reproduction	Reproduction involving the union of gametes that are typically haploid and of two kinds (male and female).
stand (forest s.)	A population (natural or planted) of trees possessing sufficient uniformity in composition, constitution and arrangement to be distinguishable from adjacent populations 'Stand' is the conventional unit for forestry management and is used interchangeably with the term 'population' (see ⇒ population).
tissue culture	A cellular mass grown and maintained *in vitro* on a solid or liquid medium. Protoplasts, cells, tissues, organs, embryos, pollen or seeds may be grown in this manner.
viability	Characteristics of seeds referring to their capacity of germination under appropriate conditions.
wind pollination	Pollination by windborne pollen.