EUROPEAN FOREST GENETIC RESOURCES PROGRAMME (EUFORGEN)

Picea abies Network

Report of the second meeting
5-7 September 1996
Hyytiälä, Finland

J. Turok *and* **V. Koski,** *compilers*

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International Plant Genetic Resources Institute

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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

Participants from 17 countries attended the second *Picea abies* (Norway spruce) Network meeting held 5-7 September 1996 in Hyytiälä, Finland (see List of Participants). They were welcomed by Prof. Veikko Koski, the host and local organizer of the meeting. He mentioned the importance of boreal forest genetic resources and the various activities on their conservation and use.

Mr A. Uotila, Director of Hyytiälä Summer Center of the University of Helsinki then introduced this well-known educational facility in Finland to the participants. Foundation of the Center, which provides practical courses to students of forestry, agronomy and environmental sciences, dates back to 1910.

The basic principles of collaboration in EUFORGEN were briefly explained by J. Turok. He reported on the progress made in other networks, described current activities in the context of the Pan-European Process on Forests and mentioned the outcomes of the International Technical Conference on Plant Genetic Resources (held in Leipzig, Germany, in June 1996). He noted that the number of countries attending this Network meeting doubled in comparison with last year.

The agenda of the meeting was approved with amendments (see Agenda).

Participants from each country then gave updates on the progress made in national genetic conservation programmes on Norway spruce since the last meeting. The extent and level of activities developed in each country during this relatively short period of time differed greatly between the individual countries. The update reports from most countries are included in this volume.

France, Hungary, Italy, Sweden and Switzerland gave introductory country reports because of their attendance for the first time at the Network meeting (see this volume). The Russian Federation sent a country report but a representative was not able to attend the meeting. A summary of the report was read at the meeting.

Prof. T. Skrøppa presented a discussion paper asking about the consequences of recent research findings on adaptive traits and their possible non-Mendelian inheritance in Norway spruce (see this volume). Prof. C. Mátyás provided a report on the outcomes of the IUFRO Conference on diversity and adaptation in forest ecosystems in a changing world (held in Vancouver, Canada, in August 1996).

As a contribution to raising public awareness about Norway spruce genetic resources, the organizers invited journalists from several Finnish newspapers. Attention was given to the problems and needs of genetic conservation in forestry in Europe and the approaches chosen at a national level.

Technical guidelines for genetic conservation of Norway spruce

Following the objectives of the Network and according to the outcomes of the previous meeting, it was agreed to produce practical management guidelines often requested by forest officers and national or regional authorities responsible for gene conservation. The decision on this task was facilitated by the fact that Norway spruce has been identified as one of the key species in many national gene conservation programmes and activities. The comparatively good knowledge on the genetic structures and diversity within and among Norway spruce populations from the whole distribution area in Europe provided an excellent precondition for producing the guidelines.

It was noted that delays have occurred in the production schedule of the guidelines, partly because of the intensive interactions among the Network members. The participants felt it was necessary to re-discuss the guidelines for *in situ* conservation on the basis of the paper written by V. Koski, and the guidelines for *ex situ* conservation on the basis of the outlines prepared by T. Skrøppa and L. Paule. Three discussion groups were created. A list of issues was compiled that later assisted the authors of the Technical Guidelines in incorporating new suggestions and comments provided by the Network members.

The advanced draft version of the Guidelines was circulated once again and reviewed after this meeting (see Workplan). After incorporation of final comments and suggestions, the Guidelines are being distributed with this Report of the second Network meeting.

Collaboration with IUFRO working party on Norway spruce

The implementation process of Strasbourg Resolution 2 and later EUFORGEN have benefited greatly from the ongoing activities of several IUFRO (International Union of Forestry Research Organizations) working parties dealing with forest gene conservation aspects. The working party 2.02.11 Norway spruce breeding and genetic resources has been promoting research and activities at the scientific and technical levels for decades. The experience and positive impact of IUFRO for launching activities within the framework of EUFORGEN was particularly valuable in the case of the Norway spruce Network.

T. Skrøppa, Chair of the working party on Norway spruce breeding and genetic resources, gave an overview of current activities and plans for the near future. He informed about the next meeting to be held in September 1997. Regular updating of the database with results and data gained through the international provenance experiments, comprehensive evaluation and better use of the data, identification of research priorities and further promotion of international research activities, as well as the initiation of a monograph on genetics and breeding of Norway spruce, are among the most important tasks of the working party. A survey of a bibliography including older literature on research results in genetic diversity and genetic resources of Norway spruce is also suggested. There are numerous unpublished and unknown manuscripts in various countries that should be shared internationally and this task hence belongs to the priorities of the working party.

The complementarity of efforts made by the EUFORGEN Norway spruce Network and the IUFRO working party was stressed during the following discussion by the participants. Most of the participants are currently involved in both structures and these existing links should be maintained. Complementing the IUFRO working party's focus on promoting and coordinating research, EUFORGEN, besides its regionally restricted mandate, is mainly focused on the implementation of research results into forestry practice, encouraging the establishment of national programmes and strategies and raising public awareness. J. Turok mentioned that other EUFORGEN networks also include, to some extent, tasks related to facilitating research projects and collaboration and suggested that the experience of the IUFRO working party on Norway spruce breeding and genetic resources and the networks be exchanged for the benefit of all. The role of FAO in executing country-driven programmes and priorities and its focus on development was also stressed.

While it will be very important for all members of the EUFORGEN Norway spruce Network to have regular access to the information contained in the research database of IUFRO on Norway spruce, one of the immediate Network tasks complementary to the research database is to collect and evaluate data about populations (collections) designated for gene conservation in European countries. This is an important task in monitoring the progress made for the implementation of Strasbourg Resolution 2.

Development of the database on gene conservation populations (collections)

To set up the 'monitoring' database, it was agreed at the last Network meeting that a list of descriptors be developed and adopted first. Each country would then have a database with 'passport' data of populations (collections) designated for both *in situ* and *ex situ* conservation of genetic resources using the minimum descriptors agreed upon by the Network. A European database would provide linkage among the national databases and would be hosted by an interested institute. Most countries will have their databases specifically designed for their own needs, containing more detailed information, but using the common set of minimum descriptors.

The list of descriptors was prepared by the Network during the period since the last meeting. T. Skrøppa compiled the list with input provided by Network members and by IPGRI. The document was presented and endorsed at the meeting (final version in this volume).

As no host institute for the European database had been identified since the last meeting, J. Turok encouraged participants of this second Network meeting to take action for the implementation of this important endeavour. Latvia and Czech Republic offered to take the responsibility for the coordination of this task of the Workplan.

Broadening of the scope of the Network

It was agreed that Norway spruce serves as a model species for other boreal, wind-pollinated conifers with wide distribution areas and that the decision on including further species should be postponed until the next Network meeting. A questionnaire will be prepared and circulated in 1997 in order to assess in a representative way the needs and priorities of each participating country for further action.

Workplan

Based on the Network objectives and the workplan developed at the first meeting, and according to the discussions at the second meeting, it was agreed that the following collaborative activities be carried out, listed in order of priority.

1. Information exchange on the country activities and Report of the meeting

It was agreed that introductory country reports from France, Hungary, Italy and Sweden, presented at the meeting, should be sent to J. Turok before **15 October 1996**. The contributions should be 5-10 single spaced pages in length and should be sent on a diskette. The introductory country reports will be included in the final Report of the meeting, together with the updates on the progress of genetic conservation activities in countries since the last meeting and the list of descriptors. J. Turok will prepare the Report for publication **by the end of the year**. It will be combined with publication of the Technical Guidelines for Norway Spruce Genetic Resources Conservation.

2. Technical Guidelines for Norway Spruce Genetic Resources Conservation

It was agreed that:

- All participants send suggestions, additional comments and documents or references on the respective chapters (as agreed) to V. Koski, T. Skrøppa and L. Paule before **10 October 1996**.
- V. Koski, T. Skrøppa and L. Paule incorporate new information and send their manuscripts to J. Turok by **31 October 1996**.
- J. Turok prepare and circulate draft Technical Guidelines with the received three contributions in standard format to all Network members and participants, contact persons for Norway spruce in other countries, chairs of all EUFORGEN Networks and to FAO Forestry Department by **5 November 1996**.
- Introduction to the Technical Guidelines be written by J. Turok and circulated along with all three contributions in standard format by **5 November 1996**.
- Ensuring that comments will have been received by **10 December 1996**, J. Turok will consult with V. Koski, T. Skrøppa and L. Paule, prepare final draft, ensure attractive layout and fast printing and distribution schedule of the Technical Guidelines by IPGRI **by the end of this year**.

The comments provided by each Network member will need interaction at a country level with all relevant institutions, scientists, forest officers and especially with managers responsible for gene conservation. It is important that the potential users of the manual have the opportunity to provide input to the development of the Technical Guidelines.

3. Development of the database on gene conservation populations (collections)

The participants agreed to set up a database for monitoring the progress made in gene conservation activities in all countries and thus for the implementation of Strasbourg Resolution 2. Further to the offer of Latvia and the Czech Republic to host the database as contribution in kind to the Programme and to the Norway spruce Network in particular, J. Turok will interact with J. Birgelis and K. Vancura, respectively, consult with V. Koski, chair of the Network, apply the experience of other networks in similar initiatives and circulate a proposal for concrete action including time frame to set up the database to all Network members and participants not later than **1 March 1997**.

4. Clarification of the terminology used by the Network

It was agreed that the list of terminology used by the Network would be included in the Technical Guidelines as an annex. J. Turok will compile the list of terminology on the basis of manuscripts received from V. Koski, T. Skrøppa and L. Paule before **31 October 1996**. The list will be circulated along with the draft Technical Guidelines by **5 November 1996**.

5. Mailing

Before **10 December 1996** each Network member will indicate the preferred type of distribution of Network publications (from IPGRI to the respective Network member for further distribution within a country; or direct from IPGRI to a list of selected organizations within a country) and the number of requested copies with particular mention of the Technical Guidelines.

6. Questionnaire on the scope of the Network

It was agreed that J. Turok circulate a questionnaire asking for opinions and suggestions on further scope, tasks and activities of the Network to all attending and corresponding members **by 31 March 1997**.

Conclusion

The tasks of the Workplan were discussed and amendments were incorporated.

J. Turok thanked V. Koski on behalf of all participants for overall organization of the meeting in a very pleasant working atmosphere.

K. Vancura from the Czech Republic offered to host the next Network meeting in March 1998.

Overview of country activities

Good information flow among countries is one of the prerequisites for genetic resources programmes that work effectively at a national level. This is why the Network has given great attention to the presentations and discussions of the reports from individual countries at its first two meetings. Participants from 18 countries altogether have so far reported on their Norway spruce genetic resources. The descriptions of countries' experiences with conservation and use, including issues related to the priority-setting process, threats, breeding, applied conservation methods, research, public awareness, constraints and future perspectives, offered very valuable reference material to a wide forest genetic resources community. The Network members will be updating this information regularly in the future.

It was also recognized and agreed that more focused information on gene conservation populations/collections should be compiled (see Development of the database, Workplan and Descriptors). Such information will be stored in the 'monitoring' database, hosted by Latvia and the Czech Republic, and linking a network of national databases.

Table 1 is an attempt to provide a summary overview of the ongoing gene conservation activities on Norway spruce in European countries. Of course, detailed figures will be provided as one of the possible outputs of the new database in the future.

The data presented in Table 1 were taken mainly from the national reports provided by the Network members. They have in some cases a probably unbalanced character and further discussion will be needed to complete this overview. For instance, the area of *in situ* populations includes only units specifically designated and managed for gene conservation purposes. The differences due to individual concepts (buffer zones around gene conservation stands in some countries) have, likewise, not been considered. Neither stands approved for seed collection nor any type of Norway spruce nature protection reserves have been included. The number of gene conservation populations and their respective areas could be useful additional information.

The term 'accession' under Seed storage includes all entries of a genebank (bulked seed lots from populations as well as seed samples collected from individual trees).

Table 1. Overview of the existing conservation activities for Norway spruce in European countries by methods (as in November 1996)

Country (area covered by Norway spruce, in ha)		*In situ* populations (total area, in ha)	*Ex situ* Conservation populations (area, ha)	Breeding units (area in ha or no.of clones) Seed orchards	Clonal archives	Seed storage: accessions (kg)
Austria	(1 870 000)	4150	–	–	–	15 accessions (318 kg)
Belgium	(200 000)	230	–	10 ha	880 clones	21 kg
Czech Rep.	(1 415 000)	–	1580	67 ha	150 clones	–
Denmark	(135 000)	–	–	600 clones	800 clones	–
Finland	(5 110 000)	1245	–	–	1600 clones	–
France	(730 000)	8550	–	80 ha	2000 clones	700 accessions
Germany†	(3 800 000)	913	416	95 ha (1600 clones)	2780 clones	413 accessions
Italy	(380 500)	3872	–	–	–	23 accessions
Latvia	(530 000)	700	90	170 ha	–	15 accessions
Lithuania	(450 000)	796	–	340 ha	16 ha	–
Norway	(3 500 000)	–	–	3000 clones	–	–
Poland	(1 940 000)	1800	620	500 clones	80 clones	50 accessions
Russia‡	(75 866 000)	67692	–	1910 ha (6072 clones)	672 clones	–
Slovakia	(520 000)	3150	80	7 ha	–	75 accessions (364 kg)
Sweden	(5 130 000)	206§	–	–	13 ha	–
Switzerland	(493 000)	700	–	–	–	–
Ukraine	(570 500)	2770	–	14 ha	2 ha	–

– no data provided.

† Germany: all data as of 1 January 1996. Only population seed lots are included under Seed storage.

‡ Figures for the Russian Federation as given by Routkowsky *et al.* (this volume) are related to the whole territory of the country.

§ The total area of gene conservation stands in Sweden, without distinction between units of the category *in situ* and *ex situ.*

Descriptors for Norway spruce genetic resources

1. Country where maintained
Name of country or three-letter official country abbreviation (ISO code).

2. Registration number
A national number identifying the genetic resource. As most countries have different systems for different institutions and/or type of units, the registration number will consist of two parts, the first one identifying the national system and the second the code number within the system.

3. Name of genetic resource
3.1 The local name under which the genetic resource is known and name of locality or municipality.
3.2 Forest region or district, where applicable.

4. Geographic location of site
4.1 Latitude
Degrees (°) and minutes (') followed by N or S.
4.2 Longitude
Degrees (°) and minutes (') followed by E or W.
4.3 Elevation
Lower altitude of site (m above sea level).
Upper altitude of site (m above sea level).

The geographic parameters refer to the site where the genetic resource is growing in the wild (*in situ* and *ex situ*), or is stored.

5. Responsible institution and ownership
5.1 Name of institution responsible for identification and maintenance, and contact address.
5.2 Ownership: forest owner, institution or ministry.

6. Type and function of genetic resource
Categories are:
- *in situ* natural population(s) or plantation:
  - nature reserve
  - national park
  - protected area
  - gene reserve forest/ stand/ population
- *ex situ* plantation:
  - one seed origin (known or unknown)
  - several seed origins, mixed
  - several seed origins, replicated, identity kept (provenance trial)
  - several families, replicated, identity kept (progeny trial)
  - several clones, replicated, identity kept (clonal trial)
- *ex situ* collection:
  - clonal archive
  - seed orchard

- seed bank
- pollen bank
- tissue bank.

7. Site information

7.1 Total area of genetic resource in hectares, with one decimal if smaller area.
7.2 Proportion of area covered by major tree species.
7.3 Ecological zone/ecoregion.
7.4 Climatic zone.
7.5 Closest or most representative meteorological station.

8. Genetic evaluations

Have the genetic resources been evaluated genetically? If "yes" specify type of evaluation: provenance, progeny or clonal test, biochemical or molecular characterizations.

9. Date of approval

Date on which the genetic resource was approved (in the format DDMMYYYY).

Remarks

Several objectives are present in the conservation of the genetic resources of a 'semi-domesticated' forest tree species like Norway spruce and different methods will have to be used. It is not possible to identify a set of common descriptors that are meaningful or can be used for all types of genetic resources. In each specific case, only a subset of the descriptors may be applicable. Similarly, it is not easy to define general terms that have the same precise definition in all situations. The term genetic resource is here used as a collective term to denote all collections that are managed with some gene conservation objective in mind, e.g. natural populations, plantations, clone banks, seed or pollen collections (see Technical Guidelines). The descriptors proposed here are intended only for such collections and not for individual families, genotypes, individual seed or pollen lots.

Only a few of the proposed descriptors are coded into a specific format. No attempts are made to produce a standardized coding system of all descriptors.

Updates on the progress of activities in countries

Genetic inventories continued in Austria

Thomas Geburek
Federal Forest Research Centre FBVA, Vienna, Austria

In 1993, a genetic inventory programme on Norway spruce was launched in Austria. Within this project, investigations of the genetic structures were regarded as essential to furnish criteria for declaration and management of genetic resources. Data on the geographic differentiation based on gene markers within the natural range were considered as important to determine spatial distribution of gene reserves.

Within the natural range of Norway spruce, 29 presumably autochthonous populations were selected in high altitudes. In each population, 100 adult specimens were sampled. All trees were genotyped at isoenzyme gene loci by using bud tissue. Genetic parameters were estimated by employing different computer programmes, such as GSED, BIOSYS and others.

A detailed description of the results will be given elsewhere. Here only the main results are summarized:

- The overall allozyme variation of Norway spruce in Austrian populations growing in high altitudes is low compared with populations outside the Alps.
- Distribution patterns of genetic variation in Austria can be estimated by the genetic differentiation measures. These parameters can vary between 0 (genetically identical demes) and 1 (not genetically identical). Among the populations studied, genetic differentiation was very small and ranged from 0.018 to 0.040. It is noteworthy that native forests were not characterized by extraordinary values.
- A certain gene encoding the glutamate·dehydrogenases was nearly fixed. Since outside the Alps polymorphisms at this gene locus are present, this may be regarded as additional evidence that the populations studied are most likely of autochthonous origin.
- The hypothetical gametic multilocus diversity has been accepted as an essential measure to characterize the ability of forest tree populations to adapt to changing environments. It is helpful for the selection of populations for conservation purposes. This measure varied between 18 and 49. The overall G_{ST} value was low, amounting to roughly 2%.
- A significant, however low, correlation between geographic and genetic distance was observed.
- Multivariant analysis could attribute 7% of the genetic variation to geographic substructure. However, even when this analysis was employed, no regional structure could be traced back.

Studies of the material tested in growth chambers should furnish additional information. This will, besides the studies of genetic variation at marker loci, enable us to analyze quantitative traits in order to provide assistance for better management of gene resource populations.

Belgium launches a series of activities

Alphonse Nanson
Station de Recherches Forestières, Gembloux, Belgium

A brief overview is given of gene conservation activities in Norway spruce in Belgium from March 1995 until October 1996. A survey of the situation in the conservation of Norway spruce genetic resources in Belgium was comprehensively presented at the first meeting of the EUFORGEN Network (Nanson 1995). Since that time some important developments took place in Belgium which should be mentioned here.

New Forest Tree Seed Centre

A Regional Forest Tree Seed Centre called Comptoir Wallon des Matériels Forestiers de Reproduction in Marche (Wallonia) was built and was officially opened on 16 February 1996. This facility, partly financed by the European Union, will contribute to the "Environmental Plan for the Sustainable Development in the Walloon Region" (Lutgen 1995).

The mission of this Forest Tree Seed Centre is mainly:

- to provide Wallonia, Belgium and other European countries with outstanding forest reproductive material, notably coming from the regional breeding programmes
- to maintain and conserve various *in situ* gene conservation units
- to participate in the *ex situ* conservation through Conservation Plantations of both phenotypically outstanding and most endangered populations.

Therefore, besides increasing productivity, quality, resistance and the adaptation potential of our forests, this Seed Centre should play a major role in genetic conservation.

A detailed description of the Forest Tree Seed Centre, its first seed collections and the history of forest tree seed procurement in Belgium is given in Nanson and Servais (1996).

In Norway spruce, 450 kg of seeds was collected in November 1995, exclusively from 10 seed stands in Belgium. The details are given in the Box, as further terms of reference.

We are hoping to use a systematic approach and thus extend such descriptions to all basic materials that once existed in Belgium. This is considered of great importance for gene conservation and tree breeding purposes.

The collection carried out for Norway spruce to a large extent has been sold to private and state-owned nurseries, the rest being stored for years with less fructification.

Assuming (at least) 11 ha of plantation per kg of seed for Norway spruce, the seed already collected corresponds to 450 kg x 11 = 4950 ha of Norway spruce plantations. A part of them should become 'regenerated conservation plantations' in order to save the genepool of these outstanding but old and hence endangered stands.

The trace of a part of these plantations, made with very often outstanding but endangered material, should be followed as mentioned in Nanson and Servais (1996), put into a database and conserved for future generations.

B006A GILBUSCHHECK (Long. 6°19'; Lat. 50°21'; 555 m; Bullange)[1, 2]. IVME[3] ~16.3; overall quotation: Good to Very good: 8.3/10. This stand, planted in 1864 by the Prussian Forest Service, is very probably the same as the famous provenance "Bullange" compared in the first international Norway Spruce provenance experiment of 1937/38. It took part also, this time without any doubt, in the 2nd international Norway Spruce provenance experiment of 1964/68 and is present in many other Belgian provenance experiments. It is a good provenance for production, though a bit early flushing. Its origin is unknown but probably from Germany or Poland.
B0271A DIEPBACH (340 m; Eupen). IVME = 16.9 m^3 ha^{-1} $year^{-1}$; overall quotation: Good: 8/10.
B0031A CEDROGNE (550 m; planted in 1890-1900; Vielsalm)[2]: considered as "Elite" for growth but rather early flushing. It also has been tested in Germany where it has very good results for growth.
B0220A GROSSY (420 m; Libin). IVME = 18.1 m^3 ha^{-1} $year^{-1}$; overall quotation: Good: 8/10.
B0020A CHAULETTE-GROSSY (420 m; Libin). IVME = 17.6 m^3 ha^{-1} $year^{-1}$; overall quotation: Good to Very Good: 8.3/10.
B0221A FONTAINE MAHET (465 m; Libin). IVME = 18.7 m^3 ha^{-1} $year^{-1}$; overall quotation: Good to Rather Good: 7.7/10.
B0222A CONTRANHEZ (425 m; Libin). IVME = 18.5 m^3 ha^{-1} $year^{-1}$; overall quotation: Good: 8/10.
B0226A BOIS JOURNAL (440 m; Nassogne). IVME = 16.3 m^3 ha^{-1} $year^{-1}$; overall quotation: Good: 8/10.
B0045A LAVAUX (420 m; planted in 1895; Neufchâteau)[2]. IVME = 19.0 m^3 ha^{-1} $year^{-1}$; overall quotation: Very Good: 9/10. With BAYAI[2] (Long. 49°46'; Lat. 5°28'; 400 m; planted in 1885; Neufchâteau), with around the same characteristics, and that unfortunately was blown down by the terrific storms of January-February 1990, LAVAUX is one of the best "Elite Seed Stands" in Belgium, for growth as well for late flushing. According to archives examined by Wauthoz[4], the origin of these stands, ex-property of Graf von Arenberg (Germany), seems to be "Thüringer Wald" where the family owned large forest tracts of Norway spruce and where a large cone kiln was settled.
B0230A COTE DES FORGES (400 m; Neufchâteau). IVME = 15.0 m^3 ha^{-1} $year^{-1}$; overall quotation: Good: 8/10.

[1] Official number and name of Belgian seed stands: B = Belgium; 006 = number of seed stands; A = *Picea abies*; 555 m = altitude; Bullange = Forest district.

[2] Many Plus trees of these stands are fortunately grafted and conserved in our 'Evolving Seed Orchard' of Fenffe (nr B0551A). Presently, around 150 of these most valuable trees are conserved in this seed orchard. Many are also conserved as open-pollinated progenies in progeny tests in the Belgian Ardenne.

[3] IVME = Maximum Annual Increment in Total Production according to Forestry Commission Tables - 10%. This IVME is usually reached in Belgium around 60 years, and then it tends to decrease slowly. Most of these stands with evaluation of IVME have been selected by Nanson.

[4] This author, Inspecteur des Eaux et Forêts, also published a well-known paper on production tables and rate of return of Norway spruce plantations in Belgium (Wauthoz 1955).

Measurements and analyses of the third international provenance experiment

The third international experiment with provenances of Norway spruce was initiated by Tyszkiewicz in 1972. It comprises 39 provenances which were planted in a trial in Belgium, in April 1979. The seedlings used for the establishment of the trial had been raised in a nursery in Groenendaal (sown in April 1974). The trial was established on a representative, acid soil type, in four randomized blocks design (lat. 50°09' N; long. 5°34' E; altitude 390 m). The site is located near Laroche in the centre of the Belgian Ardenne.

Evaluations have recently been completed for a student's thesis at the Faculté des Sciences agronomiques in Gembloux (Desteucq 1996).

Our field test contains 17 of the 20 basic Polish provenances distributed and additional four Polish provenances provided by Giertych. Furthermore, we have added some best-performing Norway spruce provenances from the whole of Europe (Belgium, France, Germany, Romania).

First results show the outstanding growth of some Polish provenances from the Istebna region (Beskidy, southern Poland) but Belgian ones as well as Westerhof (Germany) also are good. These results will be presented in detail, in collaboration with Polish colleagues, at the next IUFRO Symposium in Slovakia/Poland, in September 1997.

Similarly, the first international provenance experiment was measured and analyzed by another student in Belgium (Verstraete 1993). So, only the trials of the second international provenance experiment (1964/68, by Langlet and Krutzsch) still have to be assessed in the near future.

Besides their primary role as comparative experiments, provenance experiments constitute a gene reservoir providing genes for further breeding programmes. We hope to use some of the best material from certain provenances from these experiments for practical purposes in the future.

Provenance experiments can also serve as *ex situ* gene conservation units, and thus help to restore the original genepool of vanished provenances. For example, scions of the provenance Kolonowskie from Poland were sent from the second international provenance experiment (1964/68) in Bertrix (Belgium), to colleagues in Poland, in 1980, in order to restore the genepool of this valuable provenance that had disappeared by accidental clear-cutting in Poland.

Databases

We are involved in the development of a database system including, for the first time, conservation plantations and seed stands. This has been developed for Douglas fir but could be transferred readily to Norway spruce.

Other units will also be included (seed orchards, clonal archives, seed lots stored), within the EU/FAIR/EURIDEC (FAIR-CT95-0909) contract, including diverse countries of Europe and extending from 1996 to 1999.

In a third phase, this could be extended to other species that could serve different specific needs of forestry practice.

The general plan for further conservation will follow the topics discussed in our previous paper (Nanson 1995):

1. Construction and management of databases
2. Trace keeping in forest management
3. Long-term management of forest genetic resources.

However, it will be necessary to recruit an adequate scientist to fulfil this task.

Conclusions

Since the last survey on genetic conservation of Norway spruce in Belgium, some new events have occurred. A new Forest Tree Seed Centre was opened in Marche (Wallonia). Its primary role is to provide outstanding forest reproductive materials for afforestation in Wallonia, Belgium and Europe. Its other roles are to maintain and conserve *in situ* basic materials as seed stands, etc., as well as to participate in the network of *ex situ* conservation plantations of the most valuable and most endangered materials. The third international experimental trial on provenances of Norway spruce has been measured and will be conserved as a gene reservoir for further breeding. Finally, because of staff problems, databases foreseen for Norway spruce will be delayed. However, the general objectives of genetic conservation of Norway spruce in Belgium described in Nanson (1995) should be maintained in the long run.

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Czech Republic: Unfavourable climatic situation affects Norway spruce genetic resources

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Norway spruce originally represented only 15% in the natural forest tree species composition in the territory of the present Czech Republic. This small occurrence of spruce was limited to forest stands in the 5th forest vegetation zone (FVZ) and in higher elevations upwards (see Vancura 1995). The increase of spruce and other coniferous tree species has been due to the fashionable planting of pine and spruce since the end of 18th century up to today.

Negative consequences of this trend are the vast even-aged monocultures of coniferous species, mainly Norway spruce (55%), which constitute about 78% of the total forest area:

Actual area of Norway spruce	1 413 893 ha	(54.7%)
Total conifers	2 005 290 ha	(77.6%)
Total forest tree species	2 542 218 ha	(100%)

This fact made it possible to increase considerably the economic benefits in the period mentioned but in the long run it led to weakening of the forests and to the reduction of the forest soil quality due to its impoverishment. However, the extent of conifers in afforestation was increasing even though these negative consequences were known for many years. Norway spruce now represents as much as 50% of all seedlings produced in nurseries throughout the country – even in areas where spruce is not part of the natural forest ecosystems.

The new state forest policy adopted in the Czech Republic recommends that forest owners plant more broadleaves. Substantial modification of tree species composition is necessary for increasing stability of forest ecosystems, timber production and sustainability of other forest functions. Increasing the proportion of broadleaves to the detriment of Norway spruce is the main current forestry issue in the Czech Republic. The proportion of Norway spruce should decrease by 37%, which is about 18% difference in comparison with its present share.

Present situation

The health of Norway spruce stands still evokes serious worry, even though forest monitoring shows improvement of health conditions for the first time in a decade (based on Landsat data assessment).

Weather conditions play an important role. Extraordinarily dry and hot weather in 1996 (mainly in spring time, after the frostless winter) helped harmful insects to propagate extensively. Fortunately, this year brought a lot of precipitation and winter weather was closer to the long-term norm. But weather conditions in the last month of the year brought a new catastrophe (see Fig. 1). In the southeastern part of the Bohemian-Moravian Uplands and mainly in Krušné hory (Ore Mountains), in synergy with industrial emissions, the frequent icing destroyed thousands of cubic meters of timber. Hectares of red trees have been observed since February 1996 and the situation was called "the 2nd wave of forest dying".

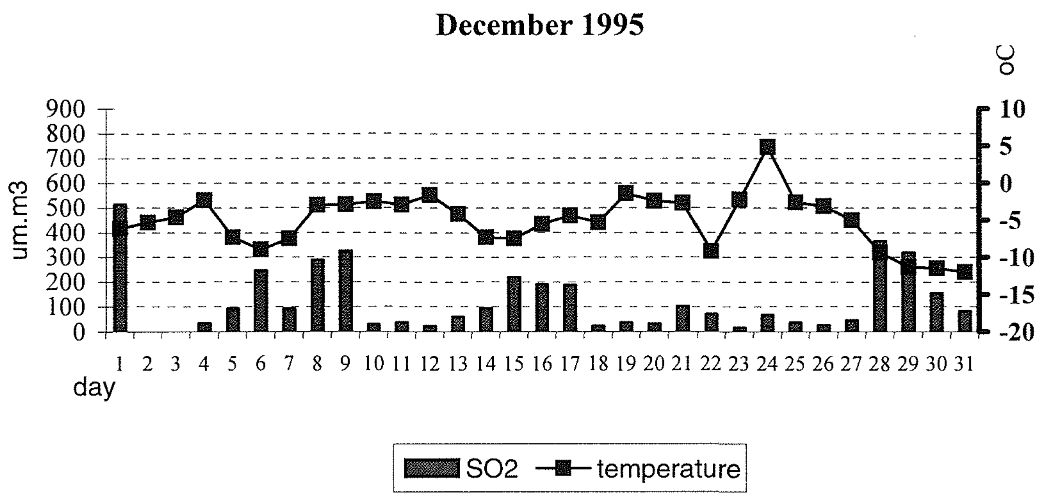

Fig. 1. Effect of stress factors (icing in relation to emissions and frost) on Norway spruce.

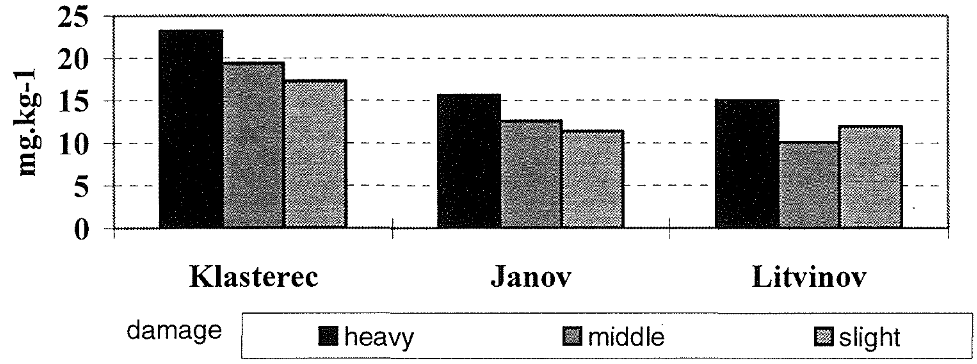

Fig. 2. Average content of fluorine in needle samples of Norway spruce.

The health development of Ore Mts. spruce stands had been promising with trees usually having needles for 4 years. But after the 1995/96 winter period most of the trees on vast areas did not regenerate. Substantial amounts of fluorine and chlorine were found in tree needles analyzed (Fig. 2). The emission load was three times higher on exposed localities, due to an unusual southeast wind. Forestry practice had to cope with a mass outbreak of nun moth (*Lymantria monacha*) in 1995 when abut 22 000 ha of spruce forest stands were infested. Together 19 771 ha of forests were under aerial treatment. A further consequence of the preceding dry seasons is continuous infestation of bark beetles, mainly in the Morava region. Total quantity of wood attacked by bark beetles (*Ips typographus* and *Pitiogenes chalcographus*) was 1 912 711 m^3. Nordic bark beetle (*Ips duplicatus* Sahlberg) infested 101 151m^3 and has become a significant pest in northern parts of Morava. There is also evidence that this species was dispersed in the area as a result of timber imports. The danger of bark beetles is enormous and damaged trees, e.g. in the Ore Mountains, will not be removed for at least 5 years. Bark beetle problems exist in the area of National Park Šumava where more than 60 000 m^3 of infested timber have been cut down since the beginning of this year.

Overview of current gene conservation measures and activities

Basic means for gene resources conservation:

		1994	1995
Gene bases:	number	135	174
	area (ha)	66 889	68 623.83
Certified forest stands approved for seed collection (ha)		103 707	106 466.72
Selected trees		3059	3059
Seed orchards (ha)		67	67
Seed plantations ('seed stands') (ha)		2 338	2 301.04

Storage of seed material in the Seed Production Plant (in Týniště n.Orl.):

	1994	1995
Norway spruce – raw seed material (kg)	230 151	10 460
Pure seed (kg)	50 300	46 810

Pure seed is stored in climatized store at 0-4°C and 75-85% relative humidity. It is stored in polythene bags inside metal or paper barrels. Moisture of seeds varies from 5 to 6%, decrease of germination is about 1-2% per year. There are enough Norway spruce seeds, so collection of this species will be minimal this year.

Reforestation (ha):

	1989	1990	1991	1992	1993	1994	1995
Total	35 016	33 615	31 516	29 600	27 698	26 897	30 128
Total conifers	28 342	27 577	26 398	24 330	22 570	20 478	21 861
Norway spruce	20 121	9 467	18 646	17 212	15 878	14 245	15 072

Changing legislation

Act No. 289/95 on Forests and Amendments to some Acts (Forest Act) were adopted in November 1995. Valid since 1 January 1996, it respects the elementary rights of forest landowners, emphasizes concern of the State for the fulfilment of all forest functions and preservation of forests. The Forest Act creates a legislative framework for fulfilment of European processes oriented to the principles of natural forest management, sustainability and biodiversity conservation. Respective decrees were prepared and adopted in spring/summer 1996.

Genetic resources are referred to in several paragraphs; such as Certified Forest Stands and Plus Trees, Handling of Seeds and Plants of Forest Tree Species, Regeneration and Tending of Forest Stands. The Forestry and Game Management Research Institute is considered a legal entity entrusted with monitoring of plus trees and forest stands approved for seed collection and other means of reproduction and with the preparation of expert opinions as well. There are also special requirements for the issue of licences, e.g. for collecting and handling reproductive material, but it seems that special supervision in this matter will be needed.

New research

A new laboratory for isoenzyme analyses is available for improving quality of present breeding programmes, monitoring and preservation of sufficient genetic variability and for further genetic and breeding activities. The Reproductive Resources Department has a long-term mandate focused on three main areas: seed science and testing, certification and registration of sources of forest reproductive material, and clonal archives.

The Biotechnology lab presently studies somatic embryogenesis, i.e. the process of differentiation of haploid and diploid cells through characteristic stages to complete plants. Despite, certain problems with clonal multiplication of coniferous species, in general this method seems to have good potential. Further research in this direction could standardize artificial seed preparation and the use of somatic embryogenesis for clonal multiplication of conifers. New embryogenic cultures were founded in August 1996.

Needs and proposals

Owing to the catastrophic development of forests in several regions during the winter 1995/96, it is recommended to continue with the research in fields which seemed to be already solved. There is no doubt that studies on forest genetics and breeding need to be a part of continuous, long-term forestry research. Besides the basic research tasks, evaluation of known provenances of Norway spruce, their conservation and use, multiplication techniques could be mentioned in connection with the situation in the Krušné hory Mountains.

Solutions to the main general problems of forestry (air pollution, damage by game (grazing), re-privatization of forest land), cooperation links (e.g. foresters/environmentalists) in respective fields and creation of a coordinating structure on genetic resources at a national level are of greatest importance, not forgetting about education and public awareness as well.

Support to international activities is also important. Connected with this item it should be mentioned that in the preparation of the FAO Technical Conference on Genetic Resources (Leipzig, June 1996) a lot of preparatory work was requested from each country and done consequently, and thus it is not very satisfactory to see that the conference *a priori* excluded the mention of forest genetic resources from the global plan of action. The activities and support on a national level in this very important area also need internationally based commitments.

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Research and further development of conservation and use in Germany

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Threats to Norway spruce genetic resources

In 1995, the annual evaluation of forest decline showed a slight decrease of the percentage of evidently damaged (loss of more than 25% of needles) Norway spruce trees over all age classes: from 24% in 1994 to 21% in 1995. As in the past, there was a significant difference in the level of damage between stands older than 60 years (36% of trees with visible damage) and stands younger than 60 years (8% of trees with visible damage). The distribution of damages remained regionally different with clear differences between northwest and east Germany. Nevertheless, a decrease of the level of damage could be observed, especially in eastern Germany. One reason for this positive development can be found in the significant change in the emission situation of air pollutants in eastern Germany since 1989.

Despite this positive development, in spring 1996 intense needle discolouration could be observed extensively in Norway spruce stands located in the central and eastern part of the Ore Mountains on an area of about 50 000 ha. All age classes were similarly affected. The extensive decline of Norway spruce stands can now be expected on about 3000 ha.

The main reason for this enormous damage was the combination of extreme winter climatic conditions (1995/96) with an unusually stable and long-term impact of air masses containing high amounts of sulphur dioxide and nitric oxide derived from the industrial area of northern Bohemia. The long-term impact was caused by frequent fogs and temporary stable weather conditions in combination with southeast to southwest winds. According to the results from a permanent monitoring plot in the affected area, acid concentration in the precipitation was about 10 times higher in the winter of 1995/96 than in 1994/95. In the winter period 1995/96, a decrease of the pH values down to 2.5 could be observed in some weekly samples.

Current status of gene conservation activities

In 1995, a report on gene conservation activities related to Norway spruce in Germany was given by Wolf (1995) including the activities until the end of 1993. The biannual activities report of the federal and state working group "Conservation of Forest Genetic Resources" for the period 1994 and 1995 was published by the Forstliche Versuchsanstalt Rheinland-Pfalz in 1996. Apart from the regular approval of Norway spruce stands for the production of selected reproductive material, a multitude of *in situ* conservation stands and the establishment of *ex situ* conservation stands and seed orchards were carried out (Tables 1, 2, 3). In 1994 and 1995, no seed collection for gene conservation purposes was reported. The propagation for conservation purposes was characterized by an increase of the number of stands sown and a significant decrease of the number of individuals propagated by cuttings.

At the end of December 1995, 315 Norway spruce stands with a total area of 913.3 ha were approved as gene conservation stands. The average area of 2.9 ha per stand seems very little compared with what forest geneticists consider the optimal size of conservation units. Nevertheless, the average size mentioned above is the actual situation in forest ecosystems in Germany which were heavily influenced by human actions. Large, continuous, autochthonous Norway spruce forests are very rare. Therefore it is necessary to conserve small-sized autochthonous Norway spruce forest stands *in situ* whenever possible.

Table 1. Conservation activities in the field

Activity		1994/95	Total (at 31 Dec 1995)
In situ			
Stands:	number	260	315
	area (ha)	766.5	913.3
Trees:	number	47	1675
Ex situ			
Stands:	number	19	402
	area (ha)	51.9	416.1
Seed orchards:	number	6	31
	area (ha)	12.9	95.0
	number of clones	180	1608
Clone collections:	number	1	13
	number of clones	154	2783

Table 2. Collecting and storage of conservation material

Activity		1994/95	Total (at 31 Dec 1995)
Seed storage			
Stands/seed orchards:	number	118	413
	amount (kg)	171.4	613.4
Trees:	number	110	3548
	amount (kg)	38.4	437.5
Pollen storage:	number	–	374
	amount (ccm)	–	5784.7

Table 3. Propagation for conservation purposes in 1994/95

Activity		1994/95
Sowing stands:	number	42
	amount (kg)	2.03
Planting trees:	number	9
	amount (kg)	0.09
Graftings:	number	360
	number of clones	70
Cuttings:	number	82040
	number of clones	4102
In vitro propagation:	number	1100
	number of clones	47

Research activities

In the period 1994/95, genetic variation in 54 stands (with samples of 50 up to 80 trees per stand) and an additional 118 single trees were investigated by biochemical methods. There was no report on physiological and morphological studies related to Norway spruce. In 1995, a practical guide on the separation methods and zymogramme evaluation "Isozyme Investigations on Norway Spruce (*Picea abies* [L.] Karst.) and Silver Fir (*Abies alba* Mill)" was published (Konnert 1995). Copies of the manual can be received from the Bayerische Landesanstalt für forstliche Saat-und Pflanzenzucht, Forstamtsplatz 1, 83317 Teisendorf, Germany.

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Outbreak of bark beetles in Lithuania

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The comprehensive report presented by Danusevicius and Gabrilavicius (1995) on Norway spruce genetic resources in Lithuania contained detailed information on the inventories, use and conservation, and theoretical and practical research results.

Since the last meeting of the EUFORGEN Network, the status of Lithuanian spruce forests has changed considerably. For the last 2 years, middle-aged and particularly mature and overmature stands have been suffering from the outbreak of bark beetles (*Ips typographus*) on a large scale. The most valuable Norway spruce genofund has been damaged to a great extent. The reasons for outbreak of the pest were unfavourable natural factors: very hot and dry summers of 1992 and 1993, which caused a decrease in the groundwater level. In addition, at the beginning of 1993, about 2 million m^3 of timber were broken by a very strong storm in spruce stands. Roots of many trees were damaged. The industrial air pollution then also negatively affected spruce forests. Weakened trees were attacked by the pest *I. typographus* and in September of 1994 the first serious damages caused by drought were noticed. The peak of *I. typographus* outbreaks and drying of spruce forests was reached in 1995. To fight against this pest, all possible mechanical, chemical and biological means were applied. According to recent forecasts, the outbreaks are expected to decrease in the nearest future.

Approximately 60 000-70 000 ha (13-15%) of middle-aged and mature Norway spruce stands were damaged by *I. typographus*, among them 15 400 ha with severe damages. The area mentioned corresponds to over 6 000 000 m^3 of timber. Therefore, sanitary clear-cuttings are being carried out. So far 12 400 ha of the damaged Norway spruce stands have been clear-cut. The Norway spruce gene fund conserved *in situ* has been greatly damaged. This is characteristic for gene conservation units of all categories. The consequences are summarized in Table 1.

Table 1. Status of *in situ* genetic conservation objects of Norway spruce (as of 1996)

	1994		1996		
Category	**No.**	**Area (ha)**	**No.**	**Area (ha)**	**Damaged trees (%)**
Strict genetic reserve	1	429	1	429	60
Genetic reserve	51	542	26	310	30-70
Seed stand	21	150	7	57	30-70
Plus trees	589		105		

It should be admitted that part of the Norway spruce genetic diversity has been lost irreversibly. Fortunately, part of it was conserved *ex situ*, in clonal archives, clonal seed orchards and progeny tests. Conservation of the Norway spruce gene fund *ex situ* will be given high importance.

At present an inventory of the remaining Norway spruce genetic resources is being carried out. They will be continuously monitored. All the management activities are aimed at restoration of damaged genetic objects wherever possible (Table 1). In genetic reserves and seed stands both natural regeneration and artificial planting with seedlings of the same origin will be applied. Instead of damaged plus trees of Norway spruce, new ones will be selected in the remaining stands. The value of the existing seed orchards, clonal archives and test plantations has significantly increased. Norway spruce plantings will be established using the seeds collected in them.

Reference

Danusevicius, J. and R. Gabrilavicius. 1995. Norway spruce (*Picea abies*) genetic resources and their conservation in Lithuania. Pp. 20-26 *in Picea abies* Network. Report of the first meeting, 16-18 March 1995, Tatra National Park, Stará Lesná, Slovakia (J. Turok, V. Koski, L. Paule and E. Frison, compilers). International Plant Genetic Resources Institute, Rome, Italy.

Table 1. Percentage of the total growing stock of tree species in different regions of Switzerland

Species	Jura	Central Plateau	Pre-Alps	Alps	Southern Alps	Switzerland
Picea abies	31.2	42.9	57.3	62.6	35.2	49.1
Abies alba	21.4	15.4	22.0	6.0	6.2	14.9
Pinus sylvestris	3.9	5.0	1.0	5.4	1.4	3.7
Larix decidua	0.4	1.2	0.4	13.0	15.2	4.9
Pinus cembra	–	–	–	1.9	0.1	0.5
Other conifers	0.3	0.8	0.2	0.0	0.1	0.3
Fagus sylvatica	30.0	20.4	13.3	6.6	13.1	16.2
Acer sp.	3.4	2.1	2.2	1.4	0.4	2.1
Fraxinus sp.	3.1	4.7	2.1	0.7	1.3	2.5
Quercus sp.	3.3	4.6	0.3	0.3	3.0	2.1
Castanea sativa	–	–	–	0.1	14.3	0.9
Other broadleaves	3.1	2.8	1.1	2.0	9.8	2.7
Total in 1000 m^3	63 574	92 785	88 139	97 481	23 148	365 128

Source: Bachofen *et al.* 1988.

stands were eliminated (Leibundgut 1986). Destruction of stands caused an increase in erosion, avalanches and flooding. Following extensive flooding in 1865, the first federal forest legislation was established in 1876. The law forbade clear-cutting in the Alps. This and the import of coal reduced the pressure on the forest stands. The devastated Norway spruce stands recovered naturally or were regenerated by planting or sowing. The material used for plantations was often not of local origin and imported from foreign countries (for example Müller 1990). In addition, plantations were established outside the natural range of Norway spruce.

The present forest law forbids clear-cutting in the whole country and protects forests as ecosystems close to nature. Particularly in the montane and subalpine vegetation zones, silviculture aims at maintaining natural stands. This is achieved by promoting natural regeneration and using natural growth patterns to obtain uneven-aged and well-structured stands.

Conservation aims

Extensive exploitation of Norway spruce stands in the past may have eliminated many local races. In addition, fragmentation and decreased size of populations may have affected genetic processes and thus genetic variation. At present, genetic variation may be influenced mainly by environmental pollution. There is growing evidence that environmental pollution causes changes in patterns of genetic variation (Scholz and Bergmann 1994).

The ability of populations to survive and reproduce in a changing environment largely relies on their genetic variation (Müller-Starck 1995a). The supply of genetic variation determines the potential of populations to generate new variation and thus to adapt to a changing environment. Conservation of as much genetic variation as possible is therefore an important factor for maintaining stable forest stands.

In Switzerland, a programme was initiated in 1987 aiming at the conservation of genetic variation of Norway spruce, silver fir and oak and it is foreseen to include rare and endangered species (OKOK Genreservate 1988). The partners of this programme are the Swiss Forest Agency, the Swiss Federal Institute for Forest, Snow and Landscape Research and the Swiss Forest Service. Because Norway spruce is common and widespread in the country and natural stands are still present, the programme aims at maintaining genetic variation *in situ* in gene reserves. *Ex situ* conservation measures are not envisaged at present.

Current status of research activities

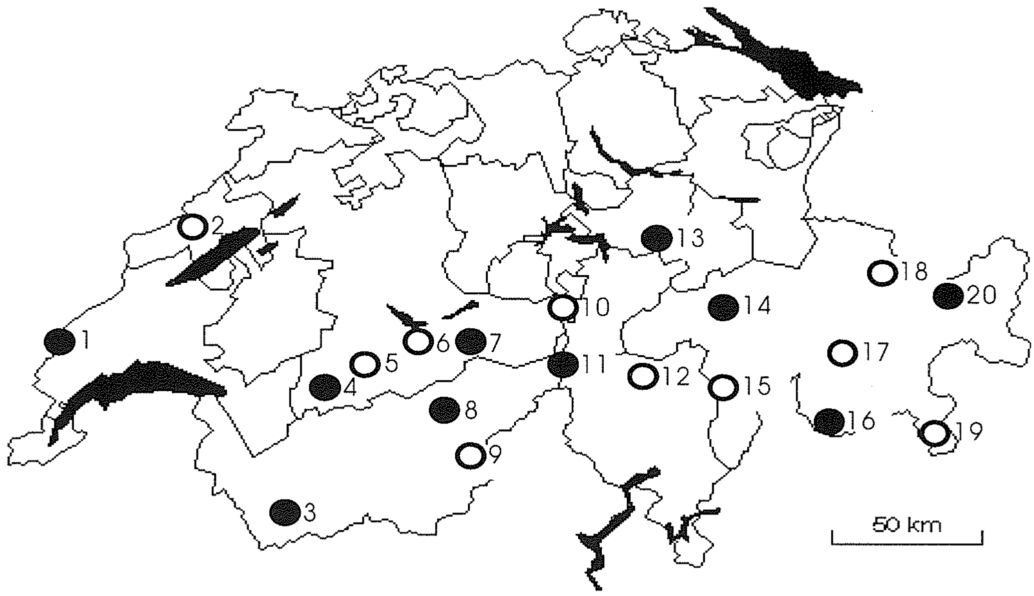
Efficient conservation of forest genetic resources largely depends on information on patterns of genetic variation in natural populations. Strong differentiation of a population from other populations may indicate its adaptive specialization or its distinct origin. On the other hand, a population revealing low differentiation from the remaining populations may well represent the species (Gregorius 1985). Such information is valuable for selecting populations for establishing gene reserves.

As a basis for establishing gene reserves, natural Norway spruce populations were investigated by isozyme gene markers. A total of 20 populations located in the montane and subalpine vegetation zones were investigated (Fig. 1) (Müller-Starck 1995b). For each of these populations, multilocus genotypes were identified at 18 loci from 100 trees. The results obtained revealed large genetic variation within populations in contrast to relatively small variation between populations. The variation observed was not smaller than in populations located at lower elevations in Germany and Italy (for references see Müller-Starck 1995b).

The populations investigated showed substantial differences in heterozygosity, number of alleles per locus and hypothetical gametic multilocus diversity. This latter measure is suggested to quantify the ability of populations to create genetic variation and thus to adapt to a changing environment (Gregorius *et al.* 1986). The highest level was found in a population of the Jura (no. 1). Additional high levels were observed in three populations distributed over the entire country (nos. 3, 14, 16) (Fig. 2).

Genetic variation observed between populations revealed evidence that populations in the west of the country (nos. 1, 2, 3) and also from regions in the southeast (nos. 15, 16) are differentiated from the remaining populations (after Müller-Starck 1995b). Differentiation of the western populations was most evident at the locus *SKDH-A* encoding shikimate dehydrogenase. In two of these populations the allele *SKDH-A4* was at least seven times more frequent than in the remaining populations. These findings are consistent with results from pollen analyses indicating that Norway spruce stands in the west of Switzerland may have a distinct origin or followed a separate colonization route (C. Burga, unpublished).

Additional investigations are being performed by studying variation in mitochondrial DNA. Analyses of two intraspecific crosses indicated that mitochondrial DNA is maternally inherited in Norway spruce (Mátyás *et al.*, unpublished). Mutations of mitochondrial DNA arising in different individuals are thus not recombined during sexual reproduction. This together with the fact that mitochondrial DNA shows a low rate of evolution in plants (Wolfe *et al.* 1987) suggests that variation in mitochondrial DNA persists over many generations and may show high levels of differentiation between populations. DNA sequence analyses of a non-coding mitochondrial DNA fragment indicated that there are at least three different mitochondrial DNA types present in Switzerland (Sperisen *et al.*, unpublished). The spatial distribution of mitochondrial DNA variation will be investigated and used to identify post-glacial migration routes.



1 Le Brassus, Risoud
2 Chaux du Millieu
3 Orsières, Fôret de Branche
4 Gstaad, Strählvorsass
5 Adelboden
6 Saxeten
7 Grindelwald, Itramenwald
8 Ried-Mörel, Aletschwald
9 Simplon
10 Engslenalp
11 Oberwald
12 Faido
13 Muotathal, Bödmerenwald
14 Brigels, Scatlè
15 San Bernardino
16 Bondo, Bosch Gras/Tens
17 Rona
18 Conters
19 Poschiavo
20 Ardez, God Grond

Fig. 1. Location of Norway spruce populations investigated by isozyme analyses (Müller-Starck 1995b). Shaded circles indicate populations selected for establishing gene reserves (Bonfils *et al.* 1996).

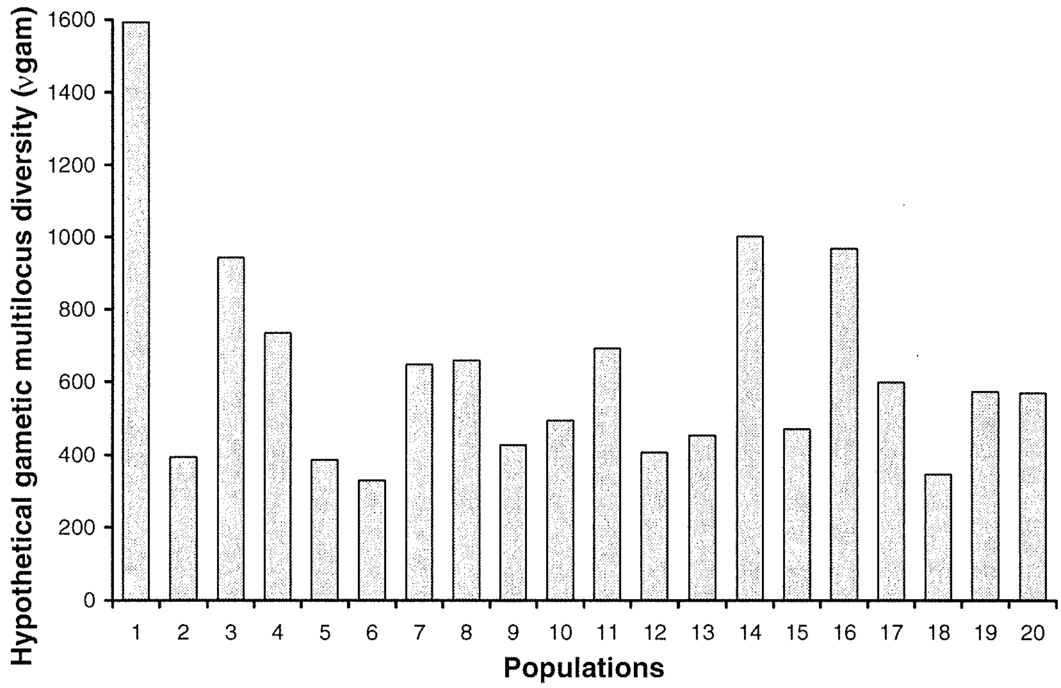


Fig. 2. Hypothetical gametic multilocus diversity (v_{gam}) of 20 Norway spruce populations in Switzerland (Müller-Starck 1995b). For locations of populations investigated see Figure 1.

Current status of conservation activities

Gene reserves

Ten of the 20 populations analyzed were selected for establishing gene reserves (Fig. 1) (Bonfils *et al.* 1996). Populations showing high numbers of alleles and high levels of heterozygosity and hypothetical gametic multilocus diversity were selected. They included populations showing strong differentiation and populations showing little differentiation. Additional criteria for the selection of populations were their autochthonous character, size, distribution within ecogeographical regions and post-glacial migration routes.

For each of these potential gene reserves contracts between the owners and the Swiss Forest Agency are under negotiation (Table 2). These contracts include the local name of the stand, the geographic location, a map of the area covered, the ownership and forest management regulations. The contracts will last 50 years. At the end of this duration they will be extended for periods of 20 years. The gene reserves will be incorporated in the general management plan of the forest owner. In case of financial losses due to the special management of the gene reserves the federal government and the cantons will compensate the owners.

The gene reserves will be under strict forest management regulations (Bonfils 1995). Introduction of foreign genetic material is forbidden and natural regeneration has to be used as far as possible to ensure transmission of all genetic information to the subsequent tree generation. The gene reserves are divided into four zones (zones 0 - 3); for each zone, management regulations are defined. In zone 0 selective thinnings are forbidden. This zone covers a relatively small area (approx. 2 ha) but ensures a selection process close to nature. Zone 1 represents the main part of the gene reserve. This zone surrounds zone 0 and is 20-100 ha in size. Within this zone traditional close-to-nature silviculture is performed to enhance natural regeneration. If natural regeneration is not possible, artificial regeneration has to rely on reproductive material from the local provenance. A zone 2 is realized if trees of foreign origin grow within the gene reserve. These trees have to be eliminated at the end of the production period. Finally, zone 3 is a buffer zone that prevents or reduces geneflow from trees of foreign provenances to the gene reserve. For this zone, no particular silvicultural regulations are defined.

Forest reserves

In addition to gene reserves, Norway spruce is also preserved in five forest reserves covering a total area of 400 ha. These forests are allowed to develop with almost no human interference, leaving the trees to reach their natural age and leaving dead wood in the forest. The forests serve as nature reserves and research populations. The structure and development of the forests are being analyzed and results from these studies will be used to establish guidelines for close-to-nature silviculture. Three of these forest reserves will be integrated in the forest gene reserves.

Seed stands

Planting trees from distant provenances may influence the patterns of genetic variation in natural stands by pollen contamination. To obtain seeds of local origin for plantations, a total of 384 Norway spruce stands were selected as seed stands and registered according the OECD regulations (Fürst, pers. comm.). These stands are distributed in all ecogeographical regions of the country.

Table 2. List of designated gene reserves with Norway spruce in Switzerland

Stand no.	Location and name of forest	Status of contract
1	Le Brassus, Risoud	accepted
3	Orsières, Forêt de Branche	under negotiation
4	Gstaad, Strählvorsass	under negotiation
7	Grindelwald, Itramenwald	in preparation
8	Ried-Mörel, Aletschwald	in preparation
11	Oberwald	in preparation
13	Muotathal, Bödmerenwald	under negotiation
14	Brigels, Scatlè	under negotiation
16	Bondo, Bosch Gras/Tens	in preparation
20	Ardez, God Grond	under negotiation

Concluding remarks

The isozyme analyses performed on 20 Norway spruce stands in Switzerland revealed significant differences in intrapopulational and interpopulational variation. These results together with information on the forest history, site conditions and autochthonous origin of the stands were used as criteria for selecting stands and their establishment as gene reserves.

The results obtained indicated that patterns of genetic variation we see in present Norway spruce populations were influenced by post-glacial migration. Similar results have been described for other plant and animal species (Hewitt 1996). These analyses indicated that many plant and animal species underwent divergent evolution in glacial refugia. Following amelioration of the climate these refugial populations expanded and, depending on the mode of migration and the route of migration taken, gave rise to the present patterns of genetic variation. To conserve as much genetic variation as possible, gene reserves should include stands derived from different glacial refugia and stands along distinct post-glacial migration routes.

Isozyme markers are likely to describe only a part of the characteristics of a provenance. For selecting stands for gene reserves it is therefore necessary to use additional information such as the forest history, site conditions and autochthony of stands. Several different types of DNA markers are currently being developed for Norway spruce. They include repetitive sequences of nuclear (Morgante *et al.* 1996) and chloroplast DNA (Vendramin, pers. comm.) and non-coding sequences of mitochondrial DNA (Sperisen *et al.* in preparation). Because these markers contain DNA of the nuclear and organelle genomes, they reveal different types of inheritance and different rates of evolution and may thus give new insights into the history and genetic structure of Norway spruce stands.

Conservation of genetic variation may not only take place in gene reserves but also by silvicultural practices applied to the total forest area. From a genetic point of view these practices would aim at promoting transmission of as much genetic variation as possible to the next generation. Nursery practices would be ideally performed in a way that no variation is lost from seed collecting to outplanting. Variation in isozymes and DNA may provide useful markers for studying genetic effects of these practices. For example, genetic effects of different modes of seed collecting, culling of seedlings in nurseries and outplanting of young trees could be analyzed. In addition, natural and artificial regeneration could be compared in juvenile stands. Results from such analyses could help to include genetic aspects in silvicultural practices.

Our experience showed that an active information policy and cooperation with the forest service is of great importance for successful implementation of any kind of gene conservation measures. In this sense establishment of gene reserves contributes not only to the conservation of genetic resources but also to the forest service's awareness of the forest diversity.

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Discussion paper

Conservation activities in *Picea abies*: genetic after-effects of the reproductive environment and their possible causes and implications

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Introduction

An understanding of the genetic mechanisms behind the observed phenotypic variability of adaptive properties is of major importance both for understanding the evolution of a species and for its breeding and gene conservation activities. For *Picea abies* (L.) Karst., studies in Norway have shown that the genetic variability of adaptive traits does not seem to be regulated only by the laws of Mendelian inheritance, but also by the climate and weather during sexual reproduction. The present contribution will review the latest results from this research and will discuss possible causes and implications.

Early and recent results

The effects were first brought to attention when it was discovered that seedlings from seed produced in seed orchards where parental clones had been transferred 6-8 degrees of latitude southwards or 500-600 m lower in altitude, do not retain the annual growth rhythm of their parents (see Johnsen and Skrøppa (1996) and references cited there). The seeds produced under warm conditions give rise to seedlings with a later flushing, an extended growth period and a delayed development of frost-hardiness during early autumn compared with seedlings from seeds of the same parents reproduced under colder conditions. Even a short forcing period in an elevated temperature in a greenhouse during pollination and early embryogenesis seem to be sufficient to induce reduced autumn frost-hardiness of the progenies (Johnsen *et al.* 1989). The effect of the parent environment is long-lasting; data indicate that they can endure for at least 17 years from seed (Edvardsen 1995). Similar effects were observed when seedlings from bulked seed lots of two seed orchards, from a cool (1987) versus a warm (1989) seed year, responded differently to photoperiodic treatments (Kohmann and Johnsen 1994). Seedlings from the cool seed year formed terminal buds at shorter nights both in growth chambers and under natural days in nurseries than did seedlings from the warm seed year.

Three new experiments were made to test whether photoperiod and temperature treatments (long and short days/high and low temperature) during male meiosis and pollen maturation could give any differences in progeny performance (Johnsen *et al.* 1996). In two independent experiments we found no evidence for altered autumn hardiness related to these pollen treatments. In a third experiment, we made crosses inside a heated greenhouse in early spring when the days were short (14 h), repeated the same crosses inside the greenhouse in May when the days were longer (18 h) and in addition made the identical crosses outside the greenhouse in May (Johnsen *et al.* 1996). No treatments were give to the male parents in this experiment. The crossing environment significantly affected the hardiness of the offspring. The progenies from the early indoor crosses were more damaged than progenies from the late indoor crosses, which in turn were more damaged than progenies from the late crosses performed outdoors. These experiments indicate that the effects of the parental environment are mainly influenced by the environmental conditions at some stages during the reproductive process taking place in the female flowers.

Practical consequences

Results from both field trials and practical plantings indicate that the observed phenomenon may have practical consequences under extreme climatic situations (Johnsen *et al.* 1989; Skrøppa, unpublished). The effects may either be positive or negative for survival and vigour of the plantation, depending on how the climatic extremes are related to the growth rhythm of the material. The situation seems to be similar to that of a provenance transfer. It will be advantageous under certain conditions (e.g. avoidance of late spring frost damage), but the opposite under other conditions (e.g. early autumn frost). The effects therefore certainly influence the adaptive properties of the offspring and will also have implications for the total growth as the duration of the growth period is influenced (Skrøppa and Magnussen 1993).

This phenomenon should also be considered for predicting the long-term effects of global warming on boreal forests.

Another implication of these results is that seedlings collected in different years in the same stand or in the same seed orchard will not always have the same adaptive properties. This will also have consequence for the provenance concept, as defined in the OECD regulations.

Possible causes

A common factor involved in seed production under warm non-native conditions is that the increased heat sum in spring induces an earlier start of the reproductive process at a shorter photoperiod compared with the cooler native environments far north and at high altitudes. Both a north to south transfer and even a strict high to low altitude transfer on the same latitude can result in a 2-hour difference in photoperiod when pollination starts in the first half of May at low elevations compared with 4 weeks later at high elevations. The differences in photoperiodic response between the seedlings from the two climatically different years 1987 and 1989 are also in agreement with the hypothesis that temperature, photoperiod or an interaction between the two, is the environmental trigger influencing the progenies by a yet unknown mechanism.

Two types of such mechanisms can be imagined. One is that selection takes place during the reproductive process, causing non-Mendelian inheritance of some alleles and resulting in segregation or transmission ratio distortion. A second mechanism is that an environmental factor at some stage during the reproductive process regulates the level of gene expression in the developing progeny. Both types of mechanisms have been shown to operate in plant species, see Skrøppa and Johnsen (1994) and references cited there.

The results from two of the experiments described above made us reject the hypothesis that male meiotic drive and pollen selection during pollen maturation in the male flowers could be a significant factor explaining the observed effects. This is the level with the greatest potential for selection. Selective events in the female flower and embryo competition remain to be tested. The selection intensities at these levels are, however, rather small and can most likely not account for the large differences observed.

The second type of mechanism, a gene regulation through a parental imprinting mechanism, can at present only be the subject of speculations in *Picea abies*. Genomic imprinting is, however, being increasingly accepted as a fundamental and widespread process that determines, in ways not predicted by the laws of Mendelian inheritance, whether a particular gene will be expressed or not (Matzke and Matzke 1993). In *Larix* spp., controlled crosses that were produced in a greenhouse and outdoors adjacent to the greenhouse showed significant differences in height. Using differential display, RT-PCR sequences have been detected as expressed in one set of progeny, but not in the other (Hutchison *et al.* 1996). The hypothesis is that this occurs through a parental imprinting mechanism, perhaps as a result of altered gene expression in the parent plant at the time of flowering.

New interpretation of provenance variation in Norway spruce

Traits related to phenology of Norway spruce provenances are clinally correlated to latitude and longitude. As much as 60-93% of the variation has been explained by these parameters for traits such as bud set in the autumn, cessation of leader growth, duration of the growth period and development of autumn frost-hardiness (Dormling 1979; Skrøppa and Magnussen 1993; Dæhlen *et al.* 1995). Our results indicate, regardless of the type of mechanism involved, that phenotypic provenance variation is not only caused by natural selection among different genotypes within populations, but also directed by environmental signals received by the female parent during sexual reproduction. Both processes seem to change phenology traits of the progeny in the same direction, and may explain why Norway spruce provenances so strongly show clinal variation along latitudinal and altitudinal gradients for traits that characterize the annual growth cycle.

Conservation of Norway spruce genetic resources

The observed effects will most likely have evolutionary consequences for Norway spruce under boreal conditions and may be one reason why the species is so highly adaptive. The importance of this factor for gene conservation activities will depend on how the mechanism operates and its pattern of inheritance, which are not known. Until more scientific information is provided, these effects should not be used as an excuse to lower the efforts in conserving the genetic resources of the species.

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Agenda

1. Welcome address and introduction

2. Brief country updates on the progress of genetic conservation activities on *Picea abies* since the last meeting (Austria, Belgium, Czech Republic, Denmark, Finland, Germany, Latvia, Lithuania, Norway, Poland, Slovakia and Ukraine)

3. Introductory country reports on the genetic resources of *Picea abies* and their conservation (France, Hungary, Italy, Russian Federation, Switzerland and Sweden)

4. Conservation activities in *Picea abies*: genetic after-effects of the reproductive environment and their possible causes and implications

5. Technical Guidelines for *Picea abies* Genetic Resources Conservation
   - 5.1 Introduction
   - 5.2 *In situ* conservation
   - 5.3 *Ex situ* populations
   - 5.4 *Ex situ* conservation in genebanks

6. Development of database for *Picea abies* genetic resources

7. Broadening of the scope of the Network to include other species of the boreal forest zone

8. Coordinated research on provenance trials

9. Collaboration with IUFRO working party on Norway spruce

10. Network matters
    - 10.1 Information from other Networks
    - 10.2 Diversity and adaptation in forest ecosystems in a changing world – report from the IUFRO Conference
    - 10.3 Mailing lists
    - 10.4 Public awareness
    - 10.5 Next Network meeting

11. Field trip: Typical forest stands with boreal tree species (Norway spruce, Scots pine) around Hyytiälä; gene reserve forest, seed orchards and field experiments in the district

12. Conclusions

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