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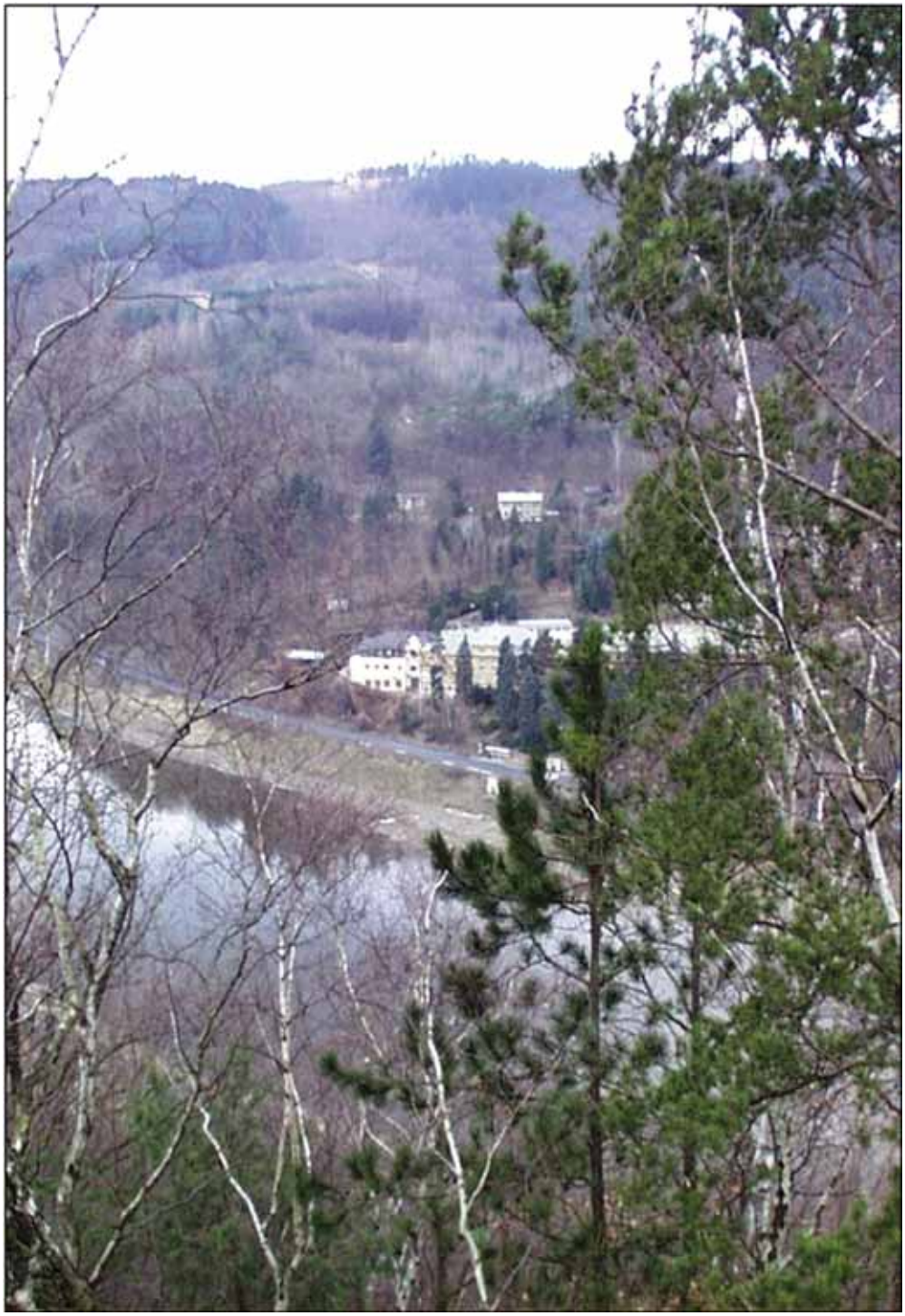

International Workshop

Breeding and improvement of forest tree species both in Sweden and the Czech Republic

Forestry and Game Management Research Institute (FGMRI)
Jíloviště–Strnady, Czech Republic April 30th, 2001

Editors
Josef Frýdl, Petr Novotný

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PREFACE AND ACKNOWLEDGEMENT

Group of research scientists from Sweden visited the Czech Republic in April 27 – May 1, 2001. Their main special involvement was concerning in coniferous tree breeding and improvement in the northern part of Sweden. In cooperation with the Czech University of Agriculture in Prague, field excursion was realized as first, during April 28 – 29, 2001. Day after this field excursion, special international workshop aimed at information about problems connected with forest tree breeding and improvement both in Sweden and the Czech Republic, was held in the Forestry and Game Management Research Institute (FGMRI) Jíloviště–Strnady, in April 30, 2001. Themes of Swedish colleagues reports have been aimed to information about sustainability in the long-term Swedish breeding programs, enhancing gain from long-term forest tree breeding while conserving genetic diversity, genetic gain in current and future seed orchards and clone mixes in Sweden, hardiness breeding of Scots pine, etc. Czech participants from both FGMRI Jíloviště–Strnady and the Czech University of Agriculture in Prague presented information about current stay of forest tree breeding and improvement in the Czech Republic both in general and within the framework of current selected specific breeding programs and some important results obtained during solution of these programs. Various other problems have been subject of discussion after presentation of main reports, too. At the end of this common workshop of Swedish and Czech specialists, there have been agreed plan of continuation of such common events in the future, as very important and useful method of common information and experiences exchange, in the frame of common cooperation.

Participants of the International Workshop „Breeding and improvement of forest tree species both in Sweden and the Czech Republic“organized by the FGMRI Jíloviště–Strnady, Department of Forest Tree Species Biology and Breeding, in April 30, 2001, are

from Sweden:

Dr. Dag Lindgren, Professor of Forest Genetics, Swedish University of Agricultural Sciences, Umeå
Scientific interests: Quantitative genetics, forest tree breeding, breeding theory

Dr. Per Ståhl, Research Director – Tree Improvement, SkogForsk, Uppsala
Scientific interests: Research with breeding of forestry trees, forest renewal, forest management, nature and environmental conservation together with general development work in Sweden and abroad

Dr. Bengt Andersson, Programme leader for conifer breeding in Northern Sweden, SkogForsk, Sävar; Professor in Forest Genetics, Swedish University of Agricultural Sciences, Umeå

Dr. Johan Westin
Scientific interests: Research in Conifer Breeding Program in Northern Sweden, SkogForsk, Sävar

Dr. Jörgen Hajek
Scientific interests: Research in Conifer Breeding Program in Northern Sweden, SkogForsk, Sävar

from the Czech Republic:

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Scientific interests: Research with forest tree species verification and identification within the framework of isozyme analyses application, including study of site conditions influence to genetic selection of forest tree species

Milan Lstibůrek, MSc., Forestry and Game Management Research Institute (FGMRI) Jíloviště–Strnady, Department of Forest Tree Species Biology and Breeding, Division of Forest Tree Species Breeding and Improvement

Scientific interests: Breeding theory, provenance research

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Scientific interests: Forest tree new variety breeding and improvement, breeding theory, forest genetics, preservation and reproduction of forest tree gene resources

We want to thank the Forestry and Game Management Research Institute (FGMRI) Jíloviště–Strnady management, in particular to Dr. Petr Zahradník (Director of FGMRI), Dr. Bohumír Lomský (Vice-Director of FGMRI), for possibility to realize workshop in FGMRI Jíloviště–Strnady. Our thanks also belong to Dr. Jaroslav Kobliha (Czech University of Agriculture in Prague, Faculty of Forestry), Milan Lstibůrek, MSc. (FGMRI) and Mr. Jiří Tomec (FGMRI) for all their help during workshop.

Dr. Josef Frýdl, Ing. Petr Novotný (Editors)

CONCEPTS AND PROBLEMS OF FOREST TREE BREEDING AND IMPROVEMENT HAVING BEEN SOLVED BY THE FORESTRY AND GAME MANAGEMENT RESEARCH INSTITUTE (FGMRI) JÍLOVIŠTĚ-STRNADY, BRIEF SURVEY OF CURRENT RESULTS

INTRODUCTORY NOTES

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INTRODUCTION

The FGMRI Department of forest tree species biology and breeding works according to the new tendencies of state forestry politics that correspond with the international settlements connected with resolutions of the world ecological and forest conferences (10th World Forestry Congress, Paris 1991, Conference about forest regeneration in Europe, Strasbourg 1991, Conference about environment, Rio de Janeiro 1992 and Helsinki 1993). These settlements, fulfilment of which was recommended to the governments of the individual countries, involved programmes of cooperation on international level especially in breeding research and in research and solution of complex problems of preservation of gene resources of forest tree species. On the base of these programmes the original tree species biodiversity could be restored and stabilized in the vast biocenology regions and above all in the areas damaged by industrialization or large exploitation (e. g. EUFORGEN programme, etc.).

The complex programme of forest tree species breeding is the principal research activity of this Department in the frame of which forest trees species variability is studied in relationship to geographical variability, adaptation abilities to the site and anthropogenic load. At studying the variability, except the classical methods (provenance trials, hybridisation projects, verification of progeny of certified reproduction material), the new methods of molecular biology (isozyme analyses, DNA analyses) are used that enable to get more reliable information about the genome of forest tree species in any stage of forest tree species development. The breeding programmes elaborated for the particular woody species are focused not only on necessity to preserve genetic variability of populations (declaration of gene bases, establishment of seed orchards and clonal archives) but also on solution of projects aimed at increase of quality, production and resistance of woody species. For the breeding also the long-term international plots are used. The breeding processes usable for biodiversity preservation are studied in details and new biotechnological methods for micropropagation of forest tree species are searched. The research of genetic manipulations is oriented to the use of transgenic forest tree species in forest breeding. This complex breeding programme includes also the partial programmes solving optimisation of using the generative and vegetative reproductions of gene resources of deciduous and coniferous tree species. Finally the conception of the Department involves the complex programme of preservation of forest diversity and prognosing of forest development.

The FGMRI is the main working place of the branch forestry research in the CR, in a long-term perspective it continuously guarantees the complex development of the research and expert activities in above mentioned forestry fields. It ensures also the information on the level of knowledge abroad, and their application in conditions of the CR. The results of the FGMRI work can be used in forming and realization of the goals of forestry policy in CR, and fulfilling of the international resolutions signed in

forestry branch. In accordance with the Pan-European process, the main attention in the FGMRI is focused on improvement of the state of forest ecosystems, and biodiversity increase in the forests, maintenance of productive forest function, and development of the non-productive forest functions. The problems mentioned are successfully solved in the FGMRI for several decades, in frame of the research projects and consultation and expert activity (the research institute was established in 1921). During the time close cooperation to other similarly oriented institutions abroad was bound.

GENETICS AND FOREST TREE BREEDING AND IMPROVEMENT

Workload and main orientation of Forest Tree Species Biology and Breeding Department have primarily been devoted to collection of basic information about economically important forest tree species, including solution of other specific problems connected with study of genetic variability, gene pool preservation and reproduction treatments, intensive breeding programmes realization with support and use of modern progressive methods of biotechnology and molecular biology. In the frame of increasing the stability and productivity of forest ecosystems, the genetic variability of forest trees species (mainly Norway spruce – *Picea abies* (L.) Karst., Scots pine - *Pinus sylvestris* L., European larch - *Larix decidua* Mill., European beech – *Fagus sylvatica* L., oaks – *Quercus* sp., ashes – *Fraxinus* sp., including some of introduced species, as e.g. Douglas fir - *Pseudotsuga menziesii* /Mirbel/ Franco), Japanese larch - *Larix leptolepis* /Sieb. et Lucc./ Gord., etc.), is studied in relationship to their geographic variability, adaptation abilities to site and civilisation load. Besides the classical methods (provenance trials, hybridisation projects), new methods of molecular biology (e.g. isozyme and DNA analyses) are used for the study of genetic variability, which enables to acquire information about the genome of forest tree species much faster. In the Czech Republic, breeding programmes elaborated for individual coniferous and broad-leaved forest tree species are focused above all on preservation of genetic variability of populations (declaration of gene bases, establishment of seed orchards and clone archives). Breeding processes that can be used for biodiversity preservation are studied in details as well as new biotechnological methods for micropropagation of forest trees species are searched. The target of breeding, as the practical application of knowledge from forest genetics in forestry practice, is to form and recommend the suitable populations and verified improved assortments of forest tree species for practical use.

To be breeding and improvement activities continuity assured, it is necessary to get on with another research of genetic variability of forest tree species (testing of progeny, provenance research), including use of modern diagnostic methods (biochemical and genetic analyses of isozymes, including DNA analyses). As well, it is necessary to get on with realisation of other hybridisation programmes being aimed mainly to heterosis effect achievement (i.e. better both qualitative and quantitative properties of hybrid progeny as compared with characteristics of parental partners). Also, another research of vegetative ways of forest tree species propagation should be realized having been aimed especially at improved and verified populations and cultivars propagation, beside other methods also within the framework of biotechnology methods use. With aim to abridge procedures of breeding material verification, it is necessary to maintain research of early diagnostics (early tests), too. As for methods to be used for evaluation of results from both measurement and assessment of tested material, including new methods of research and experimental plots establishment, more effective ways and methods will be used, commensurate beside other methods also with new applications of mathematical statistics.

Realization of projects pertinent to actual assignments of forest tree species breeding and improvement contributes for acquirement of further findings concerning genetically conditional variability of examined species, with respect to fact, that in case of some of those species first findings of this type will be achieved by this way, what so ever. In practical terms, achieved findings can be used within the framework of refinement of reproductive material zoning, e.g. it will be possible to use an information about genetically conditional traits and properties of partial populations (sources of reproductive material as seed orchards, forest stands certified for seed collection, etc.) for their selection and enlistment of the best of them to the category of tested sources of reproductive material (category T according to EU classification).

As for another practical utilisation of results achieved, there is necessary to mention proposals for using of both genetically valuable populations and verified improved varieties in forest practice, proposals of treatments aimed both at increasing of forest ecosystems stability and improvement of forest stands volume production, including proposals of treatments aimed at salvation and preservation of valuable and threatened populations in the course of maintenance of desirable level of biodiversity. Results of both current and other research activities have contributed to solution of problems connected with alterations and renewals of forest tree species composition within the framework of expected potential climatic changes.

FOREST TREE BIOTECHNOLOGY

The research tasks in relation to forest tree biotechnology are solved in the Department of Tree Species Biology and Breeding of FGMRI. Traditionally, the research in the Department is focused on establishment, verification, and standardization of explant cultivation technologies of almost all forest tree species growing in the CR including introduced ones that are important from the point of view of forest breeding. The tasks tightly connected with the endangered forest tree species micropropagation form the priority in research projects: the explant representatives are inventoried in the Bank of Explants, which was established under the subvention of the World Bank in 1996. Actually, there are 30 forest tree species and 5 endangered species important for forest biodiversity such as *Adenophora liliifolia* and *Daphne cneorum* preserved and stored in the Bank. Moreover, the Bank is utilized for international gene resources exchanges and comparative research studies.

The application of in vitro autovegetative propagation and cutting in special breeding programmes represents the further research of the Department. The individuals expressing suitable properties are widely or individually selected, propagated, and transferred in synthetic multiclonal mixed populations directly determined for forestry practice. Simultaneously they also represent a source of plant material required on the base of European international agreements by forest renewal and maintenance programmes. Establishment of synthetic populations needs steady monitoring of ontogenetic development of micropropagated material and its capability to adapt onto different natural environments. The high efficacy in the production of genetic stable populations is possible to ascertain only by the above mentioned strategies.

Micropropagation technologies elaborated on the base of research activities of the Department are utilized for reproduction of improved and selected plant material such as for late budding oaks and for genetic manipulations aimed at creation of required genotypes (the Department possesses the valid license for GMO).

MOLECULAR BIOLOGY

Genetic variability of organisms is studied in different ways. Among the most important objects of these studies in the field of forest trees are molecular genetic markers, especially isozymes and DNA. Isozyme laboratory in FGMRI (founded with benefit of the World Bank in 1997) has started its activity with investigation of *Pica abides* population from the Ore Mts. and Giant Mts. Populations of different geographic origin and tolerance to environmental stress were studied and significant differences in molecular genetic characteristics were found here. The results obtained were then utilized in further research of the *Pinus sylvestris* and *Pinus mugo* populations. Molecular genetics of broadleaves was also studied for *Alums* and *Tulia* sp.

Isozyme laboratory in FGMRI is also engaged in standardization of methodology. Genetic structure of forest trees populations in relationships to various geological, soil and climatic factors is also intensively studied and genetic identification of native trees, orchards and nurseries is pursued. In future, these research activities will continue more extensively and attention will be paid to the comparison of autochthonous and allochthonous populations in different stand conditions using isozyme analysis.

At the same time, DNA laboratory in the FGMRI is starting its activities, enabling further molecular genetic research, especially the identification and comparison of individuals and populations.

FOREST TREE SEED MANAGEMENT

The forest tree seed management research is closely related to the genetics and breeding of forest tree species. In last 10 years the research has been focused mainly on methods for the pre-sowing treatment (stratification with and without substrate) of seeds of both conifers (*Abies*, *Picea*, *Pinus* and *Larix*) and broadleaves (*Fagus*, *Acer* and *Fraxinus*) to improve seedling emergence in forest nurseries. Other studies dealt with seed-borne diseases and integrated pest management research and methods for quality testing of dormant seed (beech and fir). Since 2000 the research has concentrated on quality of seeds from seed orchards and factors influencing the quality of acorns and beechnuts during their storage. The results of the forest seed management research have been widely used in forest nurseries and at the Czech Tree Seed Plant at Týniště nad Orlicí.

There was set up a place of work at Research station at Uherské Hradiště, which dealt with broadleaved trees breeding and improvement, particularly with poplars and willows. Activities focused on testing of imported cultivars from European assortment and also on hybridisation of perspective parents, as far as balsam and black poplars are concerned. There were evaluated different characteristics in clonal test in relation to the stand, and breeding regionalization was developed including recommended breeding technology. The final test results ended in creation of Recommended clone assortment for the Czech Republic. Another next testing of new clones produced in Europe has been running now. There were put together a method of clone identification in stage of young plant and a method of generative production and rules for handling seeds. A huge breeding programme started with white poplars focused on producing and testing hybrids both suitable as ameliorating trees and good in stands under air pollution stress. The excellent clones are selected and used in reproduction in vitro.

Promising phenotypes and genotypes to be tested have been collected in the unique clonal archives at Research station Uherské Hradiště. The archives include also a gene pool of both European and extra-European willows. Number of clones is evaluated in clonal tests designed like short rotation woody crops plantations for biomass production. The extraordinary value of the archives lies in conservation of endangered native poplars.

Representative draft of results achieved and applied within the framework of Genetics and Forest Tree Species Breeding and Improvement

- The first information were gathered for classifying the selected partial populations of Norway spruce (category A stands) into the set of units certified for seed crop in accordance with the EU legislative.
- For further verifying the method of incomplete diallel hybridisation seems to be perspective according to the methodological experiences acquired from foundation and evaluation of the research plots with larch progeny from hybridisation programmes incl. the plots with the progeny from diallel hybridisation assessed in 2001. The method is of concern when a set of trees (clones) is verified by hybridisation with several, for example four testing trees (clones).
- Both high economical value and considerable adaptation ability have been certified and approved in case of both autochthonous and allochthonous populations of European larch (Sudeten ecotype).
- On the base of DBH and height growth evaluation, together with results of volume production evaluation, there have been achieved new findings concerning silver fir genetic variability in relation to selected natural forest regions. As outputs are proposals for use of silver fir reproductive material in forest practice, especially in the frame of silver fir reproductive material sources zoning.

- On the base of first results connected with European larch seed orchards progeny evaluation, it has been achieved first information available for preparation of proposals to enlist selected seed orchards among tested sources of reproductive material, in accordance with the EU legislative.
- Positive prospects of Douglas fir, grand fir and partially also noble fir have been certified and approved for the Czech Republic forest management. On the base of provenance plots evaluation, proposals to eventual import of these forest tree introduced species have been presented.
- Considerable economical value of European beech and silver fir from the Carpathian regions of the Slovak Republic, comparable with material from Hercynian–Sudeten regions of the Czech Republic, have been certified and approved by provenance tests. On the base of results achieved, it was possible to propose to import reproductive material of mentioned species from the Slovak Republic, in case of Czech local reproductive material deficiency.
- New findings about growth of both pedunculate oak (*Quercus robur* L.) and sessile oak (*Q. petraea* / Matt./ Liebl.) provenances. New findings about fructification of Slavonian ecotype of sessile oak in seed orchards. First phases of this species reproductive sources (seed orchards) verification. Further phases of another species of oaks inventory have started.
- List of recommended well-adapted clones of black and balsam poplars was published after evaluation of yield in different site conditions in the Czech Republic.
- Clones of *Populus tremula* and *Populus tremuloides* were tested in general and specific combining ability and the best parents were selected to gain high-performed progenies for reforestation.
- Principles of forest tree species reproductive material zoning have been drafted and applied as follow-up application in the Czech Republic forest management legislation.
- Conceptions for treatments of forest tree species preservation and reproduction (selection, establishment and management of gene bases, certified seed stands, elite trees, seed orchards, clonal archives) have been drafted and applied in the Czech Republic forest management legislation.
- Practical and continuous realization of treatments oriented to preservation of local autochthonous populations well-adapted to local site conditions, especially by selection, establishment and management of gene bases. In current time, there is registered almost 80,000 ha of gene bases for all important forest tree species, in the Czech Republic, in accordance with forest management legislation.
- Drafting of conception principles for treatments aimed at suitable forest tree species distribution in the Czech Republic
- Proposal of general conceptions for ensuring of suitable and valuable forest tree species reproductive material for the Czech Republic forest management
- Within the framework of molecular biology research, first results were obtained in the field of research of relationships between the genetic structure of forest trees populations and stand (environmental) conditions.
- Forest trees populations were studied using isozyme analysis. Genetic variability of several tens of *Picea abies* populations from 15 Natural Forest Areas in Czech Republic was investigated. Populations of other forest trees (*Pinus, Ulmus, Tilia* sp.) were also studied. Results from isozyme analyses of populations with forest stands damaged by industrial immissions (Ore Mts., Jizerské Mts., Giant Mts.) or pathogens (Orlické Mts.) were evaluated. Genetic stability of *Ulmus* sp. explants was measured and reproductive material from orchards and nurseries was identified using isozyme analysis.
- Realized forest seed management research results include especially improved methods for determining seed quality and health testing, determination of methods for short and long-term seed storage, determination methods for seed pre-sowing treatment (e.g. chilling without substrate), etc.

PAPERS

TREE BREEDING TOOLS (TBT)

"Arker" assisted selection

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SUMMARY

The web site "Tree Breeding Tools" is a service to make programs and other tools available for downloading. Most "tools" are related to Forest Tree Improvement and Forest Tree Seed Orchards, and in particular applications in quantitative genetic theory for tactic and strategic decisions I have been involved in. Collaborators or me (or both) have constructed most of the programs offered. It is my hope that this service will improve forest tree improvement and result in improved future forests, which hopefully will contribute to a better future World!

Programs on TBT aim at domestication of matters like:

- Group coancestry
- Group merit
- Status number
- Gene diversity
- Group merit selection
- Number of experimental sites
- Adaptation versus range (zone size)
- Effective population number (status number) of seed orchard crops
- Genetic management of seed orchards
- Linear deployment
- Selection intensity and other features related to normal distribution
- Copies of genes dropped through a genealogy
- Night-length
- Prediction of temperature and heat sums
- Gene diversity and gain as function of breeding strategy, genetic parameters, cost and time in short-term and long-term breeding, etc.

The programs together address key problems in forest tree breeding: how to combine information on environment, breeding value, genetic parameters, gene diversity, time, cost and technique into efficient tactic and strategic breeding decisions. There are tutorial examples and programs in the site (sometimes within the programs) to help understanding and learning the programs and concepts at TBT.

The web structure can be found via http://www.genfys.slu.se/staff/dagl. Tree Breeding Tools is the most important part of this structure, which can also be approached directly at *http://www.genfys.slu.se/staff/dagl/Breed_Home_Page/*

GENETIC GAIN FROM EXISTING AND FUTURE SEED ORCHARDS AND CLONE MIXES IN SWEDEN

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SUMMARY

Genetic gain in production per hectare was predicted to support decisions on the establishment of third-round seed orchards of *Pinus sylvestris*, *Pinus contorta*, *Picea abies* and *Betula pendula* in Sweden. Initially, genetic gain was calculated for existing seed orchards and clone mixes, together with the additional improvement possible by genetic thinning. Thereafter, calculations were made of the potential additional gain for the two coming rounds of seed orchards and clone mixes, and the time at which the gain could be realized for each seed zone. The predictions were based on genetic parameters summarized from hundreds of genetic field tests throughout Sweden.

The gain refers to the genetic level of the unimproved base populations (natural stands). Further, ideal functioning of seed orchards and clone mixes were assumed, e.g. panmixia, equal contributions from clones and no background pollination. As a simplification, gain is presented for only one character, i.e. production per hectare. In reality, an index of many traits is used, including, e.g. survival and stem quality. The gain in survival was also predicted for Scots pine orchards intended for localities with a harsh climate. All gain predictions took into account a sufficient genetic variability in the production populations.

In general, the genetic gain of current seed orchards is 10 – 25 %, and can reach 20 – 25 % in all new third-round orchards. The possible gain from thinning is 2 – 3 %, which is half of the theoretical level, and can be attained only in the most closely spaced orchards. The next step of improvement will yield a 35% gain for orchards established in 2015 – 2020, or as early as 2010 in some cases. Clone mix gains are of the same magnitude as for seed orchards, but can be realized sooner in commercial planting stock.

REFERENCE

Rosvall, O., Jansson, G., Andersson, B., Ericsson, T., Karlsson, B., Sonesson, J., Stener, L. G.: Predicted genetic gain from existing and future seed orchards and clone mixes in Sweden. In: Proceedings from the meeting of the Nordic Group for Management of Genetic Resources of Trees, 23–27 March 2001, Finland.

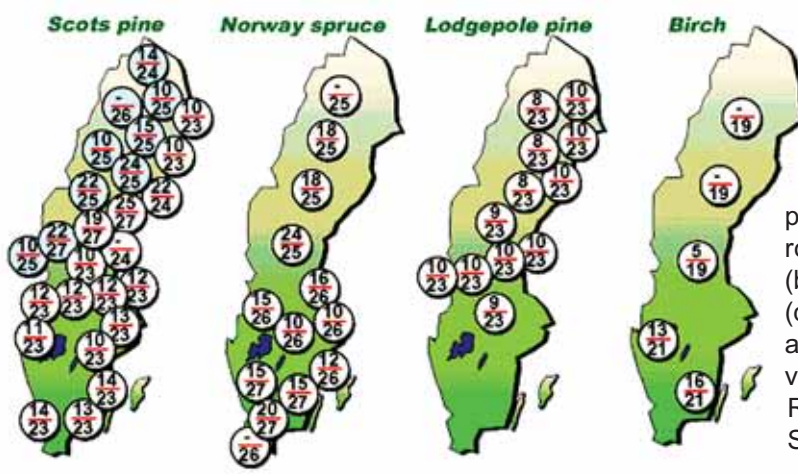

Fig. 1. Genetic gain in production per hectare (%) from present second-round seed orchards (above in circle) and potential gain from third-round seed orchards (below in circle). Blue (dashed) circles indicate additional gain in survival of 5 – 13 %. (From: Resultat, Nr. 1., 2001, SkogForsk)

DO THE HIGH GROWTH PERFORMANCES IN SOUTHERN AND SELECTED POPULATIONS HAVE SIMILAR PHYSIOLOGICAL BASES?

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INTRODUCTION

Seed sources, growth rhythm and frost hardiness

Growth rhythm and frost hardiness dynamics are important traits, with a strong influence on growth performance in boreal climates. Through the on-going process of adaptation, many tree species have developed natural populations that show enhanced fitness under specific environmental conditions. In Norway spruce (*Picea abies* (L.) Karst.) the origin of the seed source is known to influence various aspects of growth rhythm and development of frost hardiness. Therefore, in artificial reforestation the origin of the seed source may influence growth.

There are three types of seed source origin for Norway spruce. They may be: 1. local seed sources collected from trees with similar origins to the planting site, 2. transferred seed sources collected from trees of a different origin than the site (i.e. differing in latitude or altitude), 3. seed sources from clonal seed orchards composed of plus-trees, selected for superior height performance. The third alternative may be subdivided into specific seed sources produced on trees that have been transferred to environments more or less different from the original environment.

Transferred natural stand seed of Norway spruce and seed produced in seed orchards are commonly used in Swedish forestry. In Sweden transferred natural stand seed has been used on a large scale since the 1950s and seed orchard seed from the 1960s. The use of seed from non-local sources is motivated not just by seed quality considerations, but also by its potential to establish highly productive stands (Anon. 1993).

The migration history of Norway spruce after the latest glaciation involves a northward migration of spruce, from refuges in Central and Eastern Europe, to northern Fennoscandia (Schmidt-Vogt 1977). Further migration in Scandinavia occurred mainly from north to south. It is commonly stated that the migration pattern of spruce in Fennoscandia has affected growth rhythm and hardiness and that the present clones in these characters are remnants of the migration history.

When natural Norway spruce populations with different seed origins are evaluated at a set location, seed sources transferred northward show increased height growth (Worrall 1975, Skrøppa, Magnussen 1993), and later flushing (Worrall 1975) with similar survival rates (Rosvall, Eriksson 1981) compared with populations of local origin. Similarly, progeny of selected plus-trees tend to show higher growth potential (Johnsen 1989, Skrøppa, Johnsen 1999, Karlsson 1999) with the same survival rates as compared with local seed sources.

The main objective with this study of seasonal rhythm of growth and hardiness was to characterise the dynamics of growth and hardiness in local, transferred and selected seed sources.

Shoot growth in conifers

Shoot anatomy and bud morphology of most genera, and many species within the Pinaceae, have been thoroughly described and show strong similarities. A common zonation pattern exists within the apex although differences occur in the absolute size of the apex and the relative size of the cytological zones between individuals and species (Burley 1966). In the genera *Picea* and *Abies* a crown exists in the anatomy of the buds (Parke 1959, Romberger 1963), which appears to be a feature of some importance in the process of extra-organ freezing.

The initiation and development of primordia in the apical meristem have previously been described in *Pseudotsuga menziesii* by Owens (1968), in *Picea glauca* by Owens et al. (1977) and in *Picea abies* by Hejnowicz, Obarska (1995). Generally, budscales and needle primordia are initiated in the peripheral zone

of the apical meristem. A change from bud-scale initiation to needle initiation occurs at the completion of shoot elongation. The initiated primordia enlarge, after an initial period of division in all planes, through divisions in an intercalary meristem and by cell enlargements throughout the primordia. The number and shape of all foliar organs initiated in buds of mature trees can generally be determined before dormancy.

After dormancy the bud scales generally undergo no noticeable enlargement, but the needle primordia begin to mature at different times and at different rates. Maturation occurs basipetally and starts before the needles are fully elongated, simultaneously with divisions in the intercalary meristem. Maturation of needles continues throughout the entire growing season and is promoted by warm temperatures. The degree of maturation of needles affects various features, for example, the thickness of the needle cuticle, which may increase the ability of the needle to withstand drought stress in winter (Tranquillini 1979, Vanhinsberg, Colombo 1989).

The developmental stages as indicated by the morphological development before and after dormancy, are not definite. For instance, in mature trees of *Picea abies*, bud scales may be initiated both in late autumn and in spring (Hejnowicz, Obarska 1995). In one-year old seedlings of *Picea sitchensis*, with delayed bud development in autumn, a small number of needle primordia may also be initiated during the first part of the elongation phase in spring (Burley 1966).

Furthermore, developed organs may be transformed, as bud scale primordia may elongate from broad and flat needle-like structures during the change from sylleptic to proleptic free growth with increasing age (Wuehlish, Muhs 1986).

All initiated needle primordia are attached to the pith rib-meristem, through the peripheral zone, along the axis of the embryonic shoot. The role of cell division and cell elongation in lateral shoot elongation have previously been described in *Pseudotsuga menziesii* (Owens et al.1985) and in *Picea engelmannii* (Owens, Simpson 1988). Generally, the preformed undifferentiated pith cells in the pith rib-meristem start to divide and elongate after dormancy. Early shoot elongation before flushing results from a rapid increase in mitotic activity in the pith rib-meristem, whereas late shoot elongation after flushing results from cell elongation. Shoot elongation as a whole results in increased mean stem-unit length. In *Picea engelmannii*, elongation of the original pith cells has been found to account for less than 15 % of the final lateral shoot length. The remaining increase in shoot length was due to cell divisions and similar elongation in the length of the resulting daughter cells. Considering the overall similarities in shoot anatomy within the Pinaceae, the original pith cells and daughter cells seem likely to make similar relative contributions to final shoot length in *Picea abies*.

Cell division in plants takes place in meristems, where cells pass through and between the different stages of the mitotic cell cycle. After cytokinesis, the cell passes through G1 and at a specific point late in G1, the fate of the cell is decided from one of four fates; to divide, arrest, differentiate, or senesce (Jacobs 1995). In addition to the decision point, checkpoints exist between the different phases where the conditions are checked before the cell cycle proceeds. Specific regulators control the checkpoints and the presence of these regulators in non-dividing plant cells may confer upon them mitotic competence. The developmental plasticity of plants suggests that an intermediate, developmentally metastable phase (G0), between proliferation and terminal differentiation, may exist in vegetative plant cells (Jacobs 1995).

The number of cells produced in a meristem during a year is dependent on four cell cycle parameters: the frequency of cells undergoing mitosis, the rate of cell division, the size of the meristem, and the duration of cell cycle activity.

The frequency of cells undergoing mitosis, as expressed by the Mitotic Index (MI), is the most frequently and readily used cell cycle parameter (Grob, Owens 1994). In mature trees, MI has been related to the developmental stage of vegetative buds (Owens. Molder 1973) and water relations (Owens et al. 1985, Owens, Simpson 1988). Based on these relationships, and similar relationships occurring in seedlings, MI represents a powerful tool that can be used to describe cellular conditions in apical meristems throughout the seasonal growth cycle.

Fig. 1.
Embryonic lateral shoot of *Picea abies* (origin 60° 35' N) collected in a field test at Sävar (63° 54' N, 20° 33' E, alt. 10 m) on June 2, 1997. At the top is the apex, in the middle are elongating preformed needles and the pith rib-meristem, and below these is the crown. The vertical line indicates 1 mm. (Courtesy P. HÖRSTEDT, Dept. of Pathology, Univ. of Umeå, Sweden)

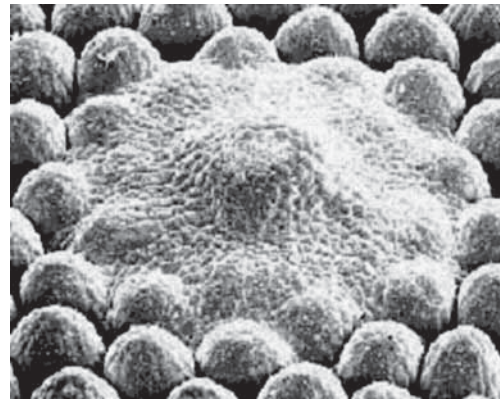

Fig. 2.
Apex of a terminal lateral shoot in *Picea abies* (Clone 115, origin 63º N) collected in a field test at Sävar (63° 54' N, 20° 33' E, alt. 10 m) in November 1995 (Courtesy P. HÖRSTEDT, Dept. of Pathology, Univ. of Umeå, Sweden)

The relationships between MI and other cell cycle parameters are not consistent, and a change in MI does not necessarily indicate changes in factors such as the rate of cell division. For instance, in roots of *Zea mays* the length of the mitosis phase is not extended in proportion to the length of the cell cycle (CLOWES 1960), whereas in roots of *Vicia faba* all stages of the cell cycle are reduced proportionally with lowered temperature (MURIN 1981). In addition, cells in different zones of a

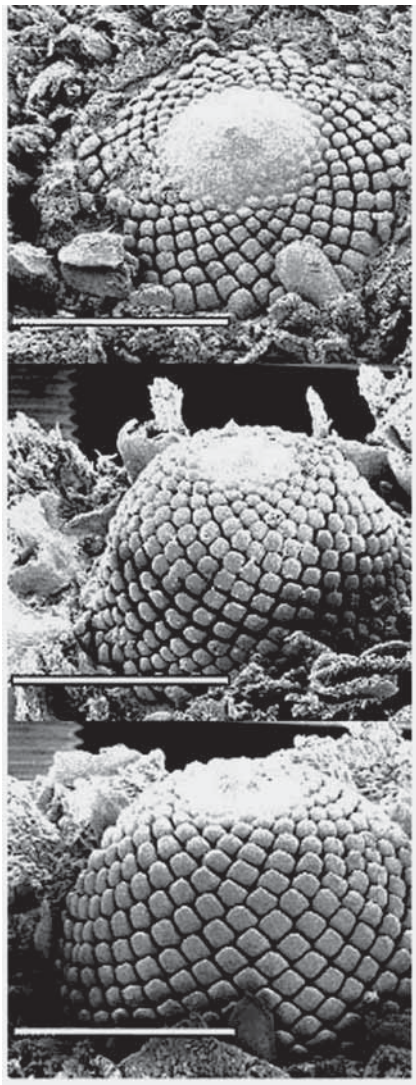

Fig. 3 a-c.
Embryonic leader shoots of *Picea abies* (origin 63 oN) collected in a field test at Sävar (63^{0}54´N, 20^{0}33´E, alt. 10 m) on August 28 (top), September 30 (middle) and October 23 (below) in 1998. The horizontal lines indicate 1 mm. (Courtesy P. Hörstedt, Dept. of Pathology, Univ. of Umea, Sweden)

meristem may show varying and independent rates of cell division and MI (Clowes 1960). Furthermore, it is obvious that large meristems tend to produce more cells than small meristems, but meristem size is in turn dependent on the rate of cell division and the frequency of cells undergoing mitosis.

The duration of cell cycle activity is species-dependent and varies between organs and tissues. In conifers with a distinct dormancy phase during winter, cell division resumes in the apical meristem in spring. In the embryonic shoot, cell division starts first in the needle primordia (Hejnowicz, Obarska 1995) then spreads to the peripheral zone, the pith rib-meristem and, lastly, the apex. In autumn, mitotic activity ceases first at the apex, then in needle primordia and internodal tissues, and finally in the youngest needle and bud scale primordia (Owens 1968, Hejnowicz, Obarska 1995). In the meristem of elongating organs, e.g. in needles and the pith rib-meristem of shoots, cell division occurs as long as the organ elongates (Owens 1968, Hejnowicz, Obarska 1995).

MATERIAL AND METHODS

The studies were performed in two non-juvenile Norway spruce field tests at Sävar (63° 54' N, 20° 33' E, altitude 10 m) in northern Sweden. In one field test seasonal changes in photochemical activity, mitotic index and cold hardiness of three clones of *Picea abies* were investigated. In the other field test seasonal changes in photochemical activity, mitotic index and cold hardiness were studied on natural and selected populations (progenies from selected plus trees). In both field tests the actual number of stem units (needles) produced by the apical meristem were studied by counting the number of needles on shoots the following year. The importance of duration of mitotic activity for production of stem-units (needles) was evaluated.

RESULTS AND DISCUSSION

Initiation of growth in spring

In early spring (April) the levels of apical mitotic activity, as expressed by the mitotic index (MI), increased rapidly in all populations. The high levels of mitotic activity appeared not to be influenced solely by temperature, as previous periods with relatively warm temperatures had no apparent effect on MI levels. The results indicate a synchronous release of a cell cycle blockage. Previous studies of MI in *Pseudotsuga menziesii* (Mirb.) Franco (Owens, Molder 1973), *Fraxinus excelsior* (Cottignies 1979) and *Abies balsamea* (Mellerowicz et al.1989) indicate that the cell cycle is blocked during autumn in the G1/S boundary.

After the short characteristic period in early spring, with high levels of apical mitotic activity

in all populations, the activity levels decreased in all populations. In mid spring, mitotic activity levels tended to increase again, following a gradual increase in temperature. The mitotic activity levels appeared to be mainly dependent on temperature. However, an effect of population origin was also seen, as northern populations showed higher MI levels than southern populations, indicating an earlier start of growth in the northern population. Furthermore, differences in MI levels indicated that growth started later in one of three clones of similar origin studied, than in the other two. The MI levels in spring appeared to reflect variation in temperature and population origins, but no effect of selection was detected. The results are in accordance with those of Cannell, Willett (1975) who showed that between both *P. sitchensis* and *P. contorta* populations, northern populations initiated apical meristematic activity before more southerly ones.

Overall, the results from spring indicate a genetic variation in initiation of growth, both among populations and among clones of similar origin. Initiation of growth tended to start later in populations of southern origins than in those of northern origins, and differences in initiation of growth were observed among clones of similar origin. After growth had been initiated, further development of growth in spring was influenced by an increase in temperature. Variation among populations in initiation of growth could not be explained solely by different temperature requirements for initiation and, therefore, other factors appeared to be involved. Generally, the different parameters that were used to show different aspects of the dynamics of growth in spring appeared to give similar results, which indicate that they may be used interchangeably.

Dehardening in spring

The results on dehardening in spring indicate a genetic variation in dehardening, both among populations and among clones of similar origin. In all populations dehardening tended to progress from very high hardiness levels in mid-April down to low summer hardiness levels in late May or early June. Populations of northern origins dehardened slightly earlier than populations of southern origins and a difference in dehardening between clones of similar origin was evident. Selection resulted in a slightly later dehardening in selected populations than in comparable natural populations. Variation among populations in initiation of dehardening could not be explained solely by differences in temperature requirements for dehardening. Thus, other factors appear to be involved. After dehardening had been initiated, further dehardening was promoted by increasing temperatures.

Conclusions regarding spring events

Taken together, the results on growth and dehardening in spring indicated genetic variation both among populations and among clones of similar origin. Initiation of growth and dehardening tended to start later in populations with southern origins than in populations with northern origins. Early initiation of growth appeared to be related to early dehardening among clones of similar origin. Furthermore, selection appeared to result in a later start of growth and a slightly later dehardening than in natural populations of similar origin. Variation among populations in initiation of growth and dehardening could not be explained solely by differences in temperature requirements, so other factors are likely involved. However, after growth and dehardening had been initiated, both processes were promoted by increases in temperature.

Cessation of growth in autumn

Apical mitotic activity in autumn, as expressed by MI, ceased earlier and declined more sharply in populations of northern origins than in populations of southern origin. Cessation of diameter growth showed no relation to the duration and level of apical MI. Among the studied clones no consistent difference in mitotic index (MI), either in period or in general levels was observed. Cessation of apical mitotic activity occurred later in selected populations than in natural populations of similar latitudinal origin, indicating there was either an effect of plus-tree selection or a long-lasting effect of the seed orchard environment (Johnsen 1989a, b) on the timing of growth cessation. Cessation of mitotic activity appeared to be influenced by population origin, whereas the effect of temperature was unclear as the

response of mitotic activity to temperature differed in spring and autumn. The results are consistent with results presented by Cannell, Willett (1975) showing that the point at which apical growth slowed down in autumn was closely correlated with latitude of seed origin. Altogether, the results on growth cessation in autumn indicated genetic variation in cessation of growth both among and within populations. Cessation of growth occurred later in populations of southern origins and then in those of northern origins, and a selection effect on cessation of growth was observed. Generally, the level of mitotic activity in autumn was influenced by temperature, as both the production of NSU and MI levels appeared to decrease with decreasing temperature. However, the influence of temperature on cessation of mitotic activity turned out as small.

Hardening

In autumn, the frost hardiness levels of needles, as expressed by the Fv/Fm-ratio after freezing, gradually increased from early September to late October. Northern populations hardened earlier than southern populations but no selection effects were observed. Furthermore, a difference in frost hardiness levels was observed among clones of similar origins, as frost hardiness developed 1 - 2 weeks later in one of the three studied clones. The rate of hardening appeared to be most rapid when the daily mean temperature fell to near or below +5 °C but was not related to the occurrence of frost. Northern populations were more frost resistant than southern populations, indicating a response to photoperiod.

Conclusions regarding autumn events

Altogether, the results on growth and frost hardiness development in autumn indicated genetic variation in cessation of growth and frost hardiness development, both among populations and among clones of similar origin. Generally, cessation of growth and frost hardiness development tended to start later in populations of southern origins than in populations of northern origins. In non-juvenile trees both the level of mitotic activity and the level of frost hardiness in autumn appeared to be influenced by temperature. However, the level of frost hardiness in needles appeared to be affected by both temperature and day length, whereas cessation of growth in buds appeared to be mostly affected by day length. Prolonged MI activity in autumn may be associated with a higher risk of frost damage to buds. In contrast, in juvenile seedlings strong correlations appeared to exist, at the population level, among growth cessation traits and frost hardiness levels.

CONCLUDING REMARKS

The results indicated genetic variation in initiation and cessation of growth, as well as in initiation of dehardening and hardening, both among populations and among clones of similar origins.

Natural populations of southern origins tended to initiate growth and dehardening later in spring and to start growth cessation and hardening later in autumn, than populations of northern origins. Natural populations transferred more than approximately 3º in latitude showed poor growth performances mainly because they had lower numbers stem-units. Based on these findings, the prolonged mitotic activity observed in southern populations seems to be of ambiguous value, since it may increase the risk of frost-related bud injuries rather than increase of the amount of stem-units produced.

Progenies of selected plus-trees showed a later start of growth and slightly later dehardening in spring. Growth cessation occurred later in juvenile seedlings of selected populations. Furthermore, in non-juvenile trees of selected populations MI activity was prolonged, compared with natural populations of similar origin. Needle frost hardiness levels in selected populations were similar to those of natural populations of similar origin. Selected populations of northern origins tended to produce more stem-units than natural populations of similar origin. Growth and hardiness performances of southern populations and of selected populations of local origin appeared, at least in part, to have a similar physiological basis.

In spring, initiation of growth and dehardening appeared to be correlated with the later occurring

bud burst both among populations and among clones, indicating that the present use of bud burst is valid and practical indicator of growth initiation. Initiation of growth and dehardening could not be solely explained by temperature.

In autumn, juvenile traits on juvenile seedling such as bud set, height growth cessation, degree of shoot lignification or frost hardiness level appeared to be possible indicators of growth cessation, at least at the population level. In non-juvenile populations, the amount of shoot growth appeared not to be correlated with duration of mitotic activity. Therefore, in non-juvenile populations, traits related to cessation of shoot growth appear to have less potential for use as indicators of growth cessation. However, it may be possible to use traits related to the duration of apical mitotic activity, e.g. bud frost hardiness, as prolonged mitotic activity could imply increased risk of bud injuries in late autumn. Initiation of growth cessation appeared to be influenced by photoperiod, and needle hardening by photoperiod and temperature. Generally, the development of needle hardiness in autumn appeared not to be fully correlated to levels of mitotic activity either among populations or among clones of similar origins.

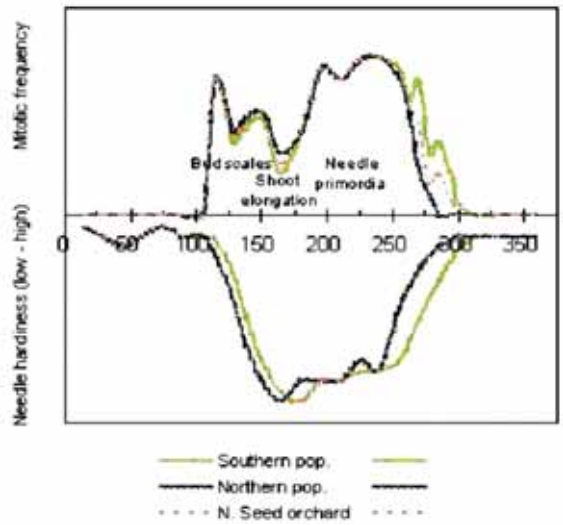

Fig. 4.
Schematic presentation of the seasonal variation in mitotic frequency and needle hardiness. Day numbers are indicated.

PRACTICAL IMPLICATIONS

- The effects of the test environment on the effects of seed transfer and selection of individual trees need to be studied. For example, prolonged mitotic activity may represent a growth potential that is mainly expressed as high numbers of stem units in favourable environments, e.g. on south facing slopes, in contrast to less favourable environments, such as flat abandoned farmland, where it may lead to low numbers of stem units.
- Breeding of Norway spruce may benefit from more distinctly defined growth traits than those traditionally used, like accumulated height growth. For instance, it may be useful to separate growth traits related to growth rhythm, e.g. duration of mitotic activity, from traits which are not, or only moderately, related to growth rhythm.
- To improve early testing, genetic correlations between similar traits in juvenile and non-juvenile trees must be identified. For example, the correlations among traits observed in juvenile seedlings, e.g. bud set and frost hardiness, with growth traits found in non-juvenile trees that are related to apical

mitotic activity e.g. duration of such activity.

- The internal factors and regulating substances involved in the seasonal development of growth and frost hardiness levels need to be characterised more fully, as the seasonal development of growth and frost hardiness levels is important for high growth performance.
- The relationship between rate of cell division and production of stem-units needs to be studied, as high growth performance and high numbers of stem-units appeared not to be correlated with prolonged mitotic activity. New tools in cell molecular biology are now available that makes it possible to measure the rate of cell division, which should help in this task.
- The relationship between needle and bud hardiness levels in non-juvenile trees and mitotic activity, e.g. duration of apical mitotic activity also needs to be investigated. This includes development of new methods for non-destructive bud hardiness measurements.

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STUDY OF SELECTION CRITERIA - LONG-TERM AND EARLY TESTS OF EUROPEAN LARCH SEED ORCHARDS

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ABSTRACT

The report presents results from evaluation of three verification plots of the long-term character and two testing plots of early tests character. These plots were established for comparison and classification of the European larch progeny from seed orchards. The experiment should result in verification of genetic value of seed orchards and classification of the most valuable units into the category of certified reproduction material resources. The experiment was established according to standard methodology, and 37 progeny planted from seed that originated from 19 seed orchards were used for comparative plantings. The two-year old non-replanted seedlings were planted in three repetitions on seven verifying plots with 50 individuals for one lot of the size 10 x 10 m. The progeny of the stand for the certified crop (provenance Opočno) was taken as the comparative standard. For the purposes of EU Project "Towards a European Larch Wood Chain", three long-term plots have been included, together with two early tests established at a very narrow spacing 0,50 x 0,50 cm, with 16 seedlings for one progeny planted in four repetitions. The number of growing individuals was recorded and height growth and stem form of the tested progeny were evaluated at 6 years after planting. Using standard mathematical and statistical methods (basic characteristics, analysis of variance (ANOVA), correlation coefficients /just for parallel plots 133, 134/), we assessed the progeny value by comparing them to the standard population. Another goal of this study was to compare and judge results received both from long-term and early tests. Based on the investigations realized on long-term plots, two seed orchards (Znojmo-Tvořihráz, Bučovice-Mouřínov) were classified like the units the progeny parameters of which are much better compared to the comparative population. The results of evaluating height growth in the early tests were not so comparable to results from long-term tests. Survival results were more or less comparable in both long-term and early tests. Given that the study trees are still quite young, our results must be considered preliminary. These initial results will be the foundation for further investigation of plantings tested on long-term plots.

INTRODUCTION

In the early 1950s, when Dr. Rubner began extensive research in the species, European larch (*Larix decidua* Mill.) forests in the Jeseníky Mts. and neighbouring areas of North Moravia and Silesia covered 3,771.94 ha. Although Dr. Rubner's report did not break out the forestland by ownership size, it is possible to conclude that smaller forest ownerships comprised 80 % of the autochthonous European larch stands of Sudeten origin and artificially planted Sudeten larch stands, with the remainder being on larger forest units (those owned and managed by larger ownerships, especially by manorial owners).

Our latest information (provided by the Institute for Forest Management at Brandýs nad Labem in 1992) states that current composition of European larch of Sudeten origin covers 8,991 ha, including both autochthonous areas (Sudeten Mountains) and cultural distribution (the remaining area of the Czech Republic). This area represents an 138% increase over the last 40 or so years. This increase corresponds with larch composition changes through the entire Czech Republic. In the 1940s, larch represented 1.6 % of the area of Czech forests, while larch comprised 3.3 % in 1992 and 3,5 % of a reduced forested area (ca 90,000 ha) today. Efforts to increase the composition of European larch continue in the Czech Republic.

Based on calculations and projections (Šindelář 1994), we expect larch to become 5 % of the total forested land.

European larch is a component in forest stands above 800 m mostly as an admixture with Norway spruce, silver fir, European beech, and in lower areas with Scots pine, sessile oak and small-leaved linden. Since European larch is a very valuable tree species growing well on sites outside of its original locations, our agency has focused on preservation and reproduction of this tree species' gene sources. In particular, we established four national nature preserves of totalling 108.03 ha, gene bases with a total area of 1,915.79 ha, forest stands certified as seed sources, registered reproductive plantings and elite trees. To prevent contamination of local populations, we do not permit the use of reproductive material from other areas, within the framework of the autochthonous area of the Sudeten ecotype of European larch.

European larch (Sudeten ecotype) is valued for its fast growth, considerable biomass production and especially for the adaptability to various environment conditions. As part of mixed stands, larch increases the forest ecosystem's stability. Although natural regeneration of larch is usually quite successful in the Czech Republic, it is necessary to employ artificial regeneration in localities where larch is not represented, or in those cases where natural regeneration is not fully successful. To satisfy these needs, there are sources of larch reproductive material, in the framework of the Czech Republic forest management. First of all, we have seed stands certified as seed sources totalling approximately 3,000 ha. Seed crop is produced not only in autochthonous areas, but also in valuable stands of artificial origin. Results of our research have documented that a considerable part of these cultural larch stands is of Sudeten origin. The characteristics of tested progeny of the planted populations were comparable with characteristics of Sudeten autochthonous progeny, especially growth intensity, total volume production and wood quality.

Seed orchards represent another measure for preservation and reproduction of gene resources of European larch (Sudeten ecotype). The total area of larch seed orchards established since the late fifties is 89.62 ha. European larch (Sudeten ecotype) seed orchards established in the area of its natural origin encompass 3.60 ha. Furthermore, there were other Sudeten larch seed orchards and clonal archives established in other areas of the Czech Republic.

As the Czech Republic joins European political and economical organizations, regulations concerning the trading of forest reproduction material must be adjusted. Reproduction material resources must be classified into four categories, and these categories must be incorporated into the present classification system, one with the long tradition in the Czech Republic. The resources marked as identified, selected and qualified according to the OECD regulations are similar to systems used in CR although "verified - tested resource" is a category, which is not considered in the Act on Forests and the connected decree no. 82/1996.

The testing of larch seed orchards' progeny and individual elite trees is subject of current research in the Czech Republic. Our goal is to verify genetically conditioned characteristics both in the larch seed orchards and in elite trees. Especially in the case of verification (testing) of larch seed orchards, it will be possible to assess the value of the larch seed orchards by comparison with standards from certificated larch forest seed stands. We are testing elite trees in order to identify which ones to use for establishment of second-generation seed orchards. Such seed orchards should gradually replace seed orchards established on the basis of phenotype selection.

MATERIAL AND METHODS

We established seven verification plots of long-term character (no. 130 – 136) established in 1994. Three of them (no. 133 A, 134 A, 136) are included together with two early tests in EU Project "Towards a European Larch Wood Chain". Two plots (no. 133 B, 134 B) also established in 1994 as early tests is represented by 37 progeny planted from the seed gathered in seed orchards and by one standard provenance – stand progeny certified for seed crop. Seed orchards, which were the sources for progeny evaluated in this study, are listed in table 1. Nineteen seed orchards were established between 1958

and 1984 in various areas of the Czech Republic. The age of seed orchards ranged between 5 and 11 years at the time of seed gathering for establishment of verification plots. Seed was gathered for 8 years (1982 to 1989) and stored in the forest seed bank in the Seed enterprise of the Forests of CR at Týniště nad Orlicí. Seed was collected in 8 seed orchards only once, in 6 seed orchards twice in various years, in 3 seed orchards three times and in 2 seed orchards four times. Except for items no. 24 and 25, the different parts of seed were investigated in all cases. The experiment included material from the repeated crop and from new progeny for assessing to what degree various proportions of seeds from the particular clones influence the growth, health state and the total value of progeny, probable various combination of hybridisation or even ecological conditions during fructification that can affect pollination, i.e. ratio of full seeds and their vitality.

As mentioned, the whole investigated material, i.e. 37 progeny of seed orchards, was planted onto three long-term verification plots which varied by the area of available plots and number of planted seedlings. Two-year old, non-transplanted ("2-0") seedlings were planted in three repetitions on seven verification plots with 50 individuals for one lot at spacing of 10 x 10 m. Two of these sites had a modified methodology, consisting of smaller spacing, lower number of plants for progeny at representing all the tested variants and smaller plots for early tests (very narrow spacing 0,50 x 0,50 cm, with 16 seedlings for one progeny planted in four repetitions). These early tests were planted in the corner of two long-term plots. The remaining four long-term verification plots were planted only with progeny from seed orchards; with 17 to 28 progeny on each plot.

Clone composition of seed orchards differs regarding the origin of plus trees; only two seed orchards (Vlašim, Mladá Vožice; Tábor, Bechyně) contain plus trees from the area of Sudeten larch. In three cases the seed orchards were established only with non-original allochthonous material (Broumov, Hvězda; Křtiny at Brno; Nové Hrady, Stropnice). In all other cases clone progeny in seed orchards were represented by plus trees from the Sudeten larch area and from other different areas outside the original area. The certified stand of category B (B-MD-514-17-2-RK) Opočno is the standard comparative population.

In autumn 1994 and 1995 the first inventories were done on all 7 verification plots (9 actually, when you consider plots no. 133 and 134 were split into A and B). From 1994 to 1998, height growth was measured on two plots destined for early testing. In 1998 we also measured stem form on these plots. We measured heights on the long-term investigation plots in autumn 1995 and 1997. In 1998, we measured stem form, too. The proportion of growing individuals is one of the criteria for the progeny adaptive ability. We calculated basic statistics, arithmetical averages and standard error of the mean, for height growth. We analysed height growth in an ANOVA based on progeny repetition. We compared our results based on the particular progeny with the coefficients of the standard progeny.

The results presented below evaluate three of the seven long-term verification plots. Evaluation of plantings for early diagnosis is also included. The validity of the early tests' predictive ability will be judged more effectively in the future, when we have results from additional ages and development stages.

RESULTS

Brief summary of evaluation and measurements of long-term plots

We examined survival and height growth in three long-term plots at the age of 6 years. Percentage of survival is very variable compared to the control, as well as in relation to the study mean. It ranges from 87 to 146 % of the mean, or from 85 to 143 % compared to the control. There was less variability in the case of height growth, which varied around the mean of 2,35 m at age 6 years. Expressed as a percentage, variability of height growth ranges from 90 to 115 % compared to control, 89 to 111 % compared to the study mean. The differences in height growth between categories were statistically significant, mostly at the 0,01 level.

Our preliminary results suggest that it is possible to approve some tested units (or seed orchards themselves) for addition to the category of tested sources of reproductive material. The criteria were the

percentage of survival and mean height growth compared to both the control and study mean. For now, our conclusions are concerned with only three progeny (three seed orchards), where we have the best results. Two of the progeny from this "best group" are from the same seed orchards, planted in two crops. In height growth, these progeny exceeded the control by 11 – 17 %; in survival percentage by 7 – 10 %.

Brief summary of evaluation and measurements of early tests

We also analysed results from measurements of early tests. Height growth was measured every year since age 2 years. At the age of 6 years, evaluation of stem form was added. Stem form, which is known to be very variable in larch, was categorized using the following classification scale: 1 – absolutely straight, 2 – slightly curved, 3 - middle curved, 4 – heavy curved, 5 – shrubby stem.

In plot no. 133 B (Milevsko, Olešná), at the age of 6 years, percentage of survival presents 54 % (1,323 surviving individuals from 2,432 seedlings planted in 1994). Number of growing individuals in relation to progeny ranged from 17 to 48 trees, compared to the survival in the control of 58 % of original number (37 trees from 64 seedlings planted in 1994). We found higher survival in plot no. 134 B (Přeštice, Nýřany), where 1,819 growing individuals (75 %) survived from 2,432 originally planted seedlings. There was a great deal of variation in the survival numbers, ranging from 34 to 56 growing individuals (i.e. 53 – 89 % of the originally planted seedlings). Survival percentage in the control was 77 %. If we created group of five best progeny for every of two plots with early tests, there is only one progeny (no. 15 – Bučovice, Mouřínov) possible to involve to both of these two groups.

As mentioned above, we also examined selected progeny in two plots with early tests at the age of 6 years. In plot no. 133 B (Milevsko, Olešná), at the age of 6 years, average heights of all progeny ranged from 185,68 cm to 331,58 cm, with a mean of 254,99 cm. In plot no. 134 B (Přeštice, Olešná), at the age of 6 years, average mean heights ranged from 197,50 cm to 384,00 cm, with a mean of 270,33 cm. The mean heights of the control progeny were 185,68 cm in plot no. 133 B and 227,65 cm in plot no. 134 B. In both cases, these average values were below the study mean. Results of ANOVA calculated for height growth are statistically highly significant for both plots with early tests, on the 0.01 level. If we created group of five best progeny for each of the two plots with early tests, various and different progeny would be involved in both of these two groups.

In the two plots with early tests, we also evaluated stem form. The category of trees with a very slightly curved stem form was the dominant category in both plots with early tests. This "sinuosity" is connected with relatively fast growth at a young age. In plot no. 133 B, average value of stem form was 2,07 and varied from 1,58 to 2,47. Results in plot no. 134 B, were slightly better; the average value of stem form was 1,50 and ranged from 1,36 to 1,78. Values from plot no. 133 B corresponded to the average value of the study. In the case of plot no. 134 B, the results were considerably below the average value of the study. Results from our ANOVA of stem form indicate that differences among individual progeny in both plots with early tests were statistically significant at the ($p \leq 0{,}01$) level. Based on these results, it was possible to choose five best progeny from each plot based on stem form characteristics. Two progeny judged as well stem formed were found in both plot no. 133 B and in plot no. 134 B. The other three progeny in each of these two groups were different each other.

As result of this preliminary selection, we identified the following four progeny (no. 1, 2, 8, 15). Progeny no. 1 and 8 were from three seed orchards established during 1969 – 1975 (units no. 2, 15 were progeny of the same seed orchard, but from different seed years).

CONCLUSIONS

Comparison of results from early tests and from parallel long-term plots

Generally, the reason why we established early tests at the possible youngest age, is our presumption that results of progeny evaluation and measurement as early tests will be indicative for information about growth and development at later stages of development. To test this presumption, we conduct early tests and long-term tests and then compare the results. Examining European larch genetics in this manner,

No. of progeny of seed orchard	Origin of progeny - seed orchard having been tested		
	Seed orchard no.	Forest Enterprise, Forest district	Local name
1	SS-MD-45-38-4-ZN	Znojmo, Znojmo	Tvořihráz
2	SS-MD-42-38-4-VY	Bučovice, Mouřínov	Vrčava
3	SS-MD-27-38-3-NA	Broumov, Hvězda	Šlégl
4	SS-MD-2-16-5-HB	Ledeč nad S., Čerňák	Čerňák
5	SS-MD-33-38-4-KM	Bystřice p. H., Lukov	Úlehla
6	SS-MD-10-16-5-BN	Vlašim, Mladá Vožice	97 B 1
7	SS-MD-53-17-2-PZ	Zbraslav nad Vlt., Slapy	Pabožek
8	SS-MD-18-16-4-LT	Litoměřice, Budyně	Šebín
9	SS-MD-87-30-3-BO	ŠLP Křtiny, Bílovice	Olomučany
10	SS-MD-45-38-4-ZN	Znojmo, Znojmo	Tvořihráz
11	SS-MD-73-29-4-ZN	Znojmo, Znojmo	Kuchařovice
12	SS-MD-29-38-4-UH	Buchlovice, Koryčany	Vršava
13	SS-MD-24-16-5-CK	Nové Hrady, H. Stropnice	Konratice
14	SS-MD-0-10-5-PZ	VÚLHM Jíloviště-Strnady	Baně
15	SS-MD-42-38-4-VY	Bučovice, Mouřínov	Vrčava
16	SS-MD-23-6-4-PJ	Spálené Poříčí, Polanka	Silov
17	SS-MD-49-28-4-NJ	Vítkov, Odry	Kletná
18	SS-MD-32-10-4-TA	Tábor, Bechyně	„U obrázku“
19	SS-MD-73-29-4-ZN	Znojmo, Znojmo	Kuchařovice
20	SS-MD-73-29-4-ZN	Znojmo, Znojmo	Kuchařovice
21	SS-MD-27-38-3-NA	Broumov, Hvězda	Šlégl
22	SS-MD-3-10-5-HB	Ledeč nad S., Čerňák	Čerňák
23	SS-MD-1-28-5-OL	Šternberk, H. Žleb	Světlov
24	SS-MD-2-16-5-HB	Ledeč nad S., Čerňák	Čerňák
25	SS-MD-2-16-5-HB	Ledeč nad S., Čerňák	Čerňák
26	SS-MD-49-28-4-NJ	Vítkov, Odry	Kletná
27	SS-MD-29-38-4-UH	Buchlovice, Koryčany	Vršava
28	SS-MD-42-38-4-VY	Bučovice, Mouřínov	Vrčava
29	SS-MD-18-16-4-LT	Litoměřice, Budyně	Šebín
30	SS-MD-42-38-4-VY	Bučovice, Mouřínov	Vrčava
31	SS-MD-18-16-4-LT	Litoměřice, Budyně	Šebín
32	SS-MD-33-38-4-KM	Bystřice p. H., Lukov	Úlehla
33	SS-MD-73-29-4-ZN	Znojmo, Znojmo	Kuchařovice
34	SS-MD-45-38-4-ZN	Znojmo, Znojmo	Tvořihráz
35	SS-MD-23-6-4-PJ	Spálené Poříčí, Polanka	Silov
36	SS-MD-24-16-5-CK	Nové Hrady, H. Stropnice	Konratice
37	SS-MD-58-15-4-PI	Písek, pl. 58	Štěkeň
38	B-MD-514-17-2-RK	Opočno	

Origin of progeny - seed orchard having been tested				
Altitude (m a. s. l.)	**Year of establishment**	**Area (ha)**	**Number of clones**	**Spacing (m)**
300	1975	3.25	70	6 x 6
350	1974	5.70	118	6 x 6
	1973	1.15	38	6 x 6
450	1960	1.03	45	4 x 4
350	1973	1.05	29	6 x 6
600	1962	1.23	37	5 x 5
	1976	1.13	36	6 x 6
165	1969	2.70	97	6 x 6
330	1984	2.11	76	6 x 6
300	1975	3.25	70	6 x 6
300	1978	2.94	36	6 x 6
490	1973	2.00	74	6 x 6
650	1973	1.04	37	6 x 6
380	1962	1.8	145	6 x 6
350	1974	5.70	118	6 x 6
485	1973	4.23	135	6 x 6
320	1976	7.00	72	6 x 6
490	1973	1.60	36	6 x 6
300	1978	2.94	36	6 x 6
300	1978	2.94	36	6 x 6
420-430	1973	1.15	38	6 x 6
450	1962	1.20	29	5 x 4
300	1958	1.20	39	8 x 8
450	1960	1.03	45	4 x 4
450	1960	1.03	45	4 x 4
320	1976	7.00	72	6 x 6
490	1973	2.00	74	6 x 6
350	1974	5.70	118	6 x 6
165	1969	2.70	97	6 x 6
350	1974	5.70	118	6 x 6
165	1969	2.70	97	6 x 6
350	1973	1.05	29	6 x 6
300	1978	2.94	36	6 x 6
300	1975	3.25	70	6 x 6
485	1973	4.23	135	6 x 6
650	1973	1.04	37	6 x 6
	1977	1.88	40	
Control standard - progeny of certified seed stand of cat. B				

Tab. 1.
Characteristics of tested European larch seed orchards

we intend to continue our study with the addition of additional information on the advanced stages of their development as soon as they will be available.

At present, we can only evaluate results of height growth of "early-test" progeny at the age of 2, 3, 4, 5 and 6 years and "long-term test" progeny at the age of 5 and 6 years. We calculated correlation coefficients for these relationships. In earlier measurements, we compared results of early tests progeny height growth at the ages of 2, 3, 4 and 5 years and results of long-term tests progeny height growth at the age of 5 years. As it is indicated in tab. 6, correlation coefficients of comparisons were statistically non-significant. For now, these results indicate a different character of height growth and development of progeny tested both as early tests and long-term tests. We must remember that these results are limited to progeny having been tested both as early and long-term tests established in the same localities using the same lots of seedlings. In attempting to explain the cause(s) of height growth dissimilarity, we might assume that there were different methodologies in the establishment of both types of research plots, considerable phenotypic variation in response to varying levels of competition and mortality, both during and after the young plantations' closing. For now, we cannot estimate if the results of evaluation and measurement of long-term tests obtained at the advanced stages of their development will have a higher predictive ability.

Given these conditions, there are some surprising results of model selection made on parallel plots of both categories. We found the best progeny (no. 1, 2, 8, 15) on research plots no. 133 and 134. These progenies' height growth, survival and stem form characteristics notably exceeded the characteristics both of the control progeny and study mean. Based on this preliminary selection, we identified these progeny as candidates for the category of tested sources of reproductive material. As we mentioned in chapter on results of long-term plots evaluation, these progeny came from three seed orchards established during 1969 – 1975 (units no. 2, 15 were progeny of the same seed orchard, but from various years of seed crop).

This report presents just the first, basic and orientation communication about our study. Findings available for practical use of early tests will be accessible by further systematic evaluation of research plots with European larch seed orchards progeny established within the framework of reproductive material testing and within the process of testing genetically conditional value of seed sources.

Based on our results, we conclude that European larch seed orchards in the Czech Republic are effective from the point of view of practically oriented selection as well as from the point of view of technical character and management of seed crop. Along with testing of seed orchards with the aim selecting and designating the best units as the category of tested source of reproductive material, the seed orchards support other research programs oriented toward investigation and evaluation of individual elite trees progeny from controlled crossings and opened pollination. The aim of these tests is the subsequent selection of the best elite trees (clones) to establish second-generation seed orchards. These new seed orchards will, in turn, foster further breeding progress and should gradually replace seed orchards and clones having been selected based on phenotypic selection.

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SUMMARY (IN CZECH)

V příspěvku jsou prezentovány výsledky hodnocení výzkumných ploch s potomstvy semenných sadů modřínu opadavého. Předmětem hodnocení byla potomstva na třech dlouhodobých plochách a na dvou plochách s časnými testy. Výzkumné plochy byly založeny s cílem porovnání vlastností a charakteristik potomstev semenných sadů a jejich následné klasifikace. Experiment by měl ověřit genetickou hodnotu semenných sadů a na základě získaných výsledků by měl umožnit navržení nejhodnotnějších jednotek do kategorie ověřených zdrojů reprodukčního materiálu. Založení série sedmi dlouhodobých ploch a dvou ploch s časnými testy s 37 potomstvy vypěstovanými z osiva sklizeného v různých letech z 19 semenných sadů bylo realizováno podle běžně používaných metodických principů. Dlouhodobé srovnávací plochy byly založeny systémem náhodného blokového uspořádání, přičemž byly dvouleté neškolkované sazenice vysazeny ve třech opakováních od každého potomstva na parcely o rozměrech 10 x 10 m při sponu 2 x 1 m. Na jedné parcele tak bylo vysazeno 50 sazenic, pro jedno potomstvo tak bylo použito 150 sazenic. Jako srovnávací standard bylo použito potomstvo uznaného porostu pro sběr osiva kategorie B (provenience Opočno). Do hodnocení realizovaného v rámci programu projektu EU "Towards a European Larch Wood Chain" byly zahrnuty tři výzkumné plochy dlouhodobého charakteru s kompletním počtem všech 37 testovaných potomstev, spolu s oběma plochami charakteru časných testů, které byly založeny na stejných lokalitách jako dvě plochy dlouhodobého charakteru. Výsadby časných testů byly realizovány rovněž systémem náhodného blokového uspořádání, se čtyřmi opakováními, při velmi hustém sponu 0,50 x 0,50 m, s počtem 16 sazenic na parcele o rozměrech 2 x 2 m. Pro jedno potomstvo tak bylo vysazeno 64 sazenic. V rámci hodnocení byl na třech dlouhodobých plochách a na dvou plochách s časnými testy v roce 1998 ve věku 6 let evidován počet rostoucích jedinců a byl měřen výškový růst spolu s hodnocením tvárnosti kmene. Výsledky hodnocení a měření všech pěti ploch byly zpracovány základními matematicko-statistickými postupy (základní charakteristiky, analýza variance), pro paralelní plochy č. 133 A-B a 134 A-B byly navíc kalkulovány i korelační koeficienty zahrnující výsledky hodnocení vztahu výsledků hodnocení na plochách s časnými testy ve věku 2 až 6 roků a výsledků na paralelních dlouhodobých plochách získanými ve věku 5 a 6 let. Získané hodnoty charakteristik potomstev semenných sadů byly porovnávány s hodnotami průměrných charakteristik srovnávacího standardního potomstva. Dalším cílem hodnocení potomstev na obou typech výzkumných ploch bylo porovnání charakteristik zjištěných u potomstev testovaných na dlouhodobých plochách s vlastnostmi potomstev hodnocených na plochách s časnými testy. Na základě výsledků zjištěných na plochách dlouhodobého charakteru byly klasifikovány dva semenné sady (Znojmo-Tvořihráz, Bučovice-Mouřínov) jako jednotky, které by přicházely v úvahu k navržení zařazení do kategorie testovaných zdrojů reprodukčního materiálu. V případě hodnocení výškového růstu potomstev na plochách s časnými testy bylo zjištěno, že získané výsledky nejsou srovnatelné s výsledky z dlouhodobých ploch, ačkoliv pokud jde o další hodnocené charakteristiky, jsou získané výsledky víceméně srovnatelné. Výsledky hodnocení je však třeba, s přihlédnutím k věku testovaných potomstev, posuzovat pouze jako předběžné. Zjištěné poznatky v současné době představují základ pro další šetření, přičemž je bude třeba konfrontovat s výsledky hodnocení v pozdějších vývojových stadiích testovaných potomstev.

	Testing of European larch seed orchards - long term research plots no.:						
	130 Lesy Steinských, Černošice	**131 Rožmitál pod Tř., Bohutín (Zdaboř)**	**132 Rožmitál pod Tř., Bohutín (Březnice)**	**133 A Milevsko, Olešná**	**134 A Přeštice, Nýřany**	**135 Nové Město, Moravec**	**136 Šenov, Petřvald**
Principles of establishment	**Randomized block system**						
Number of progeny	18	18	29	38	38	19	38
Number of repetitions	3	3	3	3	3	3	3
Number of blocks	54	54	87	114	114	57	114
Size of blocks (m)	10 x 10	10 x 10	10 x 10	10 x 10	10 x 10	10 x 10	10 x 10
Spacing (m)	2 x 1	2 x 1	2 x 1	2 x 1	2 x 1	2 x 1	2 x 1
Number of seedlings per block (pcs)	50	50	50	50	50	50	50
Number of seedlings per progeny (pcs)	150	150	150	150	150	150	150
Total number of seedlings to be planted (pcs)	2700	2700	4350	5700	5700	2850	5700
Border lines (pcs)	360	580	860	550	680	460	480
Totally planted (pcs)	3060	3280	5210	6250	6380	3310	6180
Totally planted area (ha)	0.54	0.54	0.87	1.14	1.14	0.57	1.14

Tab. 2.
Basic methodological principles for establishment of research plots for testing of European larch seed orchards (long term experiment)

Testing of European larch seed orchards - early tests - research plots no.:		
	133 B Milevsko, Olešná	**134 B Přeštice, Nýřany**
Principles of establishment	Randomized block system	
Number of progeny	38	38
Number of repetitions	4	4
Number of blocks	152	152
Size of blocks (m)	2 x 2	2 x 2
Spacing (m)	0.50 x 0.50	0.50 x 0.50
Number of seedlings per block (pcs)	16	16
Number of seedlings per progeny (pcs)	64	64
Total number of seedlings to be planted (pcs)	2432	2432
Border lines (pcs)		
Totally planted (pcs)	2432	2432
Totally planted area (ha)	608	608

Tab. 3.
Basic methodological principles for establishment of research plots for testing of European larch seed orchards (early tests)

In relation to 5 years of 133-4 A			
Plot no. 133 A-B correlation coefficients		**Plot no. 134 A-B correlation coefficients**	
A - X	0.2112	**A - X**	0.2283
B - X	0.0621	**B - X**	0.2284
C - X	-0.0723	**C - X**	0.2054
D - X	-0.0395	**D - X**	0.1697
E - X	-0.0371	**E - X**	0.1796
A - E	0.3066	**A - E**	0.5256
B - E	0.7328	**B - E**	0.7604
C - E	0.9477	**C - E**	0.9353
D - E	0.9748	**D - E**	0.9725
In relation to 6 years of 133-4 A			
Plot no. 133 A-B correlation coefficients		**Plot no. 134 A-B correlation coefficients**	
A - Y	0.2272	**A - Y**	0.1856
B - Y	0.1027	**B - Y**	0.1941
C - Y	-0.0159	**C - Y**	0.1919
D - Y	0.0115	**D - Y**	0.1622
E - Y	0.0286	**E - Y**	0.1809

A, B, C, D, E - average heights of early tests progeny at 2 - 6 years; X - average heights of paralell long-term tests at 5 years; Y - average heights of parallel long-term tests at 6 years

Tab. 4.
Correlation coefficients of relation among average heights. Critical value of F: 0,05 = 0,32; 0,01 = 0,42

Research plot no.	Character of test	Source of variation	Stat. F	Critical F		Statistical significance
				0.05	0.01	
133 A	Long-term	Repetition	91.122	2.99	4.60	++
		Progeny	7.345	1.42	1.63	++
134 A	Long-term	Repetition	0.658	2.99	4.60	NS
		Progeny	20.032	1.42	1.63	++
136	Long-term	Repetition	3.953	2.99	4.60	+
		Progeny	9.289	1.42	1.62	++
133 B	Long-term	Repetition	76.962	2.60	3.78	++
		Progeny	6.333	1.41	1.60	++
134 B	Long-term	Repetition	105.912	2.60	3.78	++
		Progeny	5.97	1.41	1.60	++

Tab. 5.
Analysis of variance - average heights in 1998 - age 6 years

Research plot no.	Character of test	Source of variation	Stat. F	Critical F		Statistical significance
				0.05	0.01	
133 A	Long-term	Repetition	2.447	2.99	4.60	NS
		Progeny	4.181	1.42	1.63	++
134 A	Long-term	Repetition	14.316	2.99	4.60	++
		Progeny	3.695	1.42	1.63	++
136	Long-term	Repetition	20.786	2.99	4.60	++
		Progeny	4.888	1.42	1.62	++
133 B	Long-term	Repetition	7.262	2.60	3.78	++
		Progeny	2.310	1.41	1.60	++
134 B	Long-term	Repetition	7.923	2.60	3.78	++
		Progeny	1.563	1.41	1.60	+

Tab. 6.
Analysis of variance - average stem form in 1998 - age 6 years

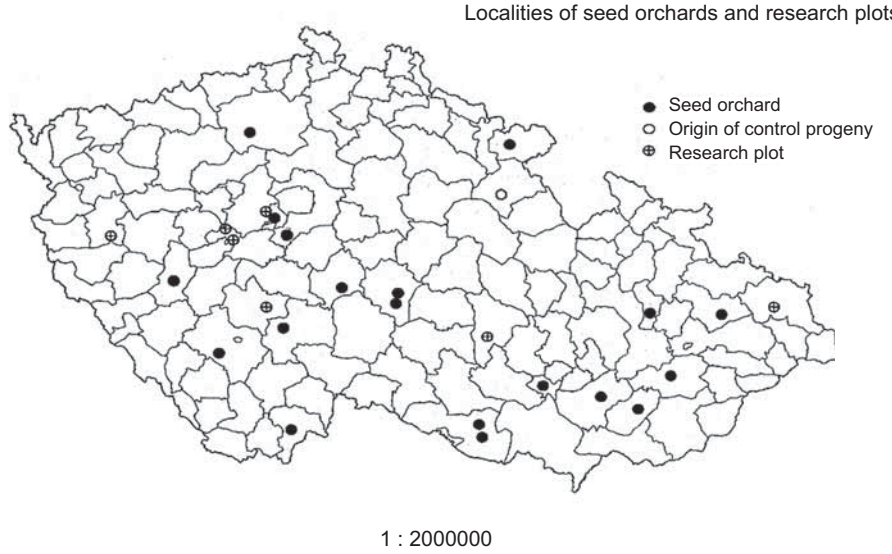
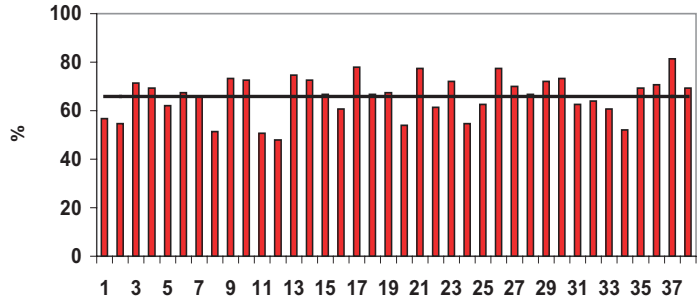
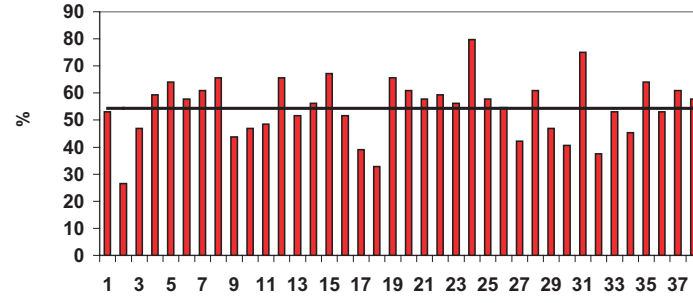


Fig. 1.
Czech Republic - testing of European larch seed orchards

LS Milevsko, Olešná, plots no. 133 A, 133 B			
Pro-geny	**Seed orchard no., /locality/, (year of crop)**	**Pro-geny**	**Seed orchard no., /locality/, (year of crop)**
1	SS-MD-45-33-4 / Znojmo, Znojmo 45 (1982)	**21**	SS-MD-27-24-3 / Broumov, Broumov 27 (1987)
2	SS-MD-42-36-4 / Bučovice, Mouřínov (1984)	**22**	SS-MD- 3-10-5 / Ledeč n. Sázavou,Čerňák (1988)
3	SS-MD-27-24-3 / Broumov, Broumov 27 (1985)	**23**	SS-MD- 1-29-4 / Šternberk, Šternberk (1988)
4	SS-MD -2-16-5 / Ledeč n.S., Čerňák (1985)	**24**	SS-MD- 2-16-5 /Ledeč n. Sázavou,Čerňák (1988)
5	SS-MD-33-41-4 / Bystřice p.H., Lukov (1985)	**25**	SS-MD- 2-16-5 /Ledeč n. Sázavou,Čerňák (1988)
6	SS-MD-10-16-5 / Vlašim, Ml. Vožice 10 (1985)	**26**	SS-MD-49-29-5 / Vítkov, Kletná (1988)
7	SS-MD-53-17-2 / Zbraslav nad Vlt., Slapy (1985)	**27**	SS-MD-29-36-4 / Buchlovice, Koryčany (1988)
8	SS-MD-18- 5-4 / Litoměřice, Budyně nad.O. (1985)	**28**	SS-MD-42-36-4 / Bučovice, Mouřínov (1988)
9	SS-MD-87-30-3 / ŠLP Křiny, Olomučany (1985)	**29**	SS-MD-18- 5-4 / Litoměřice, Budyně (1988)
10	SS-MD-45-33-4 / Znojmo, pl. 16 (1985)	**30**	SS-MD-42-36-4 / Bučovice, Mouřínov (1988)
11	SS-MD-73-33-4 / Znojmo, pl. 7, 17 (1985)	**31**	SS-MD-18- 5-5 / Litoměřice, Budyně (1988)
12	SS-MD-29-36-4 / Buchlovice, Koryčany (1985)	**32**	SS-MD-33-41-4 / Bystřice p. H., Lukov (1988)
13	SS-MD-24-15-4 / Nové Hrady, H.Stropnice (1985)	**33**	SS-MD-73-33-4 / Znojmo, Znojmo (1988)
14	SS-MD- 0-10-5 / Jíloviště (klon.archiv MD)(1985)	**34**	SS-MD-45-33-4 / Znojmo, Znojmo 45 (1988)
15	SS-MD-42-36-4 / Bučovice, Mouřínov (1985)	**35**	SS-MD-23- 6- 4 / Sp. Poříčí, Nepomuk (1988)
16	SS-MD-23- 6-4 / Sp. Poříčí, Nepomuk (1985)	**36**	SS-MD-24-15-5 / Nové Hrady, H.Stropnice (1989)
17	SS-MD-49-29-4 / Vítkov 49 (1986)	**37**	SS-MD-58-15- 4 / Písek, pl. 58 (1989)
18	SS-MD-32-10-4 / Tábor, Bechyně 32 (1986)	**38**	B-MD-514-17- 2-RK /Control.-seed stand of cat. B
19	SS-MD-45-33-4 / Znojmo, Kuchařovice (1987)	Opočno (1988)	
20	SS-MD-73-33-4 / Znojmo, Kuchařovice (1987)		



Plot no. 133 A - long term tests - % of survival 1998



Plot no. 133 B - long term tests - % of survival 1998

Fig. 2.
Plot no. 133 A,B survival in 1998 - age 6 years

LS Milevsko, Olešná, plots No. 133 A, 133 B			
Pro-geny	LS Milevsko, Olešná, plots no.133 A, 133 B Seed orchard no., /locality/, (year of crop)	Pro-geny	LS Milevsko, Olešná, plots no.133 A, 133 B Seed orchard no., /locality/, (year of crop)
1	SS-MD-45-33-4 / Znojmo, Znojmo 45 (1982)	21	SS-MD-27-24-3 / Broumov, Broumov 27 (1987)
2	SS-MD-42-36-4 / Bučovice, Mouřínov (1984)	22	SS-MD- 3-10-5 / Ledeč n. Sázavou,Čerňák (1988)
3	SS-MD-27-24-3 / Broumov, Broumov 27 (1985)	23	SS-MD- 1-29-4 / Šternberk, Šternberk (1988)
4	SS-MD -2-16-5 / Ledeč n.S., Čerňák (1985)	24	SS-MD- 2-16-5 /Ledeč n. Sázavou,Čerňák (1988)
5	SS-MD-33-41-4 / Bystřice p.H., Lukov (1985)	25	SS-MD- 2-16-5 /Ledeč n. Sázavou,Čerňák (1988)
6	SS-MD-10-16-5 / Vlašim, Ml. Vožice 10 (1985)	26	SS-MD-49-29-5 / Vítkov, Kletná (1988)
7	SS-MD-53-17-2 / Zbraslav nad Vlt., Slapy (1985)	27	SS-MD-29-36-4 / Buchlovice, Koryčany (1988)
8	SS-MD-18- 5-4 / Litoměřice, Budyně nad.O. (1985)	28	SS-MD-42-36-4 / Bučovice, Mouřínov (1988)
9	SS-MD-87-30-3 / ŠLP Křiny, Olomučany (1985)	29	SS-MD-18- 5-4 / Litoměřice, Budyně (1988)
10	SS-MD-45-33-4 / Znojmo, pl. 16 (1985)	30	SS-MD-42-36-4 / Bučovice, Mouřínov (1988)
11	SS-MD-73-33-4 / Znojmo, pl. 7, 17 (1985)	31	SS-MD-18- 5-5 / Litoměřice, Budyně (1988)
12	SS-MD-29-36-4 / Buchlovice, Koryčany (1985)	32	SS-MD-33-41-4 / Bystřice p. H., Lukov (1988)
13	SS-MD-24-15-4 / Nové Hrady, H.Stropnice (1985)	33	SS-MD-73-33-4 / Znojmo, Znojmo (1988)
14	SS-MD- 0-10-5 / Jiloviště (klon.archiv MD)(1985)	34	SS-MD-45-33-4 / Znojmo, Znojmo 45 (1988)
15	SS-MD-42-36-4 / Bučovice, Mouřínov (1985)	35	SS-MD-23- 6- 4 / Sp. Poříčí, Nepomuk (1988)
16	SS-MD-23- 6-4 / Sp. Poříčí, Nepomuk (1985)	36	SS-MD-24-15-5 / Nové Hrady, H.Stropnice (1989)
17	SS-MD-49-29-4 / Vítkov 49 (1986)	37	SS-MD-58-15- 4 / Písek, pl. 58 (1989)
18	SS-MD-32-10-4 / Tábor, Bechyně 32 (1986)	38	B-MD-514-17- 2-RK /Control.-seed stand of cat. B
19	SS-MD-45-33-4 / Znojmo, Kuchařovice (1987)	Opočno (1988)	
20	SS-MD-73-33-4 / Znojmo, Kuchařovice (1987)		

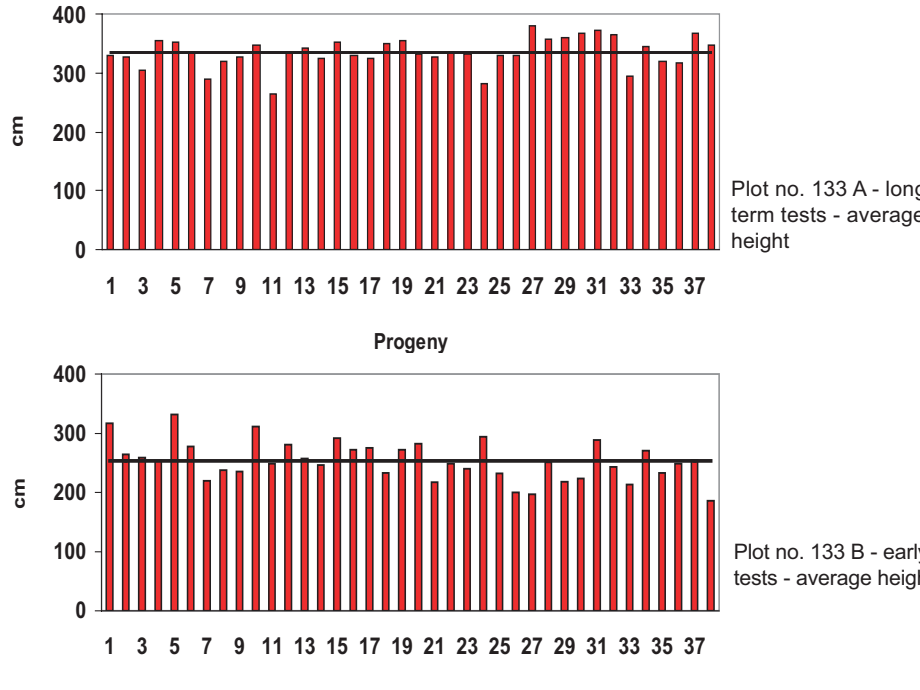
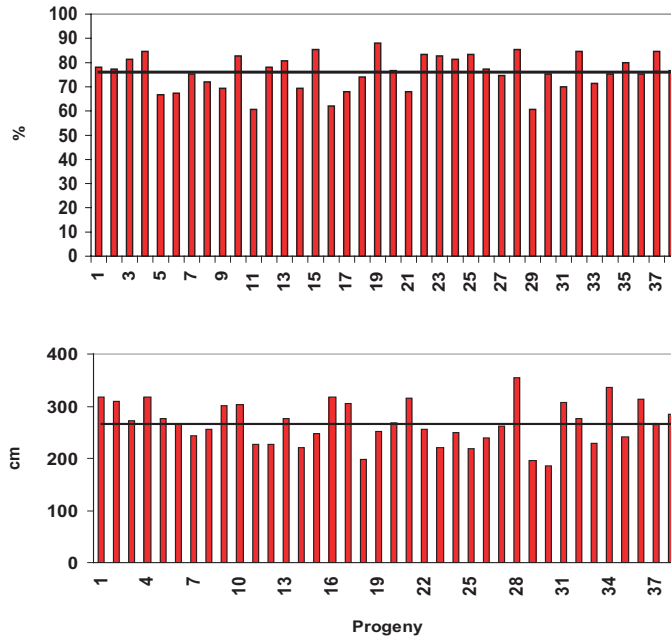


Fig. 3.
Plots no. 133 A, B - average height in 1998 - age 6 years

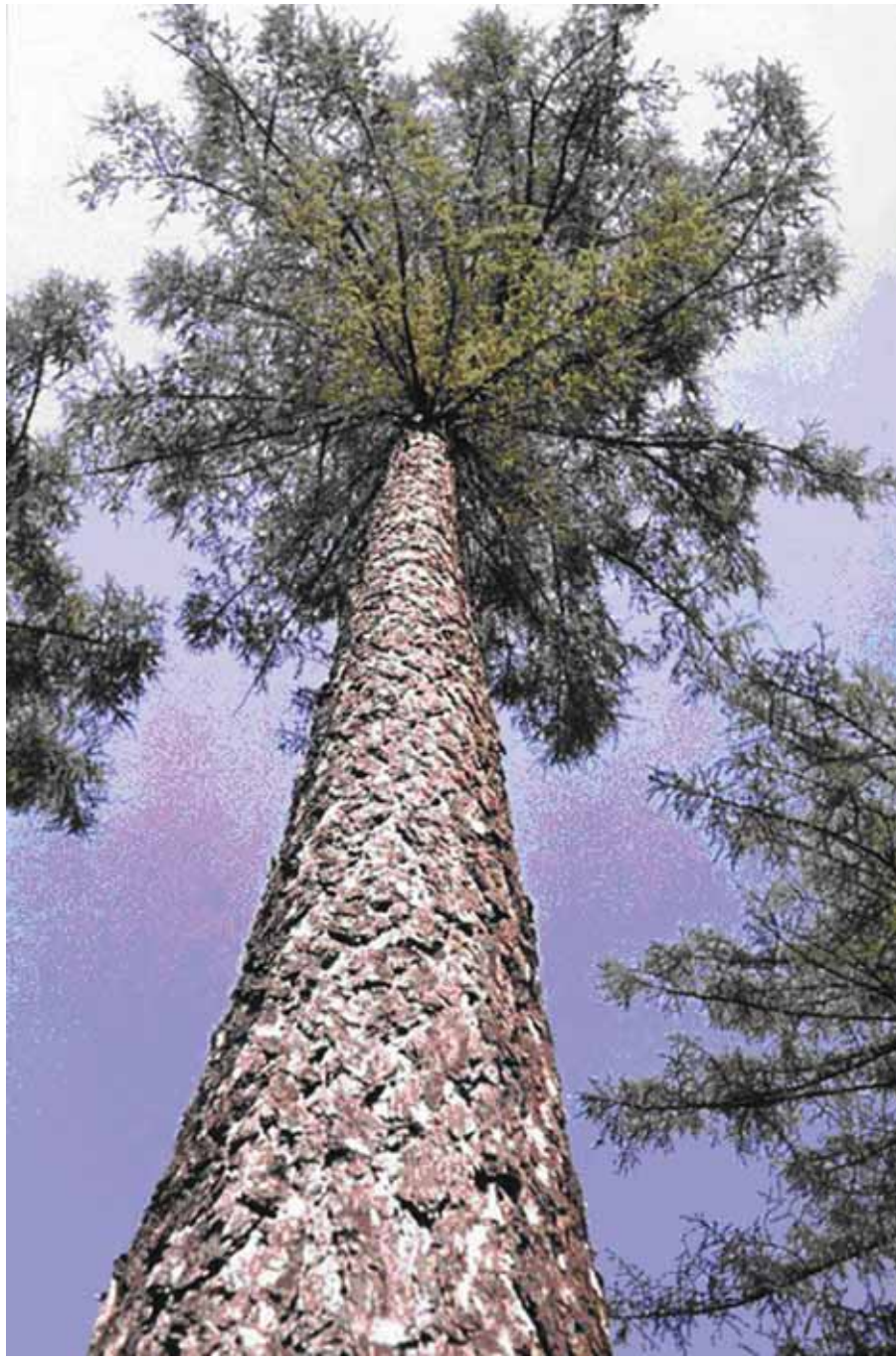
LS Přeštice, Nýřany, plot no. 134 A			
Pro-geny	**Seed orchard no., /locality/, (year of crop)**	**Pro-geny**	**Seed orchard no., /locality/, (year of crop)**
1	SS-MD-45-33-4 / Znojmo, Znojmo 45 (1982)	**21**	SS-MD-27-24-3 / Broumov, Broumov 27 (1987)
2	SS-MD-42-36-4 / Bučovice, Mouřínov (1984)	**22**	SS-MD- 3-10-5 / Ledeč n. Sázavou,Čerňák (1988)
3	SS-MD-27-24-3 / Broumov, Broumov 27 (1985)	**23**	SS-MD- 1-29-4 / Šternberk, Šternberk (1988)
4	SS-MD -2-16-5 / Ledeč n.S., Čerňák (1985)	**24**	SS-MD- 2-16-5 /Ledeč n. Sázavou,Čerňák (1988)
5	SS-MD-33-41-4 / Bystřice p.H., Lukov (1985)	**25**	SS-MD- 2-16-5 /Ledeč n. Sázavou,Čerňák (1988)
6	SS-MD-10-16-5 / Vlašim, Ml. Vožice 10 (1985)	**26**	SS-MD-49-29-5 / Vítkov, Kletná (1988)
7	SS-MD-53-17-2 / Zbraslav nad Vlt., Slapy (1985)	**27**	SS-MD-29-36-4 / Buchlovice, Koryčany (1988)
8	SS-MD-18- 5-4 / Litoměřice, Budyně nad.O. (1985)	**28**	SS-MD-42-36-4 / Bučovice, Mouřínov (1988)
9	SS-MD-87-30-3 / ŠLP Křiny, Olomučany (1985)	**29**	SS-MD-18- 5-4 / Litoměřice, Budyně (1988)
10	SS-MD-45-33-4 / Znojmo, pl. 16 (1985)	**30**	SS-MD-42-36-4 / Bučovice, Mouřínov (1988)
11	SS-MD-73-33-4 / Znojmo, pl. 7, 17 (1985)	**31**	SS-MD-18- 5-5 / Litoměřice, Budyně (1988)
12	SS-MD-29-36-4 / Buchlovice, Koryčany (1985)	**32**	SS-MD-33-41-4 / Bystřice p. H., Lukov (1988)
13	SS-MD-24-15-4 / Nové Hrady, H.Stropnice (1985)	**33**	SS-MD-73-33-4 / Znojmo, Znojmo (1988)
14	SS-MD- 0-10-5 / Jíloviště (klon.archiv MD)(1985)	**34**	SS-MD-45-33-4 / Znojmo, Znojmo 45 (1988)
15	SS-MD-42-36-4 / Bučovice, Mouřínov (1985)	**35**	SS-MD-23- 6- 4 / Sp. Poříčí, Nepomuk (1988)
16	SS-MD-23- 6-4 / Sp. Poříčí, Nepomuk (1985)	**36**	SS-MD-24-15-5 / Nové Hrady, H.Stropnice (1989)
17	SS-MD-49-29-4 / Vítkov 49 (1986)	**37**	SS-MD-58-15- 4 / Písek, pl. 58 (1989)
18	SS-MD-32-10-4 / Tábor, Bechyně 32 (1986)	**38**	B-MD-514-17- 2-RK /Control.-seed stand of cat. B
19	SS-MD-45-33-4 / Znojmo, Kuchařovice (1987)	Opočno (1988)	
20	SS-MD-73-33-4 / Znojmo, Kuchařovice (1987)		



Plot no. 134 A - long term tests - % of survival 1998

Plot no. 134 A - long term tests - average height 1998

Fig. 4.
Plot no. 134 A - average survival and height in 1998 - age 6 years



Sudeten larch (Sudeten /Jeseníky/ Mts., Zábřeh, Czech Republic)
Photo Ing. Eduard Průša

Testing of European larch seed orchards - plot no. 134 A (LS Přeštice, Nýřany) September 1997 - 5 years

June 1999 - 7 years

Testing of European larch seed orchards. Research plot no. 134 A (left), 134 B (right) (LS Přeštice, Nýřany) January 2003 - 11 years

Testing of European larch seed orchards. Research plot no. 134 A (LS Přeštice, Nýřany) January 003 - 11 years

Testing of E. larch seed orchards plot no. 136 Šenov March 1994 - establishment of plot - seedlings 2 years

Testing of E. larch seed orchards - plot no. 136 Šenov - Oct. 1994 - 2 years

Testing of E. larch seed orchards - plot no. 136 Šenov - Oct. 1995 - 3 years

Testing of E. larch seed orchards - plot no. 136 Šenov - Oct. 1997 - 5 years

Testing of E. larch seed orchards - plot no. 136 Šenov - Oct. 1999 - 7 years

Testing of E. larch seed orchards - plot no. 136 Šenov - March 2003 - 11 years

MICROPROPAGATION OF ENDANGERED SPECIES OF *DAPHNE CNEORUM*

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ABSTRACT

A protocol for micropropagation of endangered *Daphne cneorum* is reported. The modified agar medium WPM (0.2 mg l^{-1} BAP; 0.1 mg l^{-1} IBA; 200 mg l^{-1} glutamine; 200 mg l^{-1} casein hydrolysate) was used for induction of organogenesis. WPM medium with the third concentrations of nutrients and trace elements in which the IBA was replaced with 7.1 mg l^{-1} of NAA was used for rooting. Both media concentrations were proved competent in in vitro successful multiplication of three *Ulmus* species (Malá 2000). Organogenesis was induced in plant material (dormant apical buds and young seedlings cultured from seeds) collected in two different localities where *D. cneorum* survived up to the present. In total 344 multi-apex cultures (20.2 ± 5.3 shoots per culture) from first locality, and 236 multi-apex cultures (38.3 ± 12.2 shoots per culture) from the second locality were obtained after five months of cultivation. Germination capacity of *Daphne* seeds was very low. One multi-apex culture bearing 12 shoots sprouted out only in one of 10 *Daphne* seeds available, of which 26.7 ± 8.2 of shoots per culture (35 multi-apex cultures) were obtained after five months of cultivation. The plantlets were acclimatized in diluted MS medium (1 : 10) for three weeks and then were transferred into external conditions where they successfully survived the winter period and started to flower.

Abbreviations: BAP – benzylaminopurine; IBA – ß-indolebutyric acid; NAA – α-naphthalene acetic acid; WPM – woody plant medium (1981); MS – Murashige and Skoog plant medium (1962)

INTRODUCTION

The *Daphne cneorum* is an evergreen low shrub originally occurring in thermophyte and in the warm parts of mesophyte regions of the Czech Republic. It gradually disappeared during the last few decades and nowadays occurs only rarely in two localities in Central Bohemia. *Daphne* propagation by means of cuttings could be carried out but the collecting of plant material decreases the viability of donor plants and threatens the survival of remnant individuals of *Daphne* in their natural stand. The micropropagation of different species or varieties of *Daphne* is mentioned only sporadically (Cohen 1975, Cohen, Le Gal 1976, Marks, Myers 1992, 1994). Cohen (1977) considered *D. cneorum* as irresponsible to induction of organogenesis and consequently not really appropriate for micropropagation by that way. Later Christie and Brascamp (1989) and Wei et al. (1992) described the successful micropropagation of *D. cneorum*.

Following is a report of *Daphne* multiplication on the basis of the micropropagation technology, which was successfully applied for *Ulmus glabra, U. minor,* and *U. laevis* regeneration (Malá 2000). We refer an efficient protocol for induction of organogenesis on the dormant apical buds and seed-derived young seedlings and for ensuring rhizogenesis, acclimatization, and conversion of in vitro cultured plantlets to complete viable plants that could be utilized both for successful preservation and reintroduction of *D. cneorum* into its original stand.

MATERIAL AND METHODS

Plant material

Twenty shoots 3 cm long bearing an apical dormant bud from the locality A (25 km west of Prague), 10 shoots of the same form from the locality B (45 km north of Prague), all collected in February 1995, and 10 seeds (about 4 mm in size) collected at the end of May 1995 in the locality A were used for the formation of primary cultures. The shoots and seeds were surface-sterilized in 0.1% w/v $HgCl_2$ by vigorous shaking for 20 min. After three rinsing in sterile distilled water, the shoots and seeds were placed into agar medium in 100 ml Erlenmeyer flasks.

Induction of organogenesis and multiplication of shoots

Organogenesis in both the shoots and the seeds was induced in 6% agar medium (Sevetin Co., the Czech Republic) containing WPM (Lloyd, McCown 1981), which was enriched with 200 mg l^{-1} of glutamine and 200 mg l^{-1} of casein-hydrolysate, 30 g l^{-1} of sucrose, 0.2 mg l^{-1} of BAP, and 0.1 mg l^{-1} of IBA. Multiplication of shoots and growth of multi-apex cultures were achieved on the agar medium with WPM of the same composition.

All cultures were kept at 25 °C under white fluorescent light (36W/33 Philips Tubes, the Netherlands) at an intensity of 30 $\mu mol.m^{-2}.s^{-1}$ with a 16-h photoperiod for 90 days. Cultures were transferred onto fresh medium every 3 - 4 weeks. The number of shoots per culture was counted at the end of cultivation.

Rooting

Multi-apex cultures (2 - 5 shoots 2 - 3 cm long) were transferred onto WPM medium with 1/3 concentration of macro- and micronutrients, 2.83 mg l^{-1} of IBA, and placed in the dark for 7 days. After this period they were transferred to light and replanted to the same medium without IBA. Simultaneously, the influence of NAA on rooting process was compared. The IBA in the 1/3 strong WPM medium was replaced with 7.1 mg l^{-1} of NAA. There were used 25 shoots from localities A, B, and from the cultures propagated from seeds in each rooting series.

Aclimatization

The rooted plantlets were replanted to the perlite and kept in constant cultivation conditions under continuous light (30 $\mu mol\ m^{-2}\ s^{-1}$) at 20 °C for 2 weeks. Cultures were watered once a day with the medium MS (Murashige, Skoog 1962) diluted 1 : 10 with the distilled water. Then the plantlets were transplanted into the peat substrate and grown in the greenhouse, where they were acclimatized to 70% relative humidity.

Statistical analysis

One-way analysis of variance was used for the statistical evaluation of results.

RESULTS

Induction of organogenesis and multiplication of shoots

Sixteen primary explants of 20 shoots collected in the locality A and 6 explants of 10 shoots of locality B remained after 30 days of cultivation. Each of dormant buds had grown into new shoot 2.5 ± 0.6 cm long. These shoots were used for further multiplication. In 4 weeks after the first passage there had grown on the shoots originating from the locality A in average 2.8 ± 1.2 of adventitious shoots and on the shoots from the locality B in average 4.5 ± 2.6 of adventitious shoots. These newly grown shoots were used for further multiplication. After the subsequent 3 passages over the period of 3 months we had been successful in growing of multi-apex cultures with a remarkably higher number of shoots: of the cultures from locality A in average 20.2 ± 5.3 of shoots per one multi-apex culture and 38.3 ± 12.2 of shoots per one multi-apex culture from locality B. We have also observed on the cultures from these localities phenotypic differences. The cultures from locality A were characterized by light green color and by more feeble

and shorter shoots (in average 3.2 ± 0.8 cm), while those from the locality B were mainly dark green with strong and longer shoots (4.8 ± 0.6 cm). After five months´ cultivation we had obtained from 16 shoots from the locality A in total 344 multi-apex cultures and of 6 shoots from the locality B in total 236 multi-apex cultures.

Induction of organogenesis on young seedlings

Of 10 seeds there had come in one seed after 3 weeks. After the first passage within 3 weeks there had grown from the young seedling 12 shoots, which were kept as an individual clone. After further 6 passages within 6 months the number of new shoots had reached the average of 26.7 ± 8.2 per one multi-apex culture and in total there had been obtained 35 multi-apex cultures.

Rooting

In the modified WPM medium with IBA 13 cultures from the locality A had rooted within two weeks (52 %) and 17 cultures from the locality B (68 %). Of the cultures cultivated from the young seedlings 8 plants had rooted (32 %). In the medium with NAA there was no stimulation of rooting.

Aclimatization

After replanting to non-sterile peat substrate there had been lost 3 plantlets from the locality A during the process of acclimatization, one plant from locality B and one of the cultures cultivated from young seedlings. The losses were caused by the gray mold (*Botrytis cinerea*) and were not larger than 13 % from the total number of 38 acclimatized plantlets. After three weeks the acclimatized plants had been planted into the outdoor beds, where they had survived the winter period without any losses and had blossomed (Fig. 1).

Fig. 1.
Flowering plants in outdoor beds

DISCUSSION

Daphne cneorum belongs to the group of endangered species in the CR. Due to low fertility and decreased seed germination, the plants do not propagate generatively in natural conditions. Marks and Myers (1992, 1994) used for the micropropagation the induction of shoots on proximal and distal segments of stem of nanoid cultivar of *D. cneorum*, propagated in greenhouse. They had obtained of the cultures derived from the distal parts of stem after the first passage in average 3.8 of shoots and after two subsequent passages 2.2 of shoots, while the numbers of shoots from the proximal parts were lower. The micropropagation technology used in this study (induction of organogenesis from winter dormant buds and induction of organogenesis from young seedlings) had succeeded in inducing multi-apex cultures with the average higher than tenfold number of shoots per culture. There had been recorded on the clones from the different localities and from the explant cultures from young seedlings some differences in creation of shoots and its growth habit during the multiplication that can be presumably determined genetically, eventually epigenetically (differences in pH and the composition of soils analyzed for individual localities, data not published). During the process of rooting the clone differences had also approved in the number of rooted plants. For the induction of rhizogenesis in agar substrate during our experiments there had also be proven the necessity of auxin IBA in the rooting medium, although the study of Cohen (1977) and Marks and Myers (1994) describe the rooting with auxin NAA. The problems during the induction of rhizogenesis of explants of *D. cneorum* in agar were described by Christie and Brascamp (1989) and they recommend to stimulate the rooting by combination of auxins IBA and NAA in peat substrate.

The method of multiplication (including time schedules of individual passages and conditions of cultivation during the induction of organogenesis, rooting and acclimatization) that we have elaborated for the micropropagation of *D. cneorum* can be recommended for the preservation of this endangered shrub.

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IDENTIFICATION OF FOREST TREE GENE RESOURCES IN THE FGMRI ISOENZYME LABORATORY

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ABSTRACT

Isoenzyme laboratory of the FGMRI, the activity of which started in 1998, is dealing with the wide spectrum of problems. In the frame of the projects solved in the Department of Forest Tree Species Biology and Breeding isoenzyme analyses have been used for comparing for instance spruce stands from the different localities of the Krkonoše Mts. (project FACE for the Krkonoše National Park: Genetic verification of spruce stands certified for seed collection in the Krkonoše Mts., 1997 - 1998). Isoenzymes as markers for mitoring the valuable tree species populations and for verifying the genotypes of valuable populations of mountain spruce, fir and dwarf pine were used; they also served as bioindicators of forest tree species adaptivness to atmospheric immissions (Grant of the Ministry of Environment: Causes of forest ecosystems damage and prognosis of its further development including proposals of subsequent measurements in the areas under the long-term immission load, solved in 1999 – 2000). Connected with this project is the finding of significant differences by means of isoenzyme analyses among the sets of Norway spruce with different degree of immission damage in the Krušné Mts.

INTRODUCTION

In the frame of the Ministry of Agriculture assignation the populations of forest tree species, mainly Norway spruce and Scotch pine in various natural forest areas, are continuously monitored in the FGMRI isoenzyme laboratory, the methodology of isoenzyme analyses and their statistical processing is developed with the emphasis on standardization at selection of investigated populations, sampling, analyses and evaluation. These activities are used in the expert and consultative work for forest owners. Several original themes are solved within the projects of the National Agency for Agricultural Research. The projects are following:

1. Influence of forest management on forming the run-off and water quality in the forested catchments in the context of complex landscape protection against flood (Isoenzyme analyses of spruce populations in small catchments of the natural forest areas in the Moravskoslezské Beskydy Mts.)
2. Physiological and genetic quality of seed of European larch and Scotch pine from the seed orchards (Identification of clone of seed orchards with Scotch pine by means of isoenzyme analyses)
3. Stress-tolerant clone mixtures of Norway spruce for mountainous areas (Comparison of genetic properties of chosen Norway spruce clones by means of isoenzyme analyses)
4. Micropropagation of endangered populations of important woody species and determination of genetic variability of donor populations and derived explant clone by isoenzyme analyses (Isoenzyme analyses of sets and populations of Scotch elm, European white elm, smooth elm, small-leaved linden and large-leaved linden from different localities and explant cultures derived from them)
5. Genetic identification of forest tree species in various site conditions by means of isoenzyme analyses (Investigation of populations of Norway spruce, Scotch pine and European larch in various localities in CR in connection with the monitoring plots observed in the frame of the ICP Forests Programme monitoring sites. Project comprises also the investigation outside the CR, especially in Germany and Sweden)

METHODOLOGY

Verification of origin of gene resources is focused especially on the isoenzyme analyses of forest tree species stands from the comparative standards. In 1992 the comparative standards were proposed in the frame of "Methodical instruction for verification and testing of reproduction material of forest tree species" as the partial population from the certified units of forest tree species, that respond in growth, quality, resistance and in other properties and signs to the requirements for the certified resources of reproduction material. These resources, ordinary from the viewpoint of signs and properties, included in the certified unit category B, correspond to reproduction material the most represented and thus used in forest management. Moreover, the comparative standards include the stands relatively homogenous, sufficient extensive, with reproducible properties, they are characteristic for the natural forest area or its substantial part and can be taken for the standard populations. The sets of these standards (populations) involved the units of the whole extension of elevations, or forest vegetation zones, where the relevant unit is the part of the original forest ecosystems or has, if not original, suitable conditions for the needed growth and development (Set of comparative standards for economically important woody species in CR was originally proposed in the frame of "Methodical instruction for verification and testing of reproduction material of forest tree species" for 9 species of economically important woody species).

Natural forest area	Site comparative plots, elevation m	Forest administration – forest district	Number of certified unit	Age (years)
16a		1. Kácov, Louňovice	B-SM-156-16-5-BN	96
10a	up to 600	2. Milevsko, Milevsko	B-SM-159-10-3-PI	91
16b		3. Baron J.N.Nádherný, Proseč	B-SM-25-31-3-SY	105
29		4. Vítkov, Jánské Koupele	B-SM-104-29-4-OP	95
16a		1. Pelhřimov, Černovice	B-SM-5-16-5-PE	70-90
11a		2. Kolowratovy lesy, Rozvadov	B-SM-300-11-5-TC	99
27	601-900	3. Hanušovice, Staré Město	B-SM-045-27-6-SU	97
27		4. Loučná, Kouty nad Desnou a)	B-SM-168-27-8-SU	105
10a		5. Vodňany, Buzice	B-SM-013-10-3-ST	76
13		1. NP Šumava, České Žleby	B-SM-652-13-6-PT	81
13	901+	2. Železná Ruda, Hojsova Stráž	B-SM-416-13-8-KT	100
22		3. KRNAP, Černá Hora	B-SM-10-22-7-TU	94
27		4. Loučná, Kouty nad Desnou b)	B-SM-027-27-8-SU	148

List of comparative standards observed by means of isoenzyme analyses Norway spruce *Picea abies* L.
a) Červenohorské saddle
b) Praděd saddle

RESULTS

The comparative standards of Norway spruce were the first to be investigated in the frame of identification of standard populations by means of isoenzyme analyses. Until now totally 11 populations were measured by means of isoenzyme analyses, each of them is represented by one stand, i. e. by 45 individuals. The enzymatic systems glutamate dehydrogenase (GDH), glucose-6-phosphate dehydrogenase (G-6PDH), shikimic acid dehydrogenase (SDH) and 6-phosphoglutamate dehydrogenase (6-PGDH) were investigated at each from these populations, diaphorase (DIA), hexokinase (HK) etc. at several selected populations. From these enzymes the results of three enzymatic systems GDH, G-6PDH and SDH were quantitatively evaluated and statistically processed in the 1st stage (examples of graphical processing for forest administrations Kácov and Pelhřimov).

Isoenzyme analyses of Norway spruce populations from the comparative standards for verification of forest tree species reproduction material inform about further important signs of these standards which can be identified in any stage of woody species development, stand age, unlike for instance morphological ones. The results of analyses present the significant differences among these standards from the viewpoint of allelic representation, genetic diversity, etc. The results of isoenzyme analyses of standard population corresponding to comparative standards can be taken for average coefficients (next to coefficients like quality of wood, volume production, stability of forest ecosystem, etc.) that can be used for "measuring" and for comparing the great part of other stands and populations. This comparison can be applied for instance for stands in the comparable elevation differing distinctly in quality in comparison with the comparative standards, or generally in stands in the same natural forest area. This method can be also used for observing the results of cultivation measures on the molecular-genetic level and at least for controlling the origin of reproduction material.

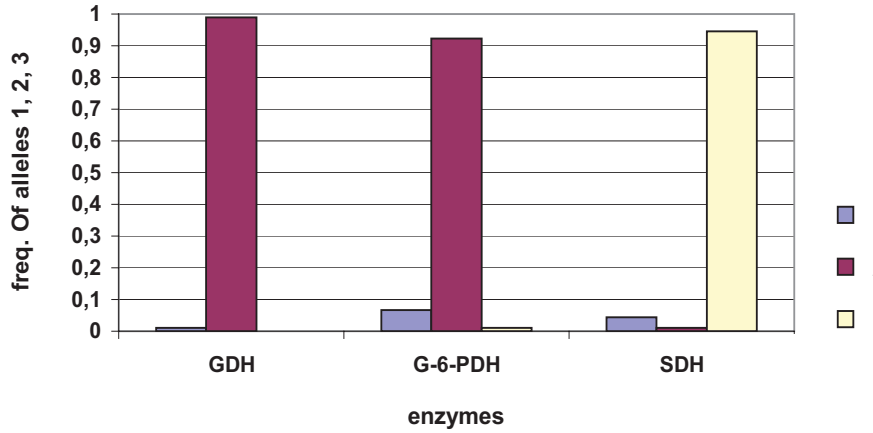

Fig. 1.
Alellic frequencies of 3 enzymes of comparative standard of Norway spruce administration Kácov, age 96 years certified unit B-SM-156-16-5-BN (2000 y.) CR Natural Forest Area 16a

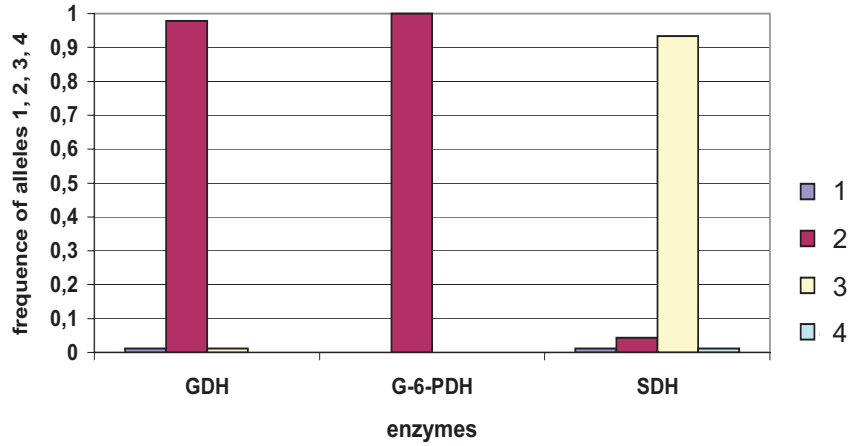

Fig. 2.
Alellic frequencies of 3 enzymes of comparative standard of Norway spruce administration Pelhřimov, age 70 - 90 years. Certified unit B-SM-5-16-5-PE (2000 y.) CR Natural Forest Area 16a

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DEVELOPMENT OF PROGENIES FROM SPONTANEOUS HYBRIDS WITHIN GENUS *ABIES*

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INTRODUCTION

Silver fir (*Abies alba* Mill.) was one of our most important forest trees. Its occurrence extremely decreased owing to its long-termed retreat under influence of changing ecological conditions. That is same with viability of recently living local populations. Silver fir is counted among domestic tree species maintenance of which is mainly connected with increasing of its resistance. It is important independently on implications of silver fir stands regeneration in Europe. Breeding of silver fir for specific resistance is not possible as in case of elms. Research has to be directed to general increasing of its viability.

Hybridization, especially interspecific hybridization, is a verified way of increasing fir general resistance and viability because hybrids within the *Abies* genus from both spontaneous or artificial hybridization distinguish themselves by heterosis not only in growth but also in increased viability against parental species. Higher tolerance to changing ecological conditions is connected with it. We can expect higher tolerance in fir hybrids in this relation not only to various obligatory stress factors including air pollution pressure but also to eventual consequences of changing in ecological conditions caused by climate warming.

We cannot rely only on gradual adaptation of domestic forest tree populations from a population genetics viewpoint with respect to great rapidity of ecological conditions changes. Genetic adaptation of domestic forest tree populations on the basis of selection and mutation processes lasts minimally ten or much more generations with sudden changes. Introduction of foreign tree species adapted to similar conditions and tree breeding using the domestic and foreign tree gene pool especially for their interspecific hybridization with consequent selection can play important role in this case.

Rohmeder and Schonbach (1959) observed that interspecific hybrids of *Abies alba*, *Abies veitchii, Abies concolor, Abies procera, Abies nordmanniana* grew more rapidly than intraspecific hybrids. Kantor and Chira (1971) were interested in hybridization of fir in former Czechoslovakia with using of *Abies alba, Abies cephalonica, Abies nordmanniana, Abies pinsapo, Abies cilicica, Abies concolor, Abies grandis* for interspecific and intraspecific hybridization.

Kobliha (1988, 1989) was interested in testing of some these hybrids. Progenies of *Abies cephalonica* from intraspecific hybridization were of excellent results. Every interspecific and intraspecific hybrid combination with genetic representation of *Abies alba* was better than *Abies alba* from free pollination. Kobliha and Pokorný (1990), Kobliha et al. (1991), Kobliha and Králík (1991) were interested in various methods of propagation of these hybrids.

Greguss (1986, 1988, 1992) and Kormutiak (1985, 1986, 1992) investigated hybridization of fir in Slovakia. Greguss (1986) mentioned that silver fir grew slower in comparison with hybrids and differentiation between hybrid progenies and progenies of individual species from free pollination increased. Kormutiak (1986) obtained similar results. He recommended primarily combinations: *Abies alba* x *Abies cephalonica*, *Abies cephalonica* x *Abies numidica*, *Abies nordmanniana* x *Abies alba*, *Abies pinsapo* x *Abies cephalonica*, *Abies pinsapo* x *Abies alba*, *Abies numidica* x *Abies nordmanniana*, *Abies numidica* x *Abies cephalonica*, *Abies concolor* x *Abies grandis* for growing in conditions of Slovakia *Abies cephalonica*. He recommended with exception of *Abies concolor* and *Abies grandis* mainly using of *Abies alba* and Mediterranean tree species. The same species already played the most important role in experiments of Kantor, Chira and Greguss.

The French research (Arbez et al. 1990) is interested in problems of Mediterranean fir species and their hybridization. French researchers also recommend using of fir progenies from self-pollination regarding to their tolerance in framework of inbreeding.

Kobliha (1994) obtained F2 generation hybrids in combination *Abies cilicica* x *Abies cephalonica*. He also realized crossings (*Abies cilicica* x *Abies cephalonica*) x *Abies alba*, *Abies pinsapo*, *Abies homolepis*, *Abies concolor* and *Abies pinsapo*, *Abies koreana*, *Abies grandis*, *Abies lowiana* x (*Abies cilicica* x *Abies cephalonica*).

MATERIAL AND METHODS

In September 1990 seeds were collected in Arboretum Kysihybel in Slovakia from 21 trees – spontaneous hybrids within genus *Abies* by colleague Greguss. Trees no. 1 - 3 and 6 - 21 incline in phenotype to *Abies cephalonica*, tree no. 4 to *Abies cilicica* and no. 5 to *Abies numidica*. These seeds were sown in Tree Breeding Station Truba in Kostelec n. Č. l. as individual progenies in spring 1991. Two-year old seedlings were planted in the nursery of the tree breeding station and then 5-year old plants were used for establishment of comparative plantation near the tree breeding station.

This plantation for progeny testing compared these progenies with *Abies alba*, *Abies nordmanniane*, *Abies balsamea*, *Abies grandis*, *Abies procera*, *Abies concolor*, *Abies gracilis* and complicated hybrid progeny *Abies koreana* x (*Abies cilicica* x *Abies cephalonica*). These 21 progenies and *Abies gracilis* were planted to the plantation always on 3 plots, *Abies alba* on 9 plots, *Abies balsamea* on 4 plots, *Abies grandis* and *Abies koreana* x (*Abies cilicica* x *Abies cephalonica*) on 2 plots, *Abies nordmanniana*, *Abies procera* and *Abies concolor* on 1 plot. Twenty-five plants were planted to the square plots (5 x 5 plants) in spacing 1.20 x 1.20 m.

Flushing of plants from progenies was observed during spring 1998 and 1999. Flushing plants were counted on control days: Apr. 24, 30, May 6, 13, 22, in 1998 and Apr. 21, 28, May 5, 13, 20, in 1999. On the beginning of 1999 height of 8-year old trees was measured.

RESULTS

Results of flushing observation in progenies from 4 control days in both years are showed in table 1 and figures 1, 2, 3, 4; 100% of plants were flushing in the 5th control day in both years. Table and figures show slower beginning of flushing in 1999, which was influenced by colder weather, but there is possible to observe similar trend of progeny flushing in both years. Fast flushing was observed in *Abies nordmanniana* and slow flushing in *Abies procera*, *Abies concolor* and *Abies koreana* x (*Abies cilicica* x *Abies cephalonica*).

Table 2 and figure 5 show results of height measurement. Mean height of plantation was 34 cm. Mean height of progenies from spontaneous hybrids moved from 22 cm to 45 cm. *Abies alba* reached 27 cm, *Abies nordmanniana* 104 cm, *Abies balsamea* 62 cm, *Abies grandis* 36 cm, *Abies procera* 53 cm, *Abies concolor* 60 cm, *Abies gracilis* 13 cm and *Abies koreana* x (*Abies cilicica* x *Abies cephalonica*) 28 cm.

Analysis of variance (table 3) showed that height of plant is very significantly influenced by its appurtenance to progeny. Duncan´s test (table 4) showed 12 homogenous subgroups with similar mean height.

Results show that *Abies gracilis* with mean height 13 cm (48% of *Abies alba* mean height) and low viability (absence of flushing in 1999) is not acceptable for our conditions. It is necessary to mention that some higher progenies: no - 23 (*Abies nordmanniana*), no. 26 (*Abies procera*) and no. 27 (*Abies concolor*) . are represented only by one plot.

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LIST OF PROGENIES:

Number	Progeny
21-21	spontaneous hybrids
22	*Abies alba*
23	*Abies nordmanniana*
24	*Abies balsamea*
25	*Abies grandis*
26	*Abies procera*
27	*Abies concolor*
28	*Abies gracilis*
30	*Abies koreana* x /*Abies cilicica* x *Abies cephalonica*/

Control days						Control days					
Progeny	**Year**	**1.**	**2.**	**3.**	**4.**	**Progeny**	**Year**	**1.**	**2.**	**3.**	**4.**
	1998	4.92	37.54	60.43	81.20	**16**	**1998**	1.96	36.59	61.82	92.66
	1999	0.15	18.19	52.31	89.99		**1999**	0.00	14.88	49.41	86.31
1	**1998**	12.8	61.67	66.83	78.58		**1999**	0.00	14.88	49.41	86.31
	1999	0.00	33.02	60.64	91.65	**17**	**1998**	2.78	40.08	72.9	97.22
2	**1998**	6.35	36.75	55.08	64.31		**1999**	0.00	8.28	31.75	88.64
	1999	0.00	23.45	51.78	90.91	**18**	**1998**	0.00	11.92	60.41	89.50
3	**1998**	9.90	48.83	60.95	88.49		**1999**	0.00	7.01	39.07	94.20
	1999	1.52	43.99	75.01	98.15	**19**	**1998**	16.39	62.78	71.11	91.94
4	**1998**	5.80	49.28	74.64	96.47		**1999**	0.00	16.89	44.00	94.22
	1999	1.33	22.50	61.00	95.83	**20**	**1998**	6.83	58.67	69.67	98.61
5	**1998**	0.00	47.22	67.67	95.94		**1999**	0.00	16.39	48.06	91.72
	1999	0.00	18.42	63.34	92.93	**21**	**1998**	3.17	69.42	77.94	100
6	**1998**	0.00	32.08	61.81	85.42		**1999**	0.00	36.57	47.69	79.63
	1999	0.00	5.39	49.06	96.30	**22**	**1998**	2.31	40.32	69.20	88.24
7	**1998**	6.72	50.89	75.56	90.44		**1999**	0.00	23.84	51.25	96.13
	1999	0.00	8.06	51.83	90.33	**23**	**1998**	20.00	92.00	92.00	100
8	**1998**	2.90	33.61	69.54	100		**1999**	0.00	56.00	100	100
	1999	0.00	11.25	47.53	91.29	**24**	**1998**	14.08	70.33	83.58	100
9	**1998**	0.00	57.14	77.18	98.41		**1999**	0.00	45.25	89.75	97.96
	1999	0.00	15.87	53.18	88.29	**25**	**1998**	0.00	8.695	49.87	78.54
10	**1998**	1.75	26.03	65.95	98.67		**1999**	0.00	8.88	43.92	94.74
	1999	0.00	2.78	43.44	87.00	**26**	**1998**	0.00	4.00	56.00	96.00
11	**1998**	5.50	65.00	77.06	94.50		**1999**	0.00	0.00	16.00	60.00
	1999	0.00	18.89	59.50	100	**27**	**1998**	0.00	0.00	65.22	100
12	**1998**	4.17	36.51	59.57	67.63		**1999**	0.00	0.00	72.00	96.00
	1999	0.00	12.00	47.13	90.20	**28**	**1998**	30.48	82.42	87.54	94.87
13	**1998**	8.39	46.10	73.66	100		**1999**				
	1999	1.39	17.26	47.63	83.75	**30**	**1998**	0.00	6.00	37.91	58.19
14	**1998**	4.76	19.86	51.97	78.61		**1999**	0.00	0.00	30.55	65.55
	1999	0.00	13.09	36.48	82.00						
15	**1998**	3.14	55.23	79.00	98.61						
	1999	0.00	29.30	53.69	96.02						

Tab. 1.
Flushing of progenies in 1998 and 1999 (percentage of flushing individuals)

Progeny	Number of individuals	Mean height (cm)
1	72	30
2	67	22
3	61	28
4	69	31
5	71	39
6	67	28
7	61	34
8	71	42
9	62	45
10	70	31
11	74	39
12	73	33
13	61	32
14	69	28
15	56	31
16	69	27
17	72	32
18	71	27
19	65	34
20	73	33
21	54	32
22	***190***	***27***
23	25	104
24	99	62
25	35	36
26	25	53
27	25	60
28	47	13
30	47	28

Tab. 2.
Mean height of progenies (progeny 22 = *Abies alba*)

Source of variability	Sum of squares	Degrees of freedom	Mean square	F-stat-	Significance	
					α = 0,05	**α = 0,01**
Repetition-	28,115.842	8	3,514.480	19.166++	1.95	2.52
Progeny	286,794.314	28	10,242.654	55.859++	1.50	1.75

Tab. 3.
Analysis of variance - dependent variable: HEIGHT 1999

Homogenous groups:	Mean height	
Group 1	28	13.4255
Group 2	2, 16, 18, 22, 30	26.3941
Group 3	16, 18, 22, 30, 6, 3, 14, 1, 4, 10, 15, 13, 21	28.8546
Group 4	18, 22, 30, 6, 3, 14, 1, 4, 10, 15, 13, 21, 17	29.2888
Group 5	30, 6, 3, 14, 1, 4, 10, 15, 13, 21, 17, 12, 20, 19, 7	30.9814
Group 6	1, 4, 10, 15, 13, 21, 17, 12, 20, 19, 7, 25	32.2247
Group 7	25, 5, 11	38.3500
Group 8	5, 11, 8	40.0463
Group 9	8, 9	43.7068
Group 10	26, 27	56.5800
Group 11	27, 24	61.7016
Group 12	23	104.0800

Tab. 4.
Duncan‘s test, classifying by homogenous subgroups

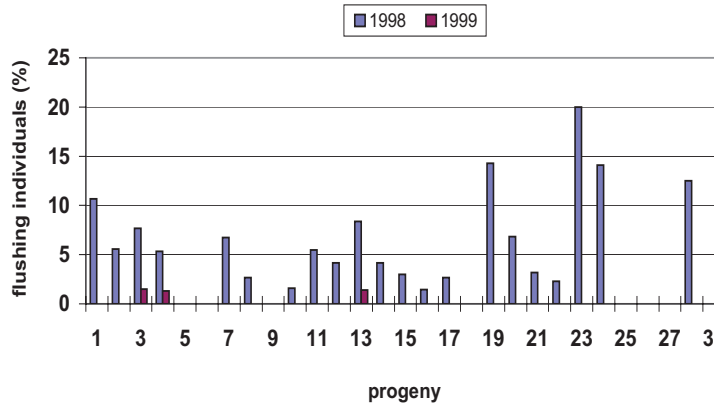

Fig. 1.
Flushing of progenies (1st control day)

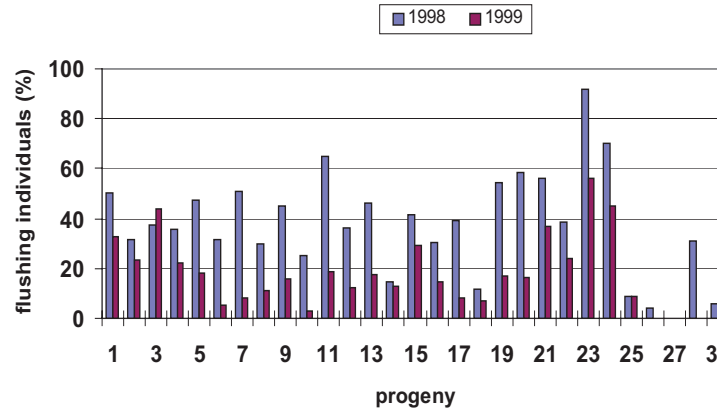

Fig. 2:
Flushing of progenies (2nd control day)

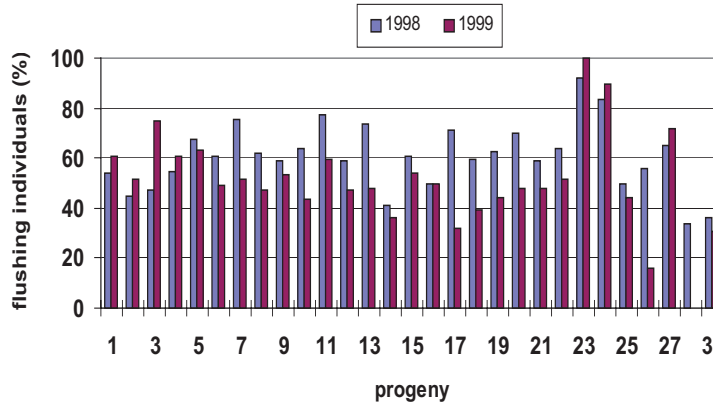

Fig. 4:
Flushing of progenies (4th control day)

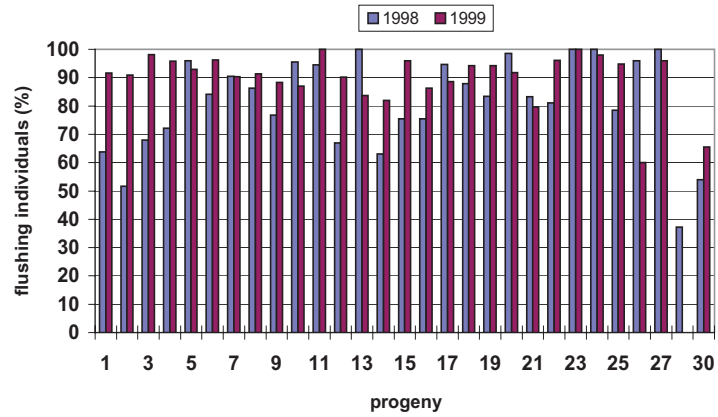

Fig. 3:
Flushing of progenies (3rd control day)

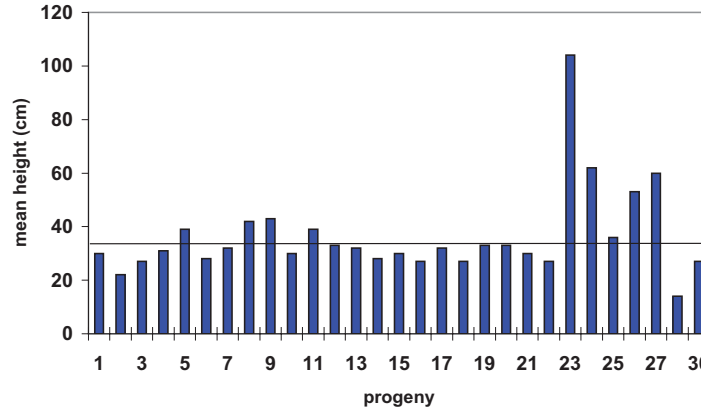

Fig. 5:
Mean height of progenies in 1999 (progeny 22 = Abies alba)

THEORETICAL ANALYSES OF THE POSSIBLE BENEFIT OF VEGETATIVE PROPAGATION IN QUALITY BIRCH

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ABSTRACT

Much effort goes into the development of vegetative propagation using micropropagation and other methods. This work analyses the profitability of vegetative propagation in forest tree breeding exemplified by birch. Such analysis was done by comparisons built on published formulas, compiled in an Excel worksheet. The worksheet enables calculations in major breeding alternatives. Inputs used in this study are genetic variance components, cost components as well as number of parents, number and size of families, size of clones, number of selected families and genotypes within a family. Outputs are gain, status number and total cost. Total investment, cost components (related to the additional genotypes and experimental plants) and heritability were found to be the most important factors affecting the efficiency of cloning in genetic testing. Cheap additional plants, high cost per additional genotype and low narrow-sense heritability make clonal option in testing more beneficial. Presence of dominance variance lowers the benefit marginally. Founders are crossed pairwise in one breeding alternative to test their full-sib families for forward selection of the best genotypes out of the best families. An option exists to vegetatively propagate each genotype before the test is planted; thus each of them is represented by number of clonal copies in a test. This is highly beneficial for testing birch. Clonal test (for backward selection of the best founders) is better compared to half-sib progeny test in a wide range of conditions provided an efficient propagation technique is available. Micropropagation is mentioned as a possibility to establish birch clonal tests. One can use this work as an introductory manual for an analysis more in depth.