LARIX
2007

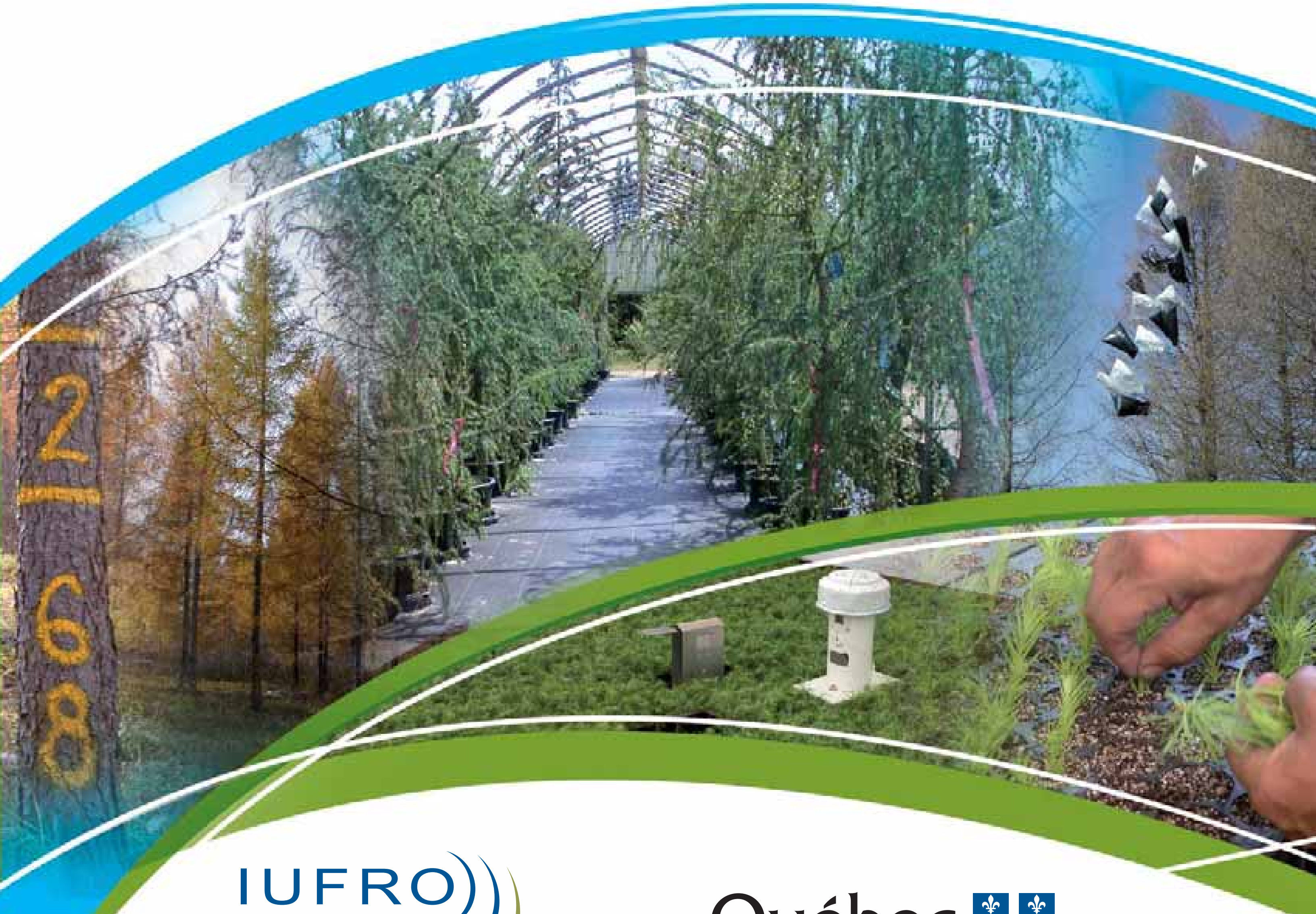
Integrated research activities for supply of improved larch to tree planting:

tree improvement, floral biology and nursery production

LARIX 2007: International Symposium of the IUFRO Working Group S2.02.07 (Larch Breeding and Genetic Resources)

Proceedings/Actes

Saint-Michel-des-Saints and Québec City, September 16-21, 2007



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Schedule of the Symposium

Larix 2007: International Symposium of the IUFRO Working Group S2.02.07:

Integrated Research Activities for Supply of Improved Larch to Tree Planting: Tree Improvement, Floral Biology and Nursery Production

Sunday Sept. 16	Monday Sept. 17	Tuesday Sept. 18	Wednesday Sept. 19	Thursday Sept. 20	Friday Sept. 21
AM **12h00 –** 1st bus departure : Montréal (P.E.Trudeau Airport) to Saint-Michel-des-Saints (≈ 2h30 drive)	Lanaudière Field Trip – **Stop #1** Typical Sugar Maple – Yellow Birch Stand **Stop #2** Tamarack Natural Stand *(Larix laricina)*	Berthierville and Batiscan Field Trip – **Stop #1** Berthier Provincial Nursery – MRNF • Indoor Orchard • Controlled Pollination • Seed Treatment Centre	**IUFRO and PCC/CPC** 4 Invited Speakers (Joint Session with the Poplar Council of Canada) (Québec City Convention Centre)	**IUFRO** Genetics Tree Breeding Silviculture of Larch and Wood Transformation I and II (Québec City Convention Centre)	**Two options:** **1)** Tree Improvement Programs at the DRF (Duchesnay) **2)** Saint-Modeste Provincial Nursery and Propagation Centre- MRNF • (full day)
(Lunch on your own)	**(Provided on tour)**	**(Provided on tour)**	**(Provided at the Convention Centre)**	**(Provided at the Convention Centre)**	**(Provided at Duchesnay or Saint-Modeste)**
PM **18h00 –** 2nd bus departure: Montréal (P.E.Trudeau Airport) to Saint-Michel-des-Saints (≈ 2h30 drive)	**Stop #3** Larch Operational Plantation (2000) **Stop #4** Larch Progeny Test and Plantation (2001)	**Stop #2** *Larix kaempferi* Seed Orchard – Batiscan Arrival in Québec City app. 17:30	**IUFRO** Plenary Session: General Topics on Larch Poster Session (Québec City Convention Centre)	**IUFRO** Tree Breeding II Nursery Production and Floral Biology (Québec City Convention Centre)	**2)** Saint-Modeste : • Larch Cutting Propagation • White Spruce Somatic Embryogenesis End of Symposium
(Dinner – Package, Auberge du Lac Taureau)	**(Dinner – Package, Auberge du Lac Taureau)**	**(Dinner on your own, Québec City)**		**(Dinner on your own, Québec City)**	
Evening Registration and Ice breaker	Working Group S2.02.07 *Business Meeting*		**IUFRO et CPC/PCC** Banquet provided at the Québec City Convention Centre		
(Auberge du Lac Taureau)	(Auberge du Lac Taureau)	(Lodging in Québec City)	(Lodging in Québec City)	(Lodging in Québec City)	

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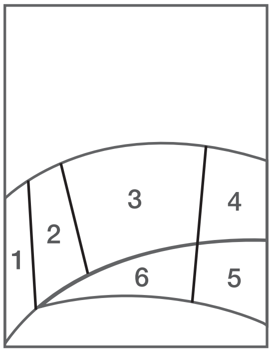

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A word from the organizers

The *Direction de la recherche forestière* (DRF) of *Forêt Québec*, in collaboration with IUFRO, the *Réseau Ligniculture Québec*, Louisiana-Pacific Ltd.– Saint-Michel Division, Smurfit-Stone Canada Packaging Inc., the *Directions régionales des forêts de Laval-Lanaudière-Laurentides et de la Mauricie* and the *Direction générale des pépinières et des stations piscicoles*, welcomes members of the IUFRO – S2.02.07 *(Larch Breeding and Genetic Resources)* working group to Québec. We hope that the Larix 2007 Symposium, the first of the working group's meetings in North America, will meet your expectations through the program activities we have organized, starting with two days of field visits.

Forêt Québec, one of the sectors within the *ministère des Ressources naturelles et de la Faune du Québec*, has the mandate of administering the various facets of sustainably managing Québec's public forests, and of contributing to the development of the forest products industry and private woodlots. Within this broad framework, the mission of the DRF is to participate in improving forest practices in Québec by undertaking research and development projects in diverse fields and by ensuring the transfer of know-how to practising foresters.

The primary aim of the IUFRO – S2.02.07 *(Larch Breeding and Genetic Resources)* working group is to facilitate communications among researchers interested in larch genetics, as well as in associated fields (physiology, wood quality, pathology, etc.), and to pursue the mission of international cooperation on larch. The principal objective of the Larix 2007 Symposium is to share advances in larch research, especially in the fields of genetics, tree improvement, flower biology and plant production, as well as in silviculture and wood processing. We hope that the program will generate dialogue among researchers and practitioners. Such meetings should also promote the launching of new cooperative projects.

This symposium aims at integrating research disciplines related to obtaining and producing improved larch varieties for reforestation. Participants will have the opportunity to discover the regions of Lanaudière and the Mauricie, and to visit natural forests as well as some larch plantations. A number of research and development projects in tree improvement and tree reproduction will be presented; among others, at two government nurseries that produce larch varieties from controlled crosses and multiplied by cuttings or somatic embryogenesis. Afterwards, participants will present the results of their research during poster and communication sessions, which will be held in Québec City as part of the *Carrefour de la recherche forestière*.

For this edition, the Larix 2007 Symposium is being held in conjunction with the 2007 annual meeting of the Poplar Council of Canada by holding the plenary session of invited speakers, a banquet at the Carrefour, as well as a visit to the *Centre d'expérimentation et de greffage de Duchesnay*, where the DRF's tree improvement programs will be presented. These two events are designed to emphasize two of our star species, poplar and larch, which have been the subject of the DRF's research programs since the early 1970s. We would like to mention that DRF is celebrating its 40th anniversary this year. Hosting events like these can be traced back to the actions of visionary pioneers such as Messrs. Gilles Vallée and Ante Stipanicic, inititiators of the research projects in tree improvement and intensive silviculture.

Everywhere in the world we see a clear tendency of meeting the increasing demand for wood through the establishment of plantations. In Québec, the *ministère des Ressources naturelles et de la Faune* Act was modified in 2005 to include principles of forest ecosystem management associated with the functional zoning of the territory. Intensive silviculture therefore meets specific production issues on a reduced area, while reducing pressures on our natural forests. In addition, in the spring of 2006 the Québec government announced a silvicultural investment program funded with a $75 M budget over four years. These investments will allow us to carry out intensive silviculture on high-potential sites, especially the establishment of fast-growing species. Holding this Larix 2007 Symposium integrates well with this thinking.

We wish you a pleasant stay in Québec, *la belle province*, and a successful and fruitful Symposium!

The Organizing Committee

Mot des organisateurs

La Direction de la recherche forestière (DRF) de Forêt Québec, en collaboration avec l'IUFRO, le Réseau Ligniculture Québec, Louisiana-Pacifique Ltée – Division Saint-Michel, Emballages Smurfit-Stone Canada Inc., les Directions régionales des forêts de Laval-Lanaudière-Laurentides et de la Mauricie, et la Direction générale des pépinières et des stations piscicoles, souhaite la bienvenue au Québec aux membres du groupe de travail de l'IUFRO – S2.02.07 (*Larch Breeding and Genetic Resources*). Nous espérons que le Symposium Larix 2007, première rencontre du groupe en Amérique du Nord, vous plaira par sa programmation particulière, débutant par deux jours de visite sur le terrain.

Le mandat de Forêt Québec, un des secteurs du ministère des Ressources naturelles et de la Faune du Québec, est de gérer les différentes facettes de l'aménagement durable des forêts publiques et de concourir au développement de l'industrie des produits forestiers et à la mise en valeur des forêts privées. Dans ce cadre, la mission de la DRF est de participer à l'amélioration de la pratique forestière au Québec en réalisant des projets de recherche et de développement dans divers domaines et en assurant l'intégration de ce savoir-faire par les forestiers.

Le groupe de travail de l'IUFRO – S2.02.07 (*Larch Breeding and Genetic Resources*) vise d'abord à favoriser la communication entre les chercheurs qui s'intéressent à la génétique des mélèzes, de même qu'aux domaines connexes (physiologie, qualité du bois, pathologie, etc.), et de poursuivre la mission de coopération internationale sur le mélèze. L'objectif principal du Symposium Larix 2007 est d'échanger sur l'avancée de la recherche sur les mélèzes, notamment dans les domaines de la génétique, de l'amélioration génétique, de la biologie florale et de la production de plants, et aussi de la sylviculture et de la transformation des bois. Nous souhaitons que le programme suscite le dialogue entre les chercheurs et les praticiens. De telles rencontres devraient également promouvoir la mise en oeuvre de nouveaux projets de coopération.

Ce symposium vise l'intégration des disciplines de recherche reliées à l'obtention et à la production de variétés améliorées de mélèzes pour le reboisement. Les participants auront l'occasion de découvrir les régions de Lanaudière et de la Mauricie et d'y visiter quelques forêts naturelles et des plantations de mélèzes. Des projets de recherche et développement en amélioration génétique et en reproduction des arbres seront présentés, entre autres, dans deux pépinières gouvernementales produisant des variétés issues de croisements dirigés et multipliées par bouturage ou embryogenèse somatique. Par la suite, les participants présenteront les résultats de leurs recherches lors des séances de communications et d'affichage, lesquelles auront lieu lors du symposium à Québec, dans le cadre du Carrefour de la recherche forestière.

Pour cette édition, le Symposium Larix 2007 s'associe à la Réunion annuelle 2007 du Conseil du peuplier du Canada pour tenir la séance plénière des conférenciers invités, un banquet lors du Carrefour ainsi qu'une visite au Centre d'expérimentation et de greffage de Duchesnay, où seront présentés les programmes d'amélioration de la DRF. Ces deux évènements mettent l'accent sur deux de nos essences « vedettes », le peuplier et le mélèze, qui font l'objet de recherches à la DRF depuis le début des années 1970. Soulignons au passage que la DRF célèbre cette année ses 40 ans. La tenue de ces évènements découle des actions de pionniers visionnaires comme MM. Gilles Vallée et Ante Stipanicic, initiateurs des projets de recherche en amélioration des arbres et en ligniculture.

Partout dans le monde, on note une tendance manifeste à combler la demande croissante en bois au moyen des plantations. Au Québec, la Loi sur le ministère des Ressources naturelles et de la Faune a été modifiée en 2005 pour y inclure les principes d'aménagement écosystémique de la forêt, associé au zonage fonctionnel du territoire. La ligniculture satisfait ainsi certains enjeux de production sur des superficies réduites, tout en diminuant la pression sur la forêt naturelle. Par ailleurs, le gouvernement du Québec a annoncé au printemps 2006 un programme d'investissements sylvicoles doté d'un budget de 75 M$ sur quatre ans. Ces investissements permettront de réaliser des travaux de sylviculture intensive sur des sites à fort potentiel, notamment la culture d'essences à croissance rapide. La tenue du Symposium 2007 s'inscrit dans cet ordre d'idée.

Nous vous souhaitons bon séjour au Québec et bon Symposium !

Le comité organisateur

Acknowledgments

We extend our thanks to the *Carrefour de la recherche forestière* and the *Direction de la recherche forestière* (DRF), who made holding the Larix 2007 Symposium possible as part of the joint seminars of the *Carrefour*. Our financial partners played a key role by providing necessary material support for this regionally based multi-day event. Thanks for your support!

In addition to the organizing committee, several persons participated in organizing the Symposium, in the regions as well as at the *Carrefour*, and we cordially thank them for their contributions: Stéphan Mercier, Maripierre Jalbert, Marie Dussault, Mireille Desponts, Jean Noël, and Pierre Gagné. Thanks also to members of the Scientific Committee, particularly to Luc Pâques, for their contribution to program development and their many ideas.

We particularly thank Pierre Périnet, Pierre Bélanger, and Marie-Louise Tardif for their collaboration and constant support, and specially Gaston Lapointe for his involvement and unfailing enthusiasm since the beginning with the Larch tree improvement project. Our thanks also are directed to DRF genetics and tree reproduction personnel, as well as to Nathalie Langlois, Sylvie Bourassa, Jessica Groleau and Guillaume Plante for their help with publications. Also, our thanks go to the other persons who participated in organizing the Symposium, particularly to the personnel of Berthier and Saint-Modeste nurseries.

We recognize the invited speakers, authors, moderators and Larix 2007 Symposium participants for their contributions.

Lastly, we wish to underline the indispensable contribution of the organizing committee members and the major support given by *Forêt* Québec managers for this event.

Martin Perron,

Chair, Organizing Committee

Remerciements

Nous remercions le Carrefour de la recherche forestière et la Direction de la recherche forestière (DRF) qui ont rendu possible la tenue du Symposium Larix 2007 dans le cadre des colloques conjoints du Carrefour. Nos partenaires financiers ont joué un rôle capital en soutenant matériellement cet événement échelonné sur plusieurs jours en région, merci pour votre appui !

En plus du comité organisateur, plusieurs personnes ont participé à l'organisation du Symposium tant en région qu'au Carrefour et nous les remercions cordialement pour leur contribution : Stéphan Mercier, Maripierre Jalbert, Marie Dussault, Mireille Desponts, Jean Noël, Pierre Gagné. Merci également aux membres du Comité scientifique, et plus particulièrement à Luc Pâques, pour leur contribution au programme et leurs avis divers.

Nous remercions particulièrement Pierre Périnet, Pierre Bélanger et Marie-Louise Tardif pour leur collaboration et leur support constant, plus spécialement Gaston Lapointe pour son engagement et son enthousiasme indéfectible pour le mélèze. Nos remerciements s'adressent également au personnel de la DRF en génétique et en reproduction des arbres, ainsi qu'à Nathalie Langlois, Sylvie Bourassa, Jessica Groleau et Guillaume Plante pour leur aide à la publication. Nos remerciements s'adressent aussi aux autres personnes ayant participé à l'organisation du Symposium, plus particulièrement au personnel des pépinière de Berthier et Saint-Modeste.

Nous sommes reconnaissants aux conférenciers(ères) invités(es), aux auteurs, aux modérateurs ainsi qu'aux participants de leurs apports au Larix 2007.

Finalement, nous désirons souligner la contribution indispensable des membres du comité organisateur et l'appui majeur des gestionnaires de Forêt Québec à cet événement.

Martin Perron,

Président du comité organisateur

Thanks to our sponsors/Merci à nos partenaires financiers !

The organisers of the Larix 2007 Symposium thank all the contributors for their valued collaboration and their support for funding./Les organisateurs du Symposium Larix 2007 tiennent à remercier chaleureusement tous les partenaires financiers de l'événement pour leur précieuse collaboration.

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Notes

Poplar Breeding Strategies and Poplar-Rust Interactions (*Melampsora larici-populina*)

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Poplar breeding strategies: lessons from the past, recent advances and perspectives for the future

Poplar breeding programs around the world have achieved substantial increases in growth and yield potential through careful combination of intra/interspecific hybridization and clonal selection. Major challenges of future poplar breeding include (1) continuous genetic gains for recognized economic traits while addressing new breeding goals, (2) optimization of large-scale deployment of the selected genetic variation to limit economical and environmental risks associated to clonal forestry, (3) bringing genomic technologies to application in conventional poplar breeding.

Breeding strategies defined for the French Poplar Breeding Program (GIS Peuplier) will be analysed in the light of significant developments of the past ten years in the following areas:

(1) Intra/interspecific hybridization: performances and heterosis level in different hybrid combinations including *Populus deltoides*, *P. trichocarpa* and *P. nigra*; respective levels of among- and within-family variations for a suite of traits of interest; relationships between intra and interspecific combining abilities.

(2) Relationships between traits of interest (growth, phenology, disease resistance, branching habit, water use efficiency, wood properties)

(3) Optimal selection age and development of stepwise selection

(4) GxE interaction

(5) Development of marker/gene-based selection: a marker-based recurrent selection to balance genetic diversity and multitrait genetic gains, when non-pedigree association genetics needs to replace pedigree populations to detect QTL, genome sequence and development of a genome-wide selection approach for an optimal construction of elite genotypes.

The breeder's efforts will not stop at designing genetically improved trees, but need to define rational deployment strategies that do not neglect the potential impacts that improved material may have on the environment and for end-users in the context of climate change and diversification of poplar cultivation schemes.

Poplar- Rust interactions: a multiscale/ multidisciplinary research approach to develop higher durability of poplar resistance to *Melampsora larici-populina* (Mlp).

Durable resistance to *Melampsora larici-populina* (Mlp) leaf rust is a major challenge for poplar breeding in Northern Europe. When no rust epidemic was evident in the natural *Populus nigra* populations, cultivation of interspecific clones carrying qualitative resistances have strongly modified both qualitative and quantitative pathogenicity components of *Mlp* populations in cultivated areas (Pinon and Frey 2005). The mixed, sexual/asexual reproduction system of *Mlp* and the high ability of dispersion of its uredospores place *Mlp* in the highest position of Mc Donald and Lande (2002) scale of evolution risk. In monoclonal poplar plantations, durability of rust resistance will depend strongly on (1) resistance mechanisms associated in a given genotype, (2) genetic diversity present in *Mlp* populations submitted to these high selection pressures and (3) possibility of recombination rate associated with sexual reproduction on larch.

Recent results on genetic control of *Mlp*-poplar interaction and multidisciplinary research projects in development will be presented under the following categories:

(1) Laboratory and field resistances in pure species (*P. deltoides, P. trichocarpa, P.nigra*), F_1/F_2 hybrids and backcrosses.

(2) Relationships between rust resistance and rust tolerance in field experiments

(3) Qualitative and quantitative resistances : frequency, genetic and molecular controls, towards cloning genetic relationships between qualitative and quantitative resistances

(4) Exapted vs natural resistances : strain-specificity, comparison of nucleotide polymorphisms and associated phenotypic variation in *P.trichocarpa* and *P.nigra*

(5) Relationships with other pest resistances in poplar

(6) Other alternatives linked to plant characteristics with limited action on pathogen populations

(7) Evaluation of selection pressures of different resistance constructions on *Mlp* populations in monoclonal plantations and clonal mixtures

Breeding strategies for durable rust resistance developed in the French Breeding Program will then be discussed.

Acknowledgements:

All results and projects represent the collective efforts of a large team of collaborators from the genetic team at INRA-UAGPF Orléans (A. Dowkiw, V. Jorge, V. Guérin, F. Lefèvre, M. Villar, L. Sanchez), the forest pathology team at INRA-IAM (J. Pinon, B. Thoirain, P. Frey, C. Husson, S. Duplessis) and INRA-Angers (M. Ménard), the genomic team at INRA-URGV (P. Faivre-Rampant, A. Bresson), the entomology team at INRA-URZF (S. Augustin), the ecophysiology team of Orleans University-LBLGC (F. Brignolas, N. Marron, R. Monclus) and of INRA-EEF (E.Dreyer). The genetic material present in these experiments has been developed by the technical team from INRA-UE Orléans and is tested in collaboration with different partners of the French national breeding program (A. Berthelot-AFOCEL; P.Baldet and V. Bourlon-CEMAGREF; O. Forestier-Pépinière forestière de Guéméné-Penfao). The author wish to acknowledge partners of POPYOMICS EU-FAIR project No.QLK5-CT-2002-00953 for collaboration on multisite evaluation of different poplar pedigrees: M. Steenackers (INBO), A. Rohde, V. Storme and W. Boerjan (VIB), N. Marron, S. Dillen and R. Ceulemans (Antwerp University), G.Taylor and A. Rae (Southampton University), M. Sabatti (Tuscia University).

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Notes

Notes

Gene Flow Between Exotic Plantations and Natural Populations of Larch and Poplar

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When genes are exchanged between native and introduced species through the process of introgressive hybridization, the genetic integrity of the native populations can be impacted. As a first step to assess the frequency and consequences of this potential exchange, we developed diagnostic molecular genetic markers and tested whether exotic genes derived from plantations of introduced tree species can be found in surrounding natural populations of poplar (*Populus balsamifera* and *P. deltoides*) and larch (*Larix laricina*). Over three consecutive years, we genotyped nearly 5000 seeds from two populations of native mother trees for each of both genera. The percentage of hybrid seeds that contained genes from different exotic species showed substantial variation among populations and species, though relatively little year-to-year variation. In larch, hybrid seed was infrequently formed (mean 2%), while in poplar, the rate of hybridization was higher and more variable (5% to 66%). Our results clearly demonstrate that hybridization between introduced and native trees can occur. The next step will be to determine whether hybrid trees establish themselves in natural populations and successfully reproduce. In addition to the implications for regulation and management, our study furnished copious DNA markers that have proved valuable for the verification of plant material used in tree breeding.

Notes

Adaptation of forest trees to climate change

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Will populations of temperate and boreal forest trees be able to adapt sufficiently rapidly to avoid extirpation during rapid climate change? What genetic factors should forest managers consider to increase the probability of maintaining healthy, viable forests for production or conservation objectives as climates warm? What can we learn from the genetic and genomic architecture of local adaptation in forest tree populations, and how will this knowledge aid our ability to predict response to climate change and inform management strategies? The high levels of genetic diversity and substantial population differentiation of forest trees for phenotypic traits related to adaptation to climate provide genetic resources for adaptation; however, the long generation length of forest trees will greatly hinder their ability to adapt. Seed transfer and deployment strategies for improved and wild stand seed lots need to be entirely re-thought, moving away from traditional views of “local is best” and towards finding and planting genotypes with temperature response curves indicating tolerance of both current and predicted future conditions in a given location. Bioclimatic models can predict the future distributions of potential habitat for species and populations, but these models are accompanied by a great deal of uncertainty, as actual migration will depend on reproduction, dispersal, and biotic interactions, thus predictions need to be empirically tested in the field. Facilitated migration of populations and species to increase levels of gene flow from milder to colder climates, and to nucleate long-distance migration event, should commence on a controlled, experimental basis. A better understanding of the genomics of complex traits such as growth and adaptation to biotic and abiotic stresses will aid in the understanding of local adaptation to temperature and genetic constraints to rapid adaptation. Isolated, peripheral populations may be important sources of genotypes adapted to extreme conditions, and thus should be of high priority for conservation.

Notes

Exotic Tree Species and Forest Management Certification

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Within a decade, forest management certification has become an essential tool used by forestry companies around the world to demonstrate to the global markets their compliance to recognized voluntary sustainable forest management standards.

These standards address a number of concerns including social, economical, and ecological aspects. Three main standards are used by the forest industry in North America: CSA Z809, FSC and SFI, while two are used in Europe: PEFC and FSC.

Natural forest management and plantation forestry are covered in the standards by specific requirements.

Each of the standard addresses exotic tree species and the use of Genetically Modified material in different ways. FSC is generally more specific and prescriptive while the other standards allow more flexibility. However, during the audit process, it appear that there is a need to clarify the status of exotic species. The auditors responsible to assess the conformity of the forest practices, often have to request on a case by case basis, a justification from the forester of the status of the exotic species.

In order to give more consistency and credibility to the audit process, a list of species considered exotic and “naturalized” should be developed and made available by the appropriate authorities.

Notes

Potentials of Multi-varietal Forestry in Tamarack Using Somatic Embryogenesis

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Introduction

Tamarack (*Larix laricina* (Du Roi) K. Koch) is one of three *Larix* species native to Canada and is a transcontinental species ranging from Newfoundland to Alaska. Tamarack is one of the fastest growing of the northern conifers (Fowells 1965) and is reported to outgrow other native conifers, at least over short rotations (Mead 1978). Owing to its fast growth rate, tamarack is considered a potentially important plantation species in eastern Canada. Current tree improvement programs are based primarily on conventional clonal seed orchard procedures (Vallée and Stipanicic 1993). Recently, with advancements in vegetative propagation techniques such as somatic embryogenesis (SE) and rooting of cuttings, alternative breeding and deployment strategies are being explored to optimize genetic gain and diversity. Multi-varietal forestry (MVF) is defined as the use of genetically tested tree varieties in plantation forestry. It is also known as clonal forestry; however, the term MVF is used to be more descriptive noting that a clone usually refers to any genotype and its ramets, while a variety refers to a clone that has been selected for certain attributes or purposes. Owing to advances in SE and cryopreservation, it is now possible to produce tested, identical conifer genotypes consistently over time, *i.e.*, tree varieties, which was not possible with seed propagated material. The implementation of MVF offers many advantages including: (1) Obtaining much greater genetic gain than any conventional tree breeding system; (2) Flexibility to rapidly deploy suitable varieties with changing breeding goals and environments; and (3) Ability to design and balance genetic gain and diversity in plantations.

The purposes of this presentation are to review genetic variability of tamarack for growth and stem quality and to explore thepossibility of implementing MVF based on somatic embryogenesis and cryopreservation.

Genetic variability from clonally replicated test

Genetic information is available at the provenance level (Cech *et al*. 1977) and at the intra-population level (Rehfeldt 1970; Park and Fowler 1982), and these studies indicated that there is significant variation at both levels. Current tree improvement programs capture among- and within-population variability using seed orchard procedures (Fowler 1986; Vallée and Stipanicic 1993). However, since vegetative propagation is achieved with relative ease, breeding and vegetative deployment strategies should be explored. Genetic gains through breeding and vegetative deployment, that include MVF and family forestry, are expected to be much larger than those from seed orchards because there are opportunities to capture greater portions of genetic variation, including non-additive genetic variance.

To design breeding and vegetative deployment strategies, it is necessary to obtain estimates of both additive and non-additive genetic variances. One such possibility is to establish a clonal field progeny test in which individuals within families are replicated vegetatively. Shaw and Hood (1985) also suggested that the use of clonal replicates in genetic testing would allow efficient utilization of the genetic variability that exists within families.

A progeny test of tamarack using clonal replicates was initiated in 1980. One objective of this study was to use clonally replicated progeny to estimate genetic parameters to aid in the development of MVF strategies. Previously reported results of 5-year height and survival can be found in Park and Fowler (1987). However, the current summary is based on the evaluation of growth and stem quality after 9 years in the field (Park 2002).

Material for the test consisted of 10 open-pollinated families from each of three natural populations of tamarack, giving a total of 30 families. Eight seedlings from each of the 30 families, for a total of 240, were randomly selected and transplanted into containers to use as donor plants. Seedlings were grown in the greenhouse for 13 weeks, and then decapitated to promote side shoots. Nine cuttings were taken from each of the ortets (clones) and rooted under intermittent mist in the greenhouse. After the rooted cuttings were grown for 20 weeks, the ramets were again decapitated to promote side shoots. After 5 weeks, 80 cuttings from each of the clones were taken and rooted in the greenhouse.

Field tests were established at two locations in the region, the Acadia Research Forest, in New Brunswick, and Trafalgar, Nova Scotia. At each location, five randomized blocks of 240 clones were planted in four-tree-row plots at 2.5 m spacing. When trees had completed 9 growing seasons in the field, height and diameter at breast height (DBH) were measured, and stem straightness was rated, using a 1 (poor) to 5 (best) scale. Tree volume was computed using the tamarack volume equation (Honer *et al.* 1983).

The variance component due to population (σ2P) was significantly large for growth traits, i.e., height, DBH, and volume, amounting to about 6% of total phenotypic variance, and exceeded that due to family (σ2F). The variance components due to clone (σ2C) were the largest genetic source of variance for all traits, ranging from 9.0 (volume) to 27.4% (stem straightness) of total phenotypic variance. The variance components due to interactions of location and clone were relatively large, but those involving population and family were small. Similarly, variance due to interactions of block and clone was relatively large but those involving populations and families were small. For stem straightness, the variance due to populations was unimportant. However, most of the variation for all the traits was due to genetic-environmental errors, that ranged from 56.5 to 69.2%.

Although open-pollinated families were used in the experiment, the use of clonal replicates provided the opportunity to partition total genetic variances into additive and non-additive genetic variances (Table 1). In general, a relatively large amount of non-additive variance was found for the traits examined. For example, 56.6% of variation in volume is accounted for by non-additive variance. Consequently, the narrow-sense heritability is relatively low. However, clonal selection is likely to be effective as the clone mean heritability is relatively high. Predicted genetic gains from a set of selection scenarios for the traits examined are presented in Table 2.

Implemetation of multi-varietal forestry

To implement MVF, effective methods of mass vegetative propagation must be available. The traditional vegetative propagation method has been rooting of cuttings, which has been effective and cost efficient for some hardwood species. In conifer species, including tamarack, however, mass propagation by rooting of cuttings is generally possible only with seedlings up to age 5, (Morgenstern *et al.* 1984). This is a serious limitation because, by the time the genetic superiority of a clone line is determined by lengthy field tests, the donor plants have become too old for further mass vegetative propagation. However, due to technological advances in in vitro vegetative propagation techniques, in particular improvement in somatic embryogenesis (SE), tamarack is considered an excellent candidate species of MVF. SE in tamatack has been achieved at a relatively high frequency (43%) when immature zygotic embryos at a precotyledonary stage were used as explants. Embryo production rate was relatively high producing over 300 somatic embryos per 0.5 g of fresh embryogenic tissue, and 48% of the somatic embryos germinated. Furthermore, over 90% of mature somatic embryos produced secondary SE when plated onto the initiation medium. (Klimaszewska *et al*. 1997).

The most important advantage of clonal propagation by SE is that embryogenic tissue can be cryopreserved in liquid nitrogen indefinitely, without changing genetic

Table 1. Estimates of additive and non-additive genetic variances, their relative percentages in parentheses, and heritabilities

Parameter	Height	DBH	Volume	Stem
Additive variance (σ^2_A)	835.8 (72.5%)	15.0 67.7%)	391113 (43.4%)	0.09 (47.4%)
Non-additive variance (σ^2_{NA})	316.7 (27.5%)	7.2 (32.3%)	510259 (56.6%)	0.10 (52.6%)
Narrow–sense heritability (h^2)	0.13	0.09	0.07	0.16
Phenotypic standard deviation for h^2	79.9	13.0	2434	0.7621
Heritability of clone means ($H^2_{\hat{C}}$)	0.59	0.54	0.63	0.87
Phenotypic standard deviation for $H^2_{\hat{C}}$	44.0	6.4	1193	0.4671

Table 2. Comparison of genetic gains as percentages of overall mean for seed orchard and clonal options, assuming a selection intensity of 1% (i=2.64) and 5% (i=2.05) for mass and clone mean selection, respectively

Trait selection[1]	Mean	Range of clone means	Mean of top 12 clones	Expected genetic gain (%)	
				Mass selection	Clone mean
Height	430 cm	235 - 529 cm	513 cm	6.4	12.4
DBH	38.8 mm	13.0 - 53.9 mm	51.0 mm	8.0	18.4
Volume	3278 cm^3	229 - 6536 cm^3	5982 cm^3	13.7	47.0
Stem	3.1	1.8 - 4.5	4.0	10.4	27.0

[1] Based on 30 ramets/clone.

makeup or loss of juvenility. This offers an opportunity to carry out lengthy genetic testing of clone lines, while the embryogenic tissues are maintained in cryopreservation. Once field testing has shown which the best clones are, the corresponding embryogenic tissue can be thawed and used for mass propagation for deployment in MVF. A generalized strategy for implementing clonal forestry using somatic embryogenesis is discussed by Park (2002).

The clonally replicated test reviewed above supports potential benefits of MVF with tamarack, as there were large genetic variances. Potential genetic gains in volume at age 9 with MVF and family forestry (i.e., vegetative deployment of tested families) are illustrated in Figure 1. For example, if we select and deploy the 10 best varieties out of 240 in the test, the mean of the selected varieties is 87% better than the average of all varieties in the test. The gain will be reduced as the number of varieties selected increases but is always greater than the family averages. Clearly, MVF has the advantage over family forestry.

As previously mentioned an advantage of MVF is being able to design and manage genetic gain and diversity. One of the common perceptions of MVF is that the deployment of tree varieties may lead to increased vulnerability to diseases and insects due to thenarrow genetic base. However, for known pests, MVF is better prepared by deploying resistant varieties. For unknown or introduced pests, protection is rather limited despite the large genetic variation existing in forest trees. Nonetheless, there is a chance that a few susceptible varieties are unknowingly deployed in a single plantation. Such risk decreases as the number of deployed varieties increases but this reduces genetic gain. Therefore, the question is "What is a safe number of varieties in a MVF planatation?" Based on various approaches, scientists generally agree that 10-30 varieties should be sufficient. Once an appropriate number of varieties is determined, the plantation can be configured as variety blocks or a random mixture (Libby 1982).

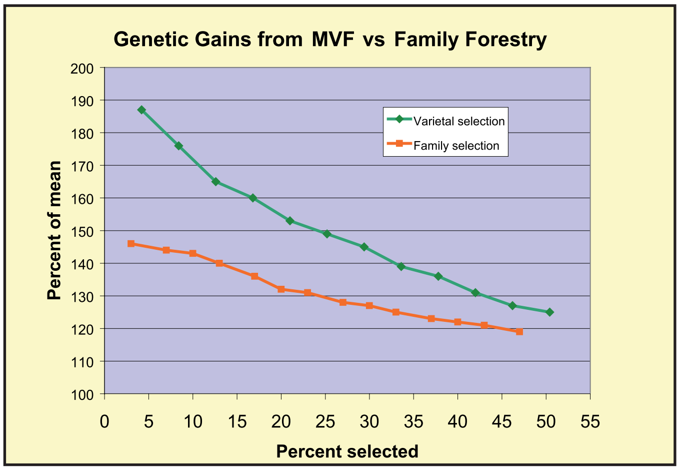

Figure 1. Realized genetic gains from MVF vs. family forestry from a clonally replicated test of tamarack evaluated at age 9. The test included a total of 240 clones representing 30 families derived from 3 natural populations of tamarack.

However, in New Brunswick, the "designed gain and diversity" approach is adopted. In this approach, a desirable level of genetic gain and diversity is determined based on the best available data. The "desirable level" depends on the test age, juvenile and mature correlation, family structure, varietal characteristics, etc. For example, when the test is young and less detail is known about the varieties, a larger number of clones would be deployed to gain a greater diversity. Using the data from the clonally replicated test, the designed gain and diversity is illustrated (Figure 2).

In Figure 2, when the 10 best varieties of 240 are selected, the volume gain is 87% better than the average of all trees in the test but the selection includes only 7 of 30 families. If we select the 60 best varieties, the gain is 49% better than average but the number of families included in the selection was increased to 22. Thus, depending on the situation, a breeder has flexibility to design and balance genetic gain and diversity.

Other variety mixture options include "mixture of varieties and seedlings", linear deployment, and species mixture. Furthermore, the diversity of MVF plantations will be managed over time as the composition of MVF plantations will be dynamic as the selection of varieties will be continuously revised based on the best available data throughout the rotation age. Also, new varieties will be introduced at each breeding cycle.

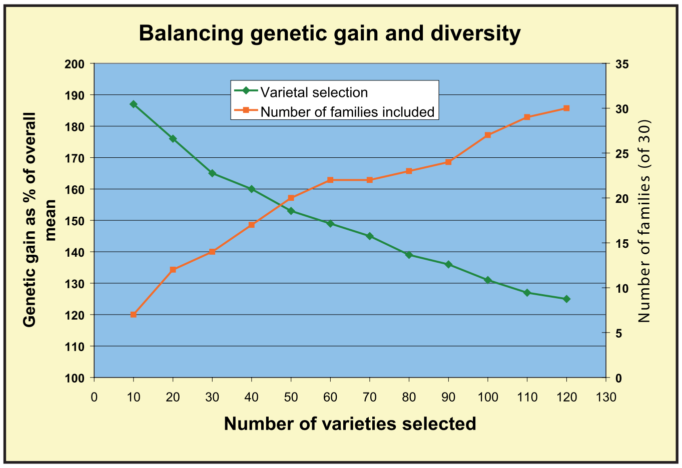

Figure 2. Designing genetic gain and plantation diversity. The green line represents the range of available genetic gain from varietal selection, while the orange line represents the diversity of variety mix in terms of the number of families included in the mixture.

Conclusions

Tamarack is a potentially important plantation species in eastern Canada because it is the fastest growing conifer in the region and it can be grown on a short-rotation. As the clonally replicated test showed, genetic gains from varietal selection will be much greater than those from conventional seed orchard breeding, because both additive and non-additive variances are captured. Tamarack can be propagated by SE. In conjunction with cryopreservation, SE can play a major role in implementing MVF with tamarack. Genetic gain and diversity of MVF plantations can be designed and managed over time using the best available data.

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Genetic variation of wood quality among Japanese larch provenances

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Japanese larch (*Larix kaempferi*) is an endemic species in Japan. Historically Japanese larch has used for forestation in the central part of Honshu Island and Hokkaido Island in Japan, and is also utilized as a pollen parent for inter-specific hybridization with *Larix gmelinii* and *Larix decidua*. Especially in western Europe the superiority of hybrid with European larch (*Larix decidua*) has been described by numerous authors for several traits of economic importance. In 1956 IUFRO international provenance trials on this species had been organized using 25 seed sources (provenances). Forty-four parallel trials were established in the world and some articles have been devoted to estimation of growth performances of provenances. Although performance on wood quality of each provenance is very important not only for forestry but also for tree breeding, the data is still limited.

In this paper, we present genetic variation of modulus of elasticity (MOE) among Japanese larch provenances in six trial stands, two in Hokkaido and four in Nagano, Japan. Several couples of better and worse provenances are common in all trial stands, and provenance X environment interaction shall be week for these provenances. Taking account of growth and tree form performances, we recommended three provenances; Sangome and Tenjin pass that are Fuji-region sources, and Kobushidake that is Kawakami-region sources.

Notes

Can F_2-hybrids be a reasonable alternative to F_1-hybrids for plantation of hybrid larch *(Larix x eurolepis)*? Influence of consanguinity levels on performance

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F_1-interspecific hybrids between European and Japanese larch show a great potential for afforestation and benefits for some combinations of a high positive heterosis for many traits. Yet, while significant progress has been achieved in sexual and vegetative mass-propagation, it remains either technically complicated or costly or both compared to mass-production of pure parental species. F_2-hybridisation seed orchards offer a good alternative for mass-production of hybrids insofar panmixis is guaranteed as in classical orchards but also if F_2-hybrid varieties conserve enough superiority compared to parental species. First results from progeny trials including a diallel mating design among F_1-hybrids with different levels of relationships will be presented.

Notes

Variability of morphological features of the larch in Siberia

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Larch (*Larix sp.* Mill.) is the main forest genus in Siberia. The systematization of larch species is based on morphological diversity, so the study of morphological features variability is actual for selecting of this forest genus (Edwards, 1956; Kruklis, Milyutin, 1977; Giertych, 1979; Abaimov *et. al.*, 1998, 2002; Chmura, Rozkowski, 2002). The purpose of my research is to investigate features of intraspecific and interspecific variability of larch in Middle Siberia comparing with others regions of Siberia.

The features of vegetative and generative organs variability of three larch species (*Larix sibirica* Ledeb, *L. gmelinii* Rupr and *L. cojanderi* Mayr) in the Middle Siberia, Yakutia and Buryatiya have been investigated. Variability of larch in Yakutia and Buryatiya have been studied for interspecific comparison of larch differentiation. Characteristics of experimental sites are presented in table 1.

During research the variability of such features as length and width of cones, number of scales in the cone, mass of 1000 seeds and parameters of seeds quality, forms of edge of seed scales, color of microstrobils, lengths of needles and the number of needles in a bunch and color of shoot have been studied. The variation of the features investigated has been obtained by statistical techniques with calculation of feature`s average value for the experimental site and feature variation coefficient. The variability of the features have been estimated on S.A. Mamayev's (1972) scale. Because of low quality of seeds some trees and some experimental site have been excluded from the investigation of these features.

The basic laws of intrapopulation and interpopulation variability have been obtained in northern and southern regions of the Middle and Eastern Siberia. Moreover, the interspecific differences between species of larch growing in Siberia have been studied.

Table 1. Characteristics of experimental sites

Species of larch	Number of experimental site	Geographical coordinates		Growth class and age of forest (year)	Administrative region
		latitude	longitude		
L. sibirica	1	67°13´	87°47´	IV, 150-200	Tajmyr region
	2	67°13´	90°32´	V, 110-180	
	3	68°26´	90°19´	IV, 280-300	
	4	58°15´	91°54´	I, 150-200	Yenisejsk region of Krasnoyarsk territory
	5	55°20´	95°43´	II, 100-150	Irbejsk region of Krasnoyarsk territory
	6	55°15´	90°10´	I, 60-100	Uzhur region of Krasnoyarsk territory
	7	55°20´	90°15´		
	8	55°25´	90°15´		
	9	54°20´	89°55´	III, 100	Republic Khakasia
	10	54°10´	90°10´	III, 60	
L. gmelinii	11	64°19´	100°07´	III, 170-180	Evenkia region
	12	62°05´	113°40´	Va, 160-170	The western regions of republic Yakutia
L. cajanderi	13	69°46´	121°58´	Va, 400	Northern regions of Yakutia
	14	69°46´	121°58´	Va, 200	
	15	62°05´	129°40´	V, 200-210	The central regions of Yakutia
	16	61°40´	130°00´	V, 160	

Table 2. Scale of variability level (Mamayev, 1972)

variability level	variation coefficient (C.V. %)
Very low	less 7
low	8 – 12
middle	13 – 20
heightened	21 – 30
high	31 – 40
Very high	more 40

The results obtained have shown that intrapopulation variability of quantitative features of generative organs is stabilized in more homogeneous environmental conditions (Table 3). The last variability size of cones have been obtained in northern populations all investigated species of larch. Intrapopulation variability of length of needles and number of needles in a bunch does not exceed of middle level in the north and south of Middle Siberia (Table 4).

The study of interpopulation variability has shown that distribution of quantitative features depends on geographical location of population and local environmental features of the region, and qualitative features are rather stable across area of larch in the Middle Siberia and Yakutia. The last size of cones have been obtained in northern populations of *L. sibirica* and *L. gmelinii*. *L. cojanderi* in northern populations has more size of cones than in southern. The small size of cones in southern populations of L. cojanderi is defined by influence hybridization of this species of larch with *L. gmelinii*, which has last size of cones than *L. cojanderi* in Yakutia. However, very often variability connected with reaction of plant on environmental conditions exceeds geographical differences between phenotyps. Furthermore, it was found that relationship of size of generative and vegetative organs with elevation is showed both northern and southern regions of Middle Siberia. With increasing the elevation, the value of these features is reduced. For example, the least values of features have been established in a mountain population on plateau Putorana (experimental site No. 2) (Table 3).

The parameters of sowing seeds quality are the most changeable (Table 5). The value of these features variability reaches “very high” and “high” levels. The greatest intrapopulation variation of these features is noted in pessimal ecological conditions in the north of

Table 3. Variability of generative organs of larch

Species of larch	Number of experimental site	Length of cones (mm)		Width of cones (mm)		The number of scales in the cone	
		$X_{cp}\pm m_x$	C.V. (%)	$X_{cp}\pm m_x$	C.V. (%)	$X_{cp}\pm m_x$	C.V. (%)
L. sibirica	1	26,6±0,6	13,0	22,3±0,6	14,4	28,1±0,7	14,2
	2	23,8±0,4	8,8	20,2±0,5	12,8	26,2±0,5	11,1
	3	27,2±0,5	10,0	27,3±0,5	10,5	27,4±0,6	11,3
	4	28,8±0,7	13,0	25,9±0,7	14,2	29,6±0,8	14,7
	5	26,3±0,6	13,2	25,4±0,6	12,8	27,6±0,7	12,9
	6	28,0±0,7	13,5	26,3±0,6	13,6	31,0±0,9	15,6
	7	26.3±0,7	14,0	27,0±0,7	13,2	28,3±0,9	17,8
	8	26,9±0,8	15,3	27,3±0,7	13,5	28,5±0,9	19,1
	9	29,3±0,6	12,0	27,6±0,5	10,0	31,8±0,8	13,3
	10	25,1±0,6	13,2	23,3±0,5	11,9	29,9±0,8	14,5
L. gmelinii	11	14,3±0,2	8,9	11,9±0,2	10,1	14,9±0,3	11,5
	12	12,9±0,3	9,5	9,4±0,2	10,9	12,5±0,4	14,4
L. cajanderi	13	14,5±0,4	10,8	15,6±0,7	17,0	17,5±0,7	15,7
	14	14,2±0,3	11,4	14,1±0,5	15,3	16,6±0,5	15,1
	15	15,7±0,3	7,8	15,2±0,3	7,4	19,4±0,5	10,0
	16	15,7±0,3	8,6	14,9±0,3	8,6	18,3±0,4	10,0

X – feature`s average value for the experimental site; m_x – error of average; C.V. – coefficient of feature variation.

Table 4. Variability of vegetative organs of *Larix sibirica*

Number of experimental site	Lengths of needles (mm)		The number of needles in a bunch	
	$X_{cp}\pm m_x$	C.V. (%)	$X_{cp}\pm m_x$	C.V. (%)
1	22,8±0,8	17,5	23,5±0,9	19,0
2	20,1±0,6	15,4	19,4±0,8	21,0
3	27,7±0,9	17,3	21,5±0,7	16,9
4	27,5±0,9	17,8	27,3±0,8	16,5
6	22,5±0,7	16,4	20,3±0,8	20,3
7	26,3±0,8	16,0	28,4±0,8	16,0
8	29,6±0,8	15,2	26,1±0,7	14,6
9	24,1±0,8	18,7	26,4±0,7	15,1
10	26,9±0,9	18,4	28,5±0,8	14,8

X – feature`s average value for the experimental site; m_x – error of average; C.V. – coefficient of feature variation.

Siberia. The more stable is mass of 1000 seeds (Table 6). Intrapopulation variation of mass of 1000 seeds is showed on middle level. The reason of such differences in parameters of sowing seeds quality is bad pollination of larches in northern regions of its area as its pollination depends on climatic conditions and so interpopulation variability of these features has latitude conformity. The values of these features are decreased at moving from north on south. However, the mass of 1000 seeds and parameters of seeds quality can be used for intraspecific diagnostic of larch. Moreover, color of microstrobils have been changed by moving from north on south. In this direction quantity of trees in population with read microstrobils have been increased.

Therefore, the larch in Siberia is characterized by great diversity of morphological traits which depends on ecological conditions of location and genetical features of larch species. The main reasons variability of larch are hybridization between different species of larch and variation of environmental conditions in population location.

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Table 5. Parameters of sowing seeds quality

Species of larch	Number of experimental site	Seeds with endosperm (%)		Germination of seeds (%)		Intensity of germination (%)	
		$X_{cp} \pm m_x$	C.V	$X_{cp} \pm m_x$	C.V.	$X_{cp} \pm m_x$	C.V.
L. sibirica	1	16,5±2,0	42,0	16,1±1,9	41,6	15,2±1,8	41,4
	2	16,6±3,2	84,5	14,4±3,0	90,2	12,0±2,8	100
	3	7,9±1,0	57,9	6,6±0,9	64,3	5,4±0,9	72,8
	4	33,7±3,7	53,5	36,1±3,9	52,6	36,5±3,9	52,3
	6	47,2±3,0	35,6	46,9±3,0	35,5	45,6±2,9	35,4
	7	47,9±2,8	32,4	47,8±2,8	32,3	46,5±2,8	32,3
	8	50,3±2,4	25,7	50,2±2,4	25,8	48,9±2,3	26,0
L. gmelinii	11	19,3±1,6	41,2	18,6±1,5	40,7	17,7±1,5	41,2
L. cajanderi	16	33,0±3,0	41,3	31,7±3,1	44,3	29,9±3,1	46,8

X – feature`s average value for the experimental site; m_x – error of average; C.V. – coefficient of feature variation.

Table 6. Mass of 1000 seeds variability

Species of larch	Number of experimental site	Mass of 1000 seeds in generally		Mass of 1000 seeds which are able to growth	
		$X_{cp} \pm m_x$	C.V. (%)	$X_{cp} \pm m_x$	C.V. (%)
L. sibirica	1	4,7±0,2	17,0	6,0±0,5	22,2
	2	3,8±0,1	12,0	4,8±0,2	13,1
	3	5,2±0,2	16,5	7,3±0,3	16,1
	4	8,8±0,4	19,3	10,7±0,3	13,6
	6	9,3±0,3	15,1	10,9±0,3	14,8
	7	8,6±0,3	15,5	10,3±0,3	13,4
	8	9,1±0,3	17,6	10,9±0,3	17,3
L. gmelinii	11	1,8±0,1	17,4	2,4±0,1	15,7
	12	1,2±0,1	19,7	-	-
L. cajanderi	15	2,3±0,1	23,9	-	-
	16	2,1±0,2	28,0	-	-

X – feature`s average value for the experimental site; m_x – error of average; C.V. – coefficient of feature variation.

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Notes

Notes

Gene diversity and differentiation of siberian larch (*larix sibirica* ledeb.) populations in the middle siberia

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On the basis of 22 genes coding allozyme diversity of 13 enzymes (MDH, IDH, G-6-PD, 6-PGD, SKDH, GOT, LAP, PGI, FDH, PGM, GDH, PEPCA, SOD), the levels of genetic variation and differentiation of Siberian larch (*Larix sibirica* Ledeb.) populations from Uzhur region and from Taimyr peninsula (Krasnoyarsk Territory) were determinated. It was established that 54.55% of gene loci assayed were polymorphic. The mean number of alleles per locus, the average observed and expected heterozygosities, effective number of alleles were equal to 1.82, 0.0966, 0.0983 and 1.20, respectively. More than 92% of the total variation resided within populations and only 7.14% of the variation distributed among populations ($F_{st} = 0.0714$). The mean genetic distance (D) between populations (Nei, 1972) ranged from 0.0014 to 0.0208 and equalled on the average 0.0104. Obtained estimates of D indicate little differentiation of genetic variation among populations studied. It was established that populations situated at Taimyr peninsula (northern part of *L. sibirica* area at studied territory) were more differentiated in comparison with others populations tested. The most significant differences in allele frequencies of loci analysed were revealed between populations Irbo and Sukharikha.

Introduction

Some 263.4 million hectares or circa 37 percent of the forest area in Russia is occupied by forests that are either pure larch forests or mixed ones, i.e. with trees of the genus *Larix* in their species composition. Growing stock of all larch taxons is as high as about 22.9 billion m^3, what makes 30.4 percent of the total growing stock of Russian forests.

The largest massives of larch forest are met in Siberia and the Far East. In fact, some 97 percent of the total area of larch forests in Russia is covered with Siberian taxons of the genus *Larix: L. sibirica, L. gmelinii, L. cajanderi* (Abaimov *et al.,* 1998).

Siberian larch is the main forestforming species in the Middle Siberia. Larix sibirica stands occupy nearly 14 percent of the larch forest area of Russia. The largest concentration of this species is in the continental mountain regions of southern Siberia. In the Priangarie region (Krasnoyarsk Territory) Siberian larch grows under favorable climatic conditions in composition with pine and spruce.

Lack of data for the other regions of the natural species range does not permit to estimate the genetic potential and intraspecific differentiation of *L. sibirica* while the knowledge of the level and distribution of genetic variation through the species range is necessary to elaborate the concepts for the exploitation and restoration of *L. sibirica* forests as well as for the concervation of their genetic diversity. The objective of this work was to study the genetic diversity, structure and differentiation of *L. sibirica* populations from Uzhur and Taimyr regions of Krasnoyarsk territory.

Material and Methods

Seeds material for electrophoretic studies were collected from 96 trees in five natural populations of *L. sibirica* from Uzhur forest-steppes zone and from Taimyr peninsula (Krasnoyarsk Territory) (Table 1).

We used seeds collected from individual trees. Seeds were soaked in distilled water for 24 h. The endosperm was separated from the embryo and grinded in one or two drops of an extracting buffer (0.05 M Tris-HCl pH 7.7 with the addition of 3% PVP-40000, 0.05% β-mercaptoethanol, 0.02% EDTA-Na$_2$, 0.06% dithiothreitol). For each tree, from 7-8 to 40-50 endosperms were examineted.

Table 1. Locations of populations of Siberian larch analyzed in this study

Population	Geographical coordinates		Elevation (m)	Growth class and age of forest (year)	Administrative region
	latitude	longitude			
Population No. 1 (Uzhur)	55°15’ N	90°10’ W	500-600	I, 60-100	Uzhur region of Krasnoyarsk territory
Population No. 2 (Uzhur)	55°20’ N	90°15’ W	500-600		
Population No. 3 (Gorbiochin)	67° 13’ N	90° 32’ W	124-470	V, 110-180	Taimyr peninsula, Krasnoyarsk territory
Population No. 4 (Sukharikha)	67° 13’ N	87° 47’ W	110	IV, 150-200	
Population No. 5 (Irbo)	68° 26’ N	90° 19’ W	95	IV, 280-300	

The extracted proteins were elecrtophoretically fractionated in 13% starch gels in three buffer systems: I - tris-citrate, pH 6.2 (Adams, Joly, 1980), II - tris-citrate, pH 8.5 / LiOH-borate, pH 8.1 (Ridgway *et al.*, 1970), III - Tris-EDTA-Boric acid, pH 8.6 (Markert, Faulhaber, 1965). The gel and electrode buffers were as recommended for the systems. Enzymes were detected according to standard protocols (Brewer, 1970; Vallejos, 1983; Shaw, Prasad, 1970; Manchenko, 1994) with minor modifications. The enzymes under study and the corresponding buffer systems are shown in Table 2.

Enzymes, loci, and alleles were designated as described by Prakash *et al.* (1969). Loci were numbered according to the electrophoretic mobility of their products. Thus, the locus coding the most mobile enzyme was labelled 1 and locus coding an enzyme with more low mobility was labeled 2, etc. Within each locus, the most frequent allozyme and the corresponding allele were designated as 100. Other allozymes of the locus were designated according to their electrophoretic mobility with reference to allozyme 100. Alleles that coded phenotypically undetectable allozymes were designated as n (null alleles).

To estimate the levels of genetic variability in the populations studied the main genetic parameters such as percentage of polymorphic loci at 95% (P_{95}) and at 99% (P_{99}) criteria, mean number of alleles per locus (A), the mean observed heterozygosity (H_o), the mean expected heterozygosity (H_e) and the effective number of alleles (n_e) were calculated. The chi-square "goodness-of-fit" tests were used to determine if observed genotype frequencies were in accordance with expectations under Hardy-Weinberg equilibrium. The distribution of genetic variation within and between populations was evaluated using Wright's F-statistics (Guries, Ledig, 1982). The genetic distance (D) among the populations was estimated by Nei's method (Nei, 1972). Calculations based on genetic data were performed using the PopGen computer program (Yeh *et al.*, 1999).

Results and Discussion

Genetic Variability within Population

Forty allelic variants at the 22 loci were revealed in the course of the study of the 13 enzyme systems in natural populations of *L. sibirica* from Uzhur and Taimyr regions. Loci Mdh-2, Got-3, Lap-1, Idh, Pgi-1, Pgm-2, Gdh, Pepca, G-6pd, Sod-1 were monomorphic in all populations. The remaining loci (Mdh-1, Mdh-3, Mdh-4, 6-Pgd-1, 6-Pgd-2, Got-1, Got-2, Lap-2, Pgi-2, Pgm-1, Fdh, Sdh-2) were polymorphic at least in one population. Eleven loci had two (Mdh-1, Mdh-4, 6-Pgd-1, Got-1, Got-2, Pgi-2, Fdh, Sdh-2) or three (6-Pgd-2, Lap-2, Pgm-1) alleles. The locus of Mdh-3 had the highest number of alleles (4 alleles). The allele frequencies are listed in Table 3. The most common alleles of the polymorphic loci were as a rule the same in all populations.

On the basis of allelic frequencies the main parameters of genetic variability were calculated (Table 4). As seen in table 4 the values of all parameters varied among the populations. The percentage of polymorphic loci at 95% criterion (P_{95}) ranged from 18.18% to 27.27% and at 99% criterion (P_{99}) from 22.73% to 40.91%. The mean number of alleles per locus (A) ranged from 1.36 to 1.59. The mean value of observed heterozygosity (H_o) varied from 0.077 to 0.117. The mean expected heterozygosity (H_e) was higher than the mean observed heterozygosity and ranged from 0.083 to 0.113. The effective number of alleles (n_e) ranged from 1.16 to 1.24. As a whole for five populations studied of *L. sibirica* the percentage of polymorphic loci at 99% polymorphism criterion composed 54.55%, the mean number of alleles per locus, the mean observed heterozygosity, the mean expected heterozygosity and the effective number of alleles were equal to 1.82, 0.097, 0.098 and 1.20, respectively.

The mean value of expected heterozygosity ($H_e = 0.098$) obtained in our study was lower than those computed for the genus *Larix* ($H_e = 0.142$) on the basis of published data for 13 *Larix* species (Fins and Seeb, 1986; Cheliak

Table 2. Ensymes (enzyme classification and nomenclature, 1962) and buffer systems used in this work

Enzyme	Designation	EC	Buffer system
Malate dehydrogenase	MDH	1.1.1.37	I
6-Phosphogluconate dehydrogenase	6-PGD	1.1.1.44	I
Isocitrate dehydrogenase	IDH	1.1.1.42	I
Shikimate dehydrogenase	SKDH	1.1.1.25	I
Leucine aminopeptidase	LAP	3.4.11.1	II
Glutamate-oxaloacetate transaminase	GOT	2.6.1.1	II
Phosphoglucoisomerase	PGI	5.3.1.9	II
Formate dehydrogenase	FDH	1.2.1.2	II
Glutamate dehydrogenase	GDH	1.4.1.2.	III
Glucose-6-phosphate dehydrogenase	G-6-PD	1.1.1.49.	III
Phosphoglucomutase	PGM	2.7.5.1	III
Superoxide dismutase	SOD	1.15.1.1.	III
Phosphoenolpiruvate carboxilase	PEPCA	4.1.1.31	III

et al, 1988; Shurkhal *et al*, 1989; Lewandowski *et al*, 1991; Liu and Knowles, 1991; Ying and Morgenstern, 1991; Semerikov and Matveev, 1995; Potenko and Razumov, 1996; Goncharenko and Silin, 1997; Shigapov *et al*, 1998; Semerikov *et al*, 1999) and for 42 conifer species (H_e = 0.145) (Krutovskii *et al*, 1989). The mean observed heterozygosity (H_o = 0.097) was somewhat lower than average estimates of this parameter both for the 13 *Larix* species (H_o = 0.138) and for 42 species of conifer (H_o = 0.152) reviewed of Krutovskii *et al* (1989).

Populations of *L. sibirica* from Uzhur forest-steppes zone had lower as a whole the average estimates the expected heterozygosity and the effective numbers of alleles in comparison with the populations from Taimyr

Table 3. Allele frequencies for 22 loci in *L. sibirica* populations studied

Locus	Allele	Populations				
		Uzhur 1	Uzhur 2	Gorbiochin	Sukharikha	Irbo
Mdh-1	100	1.000	0.983	0.969	1.000	1.000
	93	-	0.017	0.031	-	-
Mdh-2	100	-1.000	1.000	1.000	1.000	1.000
Mdh-3	113	0.048	0.052	0.031	-	0.072
	100	0.484	0.517	0.469	0.462	0.571
	68	0.242	0.259	0.219	0.269	0.143
	52	0.097	-	-	0.077	0.214
	24	0.129	0.172	0.281	0.192	-
Mdh-4	200	-	0.017	-	-	0.071
	100	1.000	0.983	1.000	1.000	0.929
6-Pgd-1	117	-	-	-	-	0.071
	100	1.000	1.000	1.000	1.000	0.929
6-Pgd-2	116	0.145	0.241	0.063	0.231	0.429
	100	0.726	0.638	0.874	0.538	0.429
	85	0.129	0.121	0.063	0.231	0.142
Got-1	107	0.274	0.293	0.062	0.346	0.214
	100	0.726	0.707	0.938	0.654	0.786
Got-2	111	0.113	0.103	0.312	-	0.429
	100	0.887	0.897	0.688	1.000	0.571
Got-3	100	1.000	1.000	1.000	1.000	1.000
Lap-1	100	1.000	1.000	1.000	1.000	1.000
Lap-2	100	0.984	0.983	1.000	1.000	1.000
	98	0.016	-	-	-	-
	Null	-	0.017	-	-	-
Idh	100	1.000	1.000	1.000	1.000	1.000
Pgi-1	100	1.000	1.000	1.000	1.000	1.000
Pgi-2	100	1.000	0.983	1.000	1.000	1.000
	92	-	0.017	-	-	-
Pgm-1	107	0.048	0.017	0.094	0.346	-
	100	0.936	0.845	0.906	0.654	1.000
	90	0.016	0.138	-	-	-
Pgm-2	100	1.000	1.000	1.000	1.000	1.000
Fdh	100	0.984	1.000	1.000	1.000	1.000
	78	0.016	-	-	-	-
Gdh	100	1.000	1.000	1.000	1.000	1.000
Skdh-2	100	0.984	1.000	0.938	0.962	1.000
	76	0.016	-	0.062	0.038	-
Pepca	100	1.000	1.000	1.000	1.000	1.000
G-6-pd	100	1.000	1.000	1.000	1.000	1.000
Sod-1	100	1.000	1.000	1.000	1.000	1.000

peninsula ($H_e = 0.096$; $n_e = 1.19$ and $H_e = 0.105$; $n_e = 1.21$, respectively). At the some time the mean number of alleles per locus in total for larch populations from Uzhur was significantly higher than those in the populations from Taimyr (A = 1.77 and A = 1.59, respectively) mostly at the expence of rare alleles. The mean values P_{95} and P_{99} for two larch populations from Uzhur region were 22.73% and 50.00%, respectively, for the populations from Taimyr peninsula the values of these parameters were 22.73% and 40.91%, respectively.

Organization of genetic variation

Population structure of *L. sibirica* from Uzhur and Taimyr regions was analysed by means of Wright's F-statistics (Guries, Ledig, 1982). Inbreeding coefficients of F_{is} and F_{it} estimate the deviations of genotype frequencies from Hardy-Weinberg equilibrium of individuals in each sample population and in the species on the whole, respectively, whereas F_{st} estimates the degree of genetic differentiation among populations.

The F_{is} values ranged among polymorphic loci from -0.1225 (Mdh-3) to 0.3441 (Pgm-1) and averaged -0.05450. Obtained value was negative. This means that within populations there was 5.45% excess of heterozygotes relative to Hardy-Weinberg expectation. Value of F_{it}, which characterizes individual inbreeding relative to the species, was on average 0.0208. Positive F_{it} value of this coefficients is indicative of 2.08% deficiency of heterozygotes in *L. sibirica* as a whole (Table 5).

The value F_{st}, which was calculated as a weighted mean of F_{st} for all the populations investigated, ranged among polymorphic loci from 0.0118 to 0.1569. The highest contribution to the among-population variability was made by the Got-2, Pgm-1, 6-Pgd-1 and 6-Pgd-2 loci, while the contribution of the Lap-1 locus was the smallest. The average value of F_{st} over all loci assayed was equal to 0.0714. This testifies that only 7.14% of the total genetic variation was attributable to the differences among the populations, remaining variation (about 92%) resided within populations.

Obtained value of F_{st} for *L. sibirica* populations from Uzhur and Taimyr is indicative of absence significant subdivision of populations from these regions. Value of Fst was within the limits of F_{st} values ($F_{st} = 0.020 – 0.086$) established for other species of *Larix : L. occidentalis* (Fins and Seeb, 1986), *L. laricina* (Cheliak *et al.*, 1988; Liu and Knowles, 1991; Ying and Morgenstern, 1991), *L. sibirica* (Semerikov and Matveev, 1995; Semerikov *et al.*, 1999), *L. decidua* (Lewandowski and Meinartowicz, 1991; Maier, 1992), *L. sukaczewii* (Shigapov *et al.*, 1998).

The degree of genetic differentiation among *L. sibirica* populations studied was quantified using Nei's genetic distance D (Nei, 1972). Estimated from the allele frequencies of the 22 loci, the genetic distances D between populations are listed in the Table 6. As the Table 6 demonstrates, the mean genetic distance between populations ranged from 0.0014 to 0.0208 and equalled on the average 0.0104. The maximum value of D was found between populations from Taimyr: Irbo and Sukharikha, the minimum value was detected between located side by side populations of *L. sibirica* from Uzhur region. The analysis of genetic distances was shown that populations studied from Taimyr peninsula were more differentiated (D = 0.0179) than distant with one another geographically populations from Uzhur region and populations from Taimyr (0.0083).

Obtained estimates of F_{st} and D are indicative of weak subdivision and low level of differentiation among *L. sibirica* populations studied in Uzhur and Taimyr regions of Krasnoyarsk territory.

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Table 4. Genetic variability at 22 loci in populations studied of *L. Sibirica* (standart deviation in parentheses

Populations	Percentage of polymorphic loci		Mean heterozygosity		Mean number of alleles per locus	Effective number of alleles
			Observed	Expected		
	P_{95}	P_{99}	H_o	H_e	A_{99}	n_e
Uzhur 1	22.73	36.36	0.0953 (0.2017)	0.0892 (0.1853)	1.5909 (1.0075)	1.1834 (0.4806)
Uzhur 2	22.73	40.91	0.0956 (0.1882)	0.0997 (0.1913)	1.5909 (0.8541)	1.1932 (0.4377)
Gorbiochin	27.27	31.82	0.0767 (0.1670)	0.0832 (0.1713)	1.4545 (0.8004)	1.1572 (0.4204)
Sukharikha	18.18	22.73	0.1154 (0.2673)	0.1066 (0.2269)	1.3636 (0.7895)	1.2410 (0.5605)
Irbo	27.27	27.27	0.1169 (0.2275)	0.1129 (0.2210)	1.4091 (0.7964)	1.2212 (0.4846)
In total for the species	36.4	54.55	0.0966 (0.1942)	0.0983 (0.1879)	1.8182 (1.0065)	1.1966 (0.4665)

Table 5. Estimates of Wright's F-statistics coefficients calculated for each locus for all *L. sibirica* populations studies

Locus	F_{is}	F_{it}	F_{ST}
Mdh-1	-0.0270	-0.0098	0.0167
Mdh-3	-0.1225	-0.0907	0.0284
Mdh-4	-0.0648	-0.0181	0.0439
6-Pgd-1	-0.0769	-0.0145	0.0580
6-Pgd-2	-0.1046	-0.0169	0.0794
Got-1	-0.0973	-0.0399	0.0523
Got-2	-0.0566	0.1092	0.1569
Lap-2	-0.0170	-0.0050	0.0118
Pgi-2	-0.0175	-0.0035	0.0138
Pgm-1	0.3441	0.4358	0.1399
Fdh	-0.0164	-0.0032	0.0129
Skdh-2	-0.0507	-0.0240	0.0254
Mean	-0.0545	0.0208	0.0714

Table 6. Genetic distances D (Nei, 1972) between populations of *L. Sibirica* based upon data from the 22 loci

Populations	Uzhur 1	Uzhur 2	Gorbiochin	Sukharikha
Uzhur 1	***			
Uzhur 2	0.0014	***		
Gorbiochin	0.0061	0.0085	***	
Sukharikha	0.0067	0.0058	0.0169	***
Irbo	0.0114	0.0116	0.0151	0.0208

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Interspecific Hybridization of Larch in Russia

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The reproductive isolation is weakly expressed between species of the genus *Larix*, including larch species in Russia. Therefore natural interspecific hybrids are formed very often within the border of areas of larch species.

Larix x czekanowskii Szafer (*L. sibirica* Ledeb. x *L. gmelinii* (Rupr.) Rupr.) is most known introgressive interspecific complex in the genus *Larix*. Approximately, the *L. x czekanowskii* forests growing in Russia occupy some 9,6 mln. ha (or about 11% of the area, covered with parent species).

Ecology of *L. x czekanowskii* since other hybrid complexes were not studied in this respect. Specific relating of these or those hybrid forms of *L. x czekanowskii* to different sites is revealed. For example, at the Baikalsky ridge (the north-western Baikal shore). *L. gmelinii* and its allied hybrids preval somehow when moving towards south-west along the Baikal in the upperand middle mountain belt. To the contrary, *L. sibirica* dominates moving to the north-east in the lower slope belt and at the Baikal lake shore. A comparison of two larch hybrid population can illustrate the described regularity. One of them (very open larch forest at the upper timberline, 30 km nord-west of the village of Baikalsk) consists up to 25% of *L. gmelinii* and up to 75% of its allied hybrids. However, composition of another population (larch forest of the cowberry-Alpenrose type, near some village) is following hybrids allied to *L. sibirica* make 10%, hybrids with equal ratio of both parents species characters make 39%, hybrids allied to *L. gmelinii* – 48%, and *L. gmelinii* itself – 3%. Thus, altitudinal population consists entirely of *L. gmelinii* and its allied hybrids, and in the forests growing in the Baikal shore these species form only 51% of the population (Kruklis, Milyutin, 1977).

Even more clear regularities between site condition and structure of hybrid populations are observed in Zabaikal'e. *L. sibirica* and *L. gmelinii* contact each other in this region according to sites clearly demarcated in ecological regard: *L. gmelinii* is met in cold bottoms of small valleys, on bogged terraces or on the northern slopes with frozen ground, while *L. sibirica* occurs in well drained larger river valleys, along the southern mountain slopes, etc. For example, in environs of the town of Petrovsk-Zabaikalsky (Chita province), hybrids allied to *L. gmelinii* are met only in the wet ravines at the eastern border of the *L. sibirica* range of distribution. *L. gmelinii* is absent on the southern slope near Osinovka village (Khantai-Chikoi plateau, Chita province), while it makes 77% in the tree stand on the north-western slope, and as much as 88% on the northern one. Obviously, in Zabaikal'e region with its sharp variations of microclimatic conditions, the dependence between larch species as well as their hybrids and site conditions is seen especially clearly.

L. x czekanowskii is characterized most often by the unusual combinations of the features of parent species. New features, for example, cones with seed scales turned back are observed sometimes. Nevertheless, they are met only in some populations and relatively seldom (2-13%). In terms of the seed-scale upper edge form, the populations of *L. x czekanowskii* – in which all its types are met in different combinations – are most differenciated. Trees with round apper edge of seed-scales prevail (57-97%) in stands similar to *L. sibirica*, while with weakly emarginate, emarginate and straight-cut (50-98%) are met in populations where features of *L. gmelinii* dominate. Hovewer, any regularity in frequencies of trees with these or those features were not revealed. In *L. x czekanowskii* populations all shapes of seed scale surface are present, however trees with cones having spoon-shaped scales (57-100%) prevail in most cases. Similar trees make the majority even in populations close to the area occupied by *L. gmelinii*. Likely the spoon-shaped seed scale is more stable than other ones. At *L. sibirica* and *L. gmelinii* hybridization the genes of *L. sibirica* which control this feature seemingly remains in genotypes of the hybrid complex longer than other genes.

Somatic heterosis in *L. x czekanowskii* is a known phenomenon, however a share of heterose plants does not exceed a few percent in natural populations. Population of *L. x czekanowskii* are characterized also by another types of heterosis: reproductive and adaptive. Reproductive heterosis is characterized by the great seed productivity (about 120 kg/ha). Adaptive heterosis is a better adaptation of hybrid populations to changable environmental conditions (Kruklis, Milyutin, 1977).

Some pecularities of *L. x czekanowskii* forests are assocated with the "introgression of formation" (Bobrov, 1972). For example, on the North Baikal coast *L. sibirica* with some hybrids can grow in stands with *Pinus pumila*, which is usually typical for *L. gmelinii*.

Western border of area of *L. sibirica* has contact with the area of *L. sukaczewii* Dylis in the Polar Urals and basin of the Ob River. Hybrid populations of these two species are forming in these regions. Hybrid forms allied to *L. sukaczewii* grow better in warm and dry sites, while the forms allied to *L. sibirica* are met cold and wet sites (Iroshnikov, 1980 *et al.*). *L. gmelinii* and *L. cajanderi* Mayr hybridize in their contact zone, east of area *L. gmelinii*. This hybrid zone covers some 7% of the area of both parent species. (Abaimov *et al.*, 1998).

Besides, hybrid complexes of larch: *L. gmelinii x L. x maritima* Sukacz. x *L. olgensis* A.Henry (= *L. x amurensis* Kolesn.), *L. olgensis x L. principis-rupprechtii* Mayr (= *L. x lubarskii* Sukacz.), *L. x lubarskii x L. olgensis* (= *L. x komarovii* Kolesn.), *L. gmelinii x L. kamtschatica* (Rupr.) Carr. (= *L. x maritima*), *L. kamtschatica x L. x maritima* (= *L. middendorffii* Kolesn.) are distinguished in the Russian Far East. These hybrid complexes are studied insufficient.

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Perspectives of androgesis in vitro using in *Larix sibirica*

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Siberian larch (*Larix sibirica* Ledeb.) is one of the main conifer species growing in East Siberia forests and has a great value for forestry. However, its use for reforestation is limited because of low seed yield and bad quality of seeds. In addition, Siberian larch trees are damaged by larch bud midge (*Dazyneura rozkovii* Mam. Et Nik.) that influences buds formation negatively. One of the ways to overcome this problem is tissue culture using. Androgenesis in vitro (microsporial/pollen embryogenesis) is unique phenomenon which leads to the formation of new plant organism from male haploid generative cell. Unfortunately, all attempts to obtain plantlets from microspore or pollen in conifer species were unsuccessful.

In our experiments, it was found that concentration of macroelements, and particularly NH_4NO_3, has a high importance for induction of andregenesis of Siberian larch. Decreasing of NH_4NO_3 concentration in two times resulted in more active androcline calluses formation. So, it is more reasonable to use ½ MS and 99 mediums as well as modified MS medium (where the concentration of this element is decreased) for induction of androgenesis in this species.

Cytological analysis have shown that hormone concentration in the nutrient medium have a high influence on embryiod formation pathways and embryoids morphology. Using auxins only (2,4-D in concentration 0,2-0,5 mg/l) resulted in the embryoid formation directly from microspore. It is interesting to note that such embryoids were similar to embryos of angiosperm plants. Using auxins in combination with cytokynins led to the callus tissue formation in which the embryo-like structures were found. Such embryoids looked like both zygotic and somatic larch embryos at the early stage of development.

Overcoming the problem of microspore-derived callus proliferation and embryoids maturation will help to solve a range of fundamental questions (such as cell totypotency) as well as multiply valuable genotypes of Siberian larch.

Notes

Growth and adaptation of clonal larchs propagated by somatic embryogenesis : three series of clonal tests in four Québec bioclimatic domains

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In Québec, *Larix decidua, L. kaempferi* and their hybrid (*L.* × *marschlinsii*) are introduced larch used for intensive silviculture, especially *L. x marchlinsii* (H_1). However, large scale production of H_1 seeds is limited by pre- and post-zygotic factors, as well as asynchrony in the flowering seasons of the parental species. With such production problems, somatic embryogenesis (SE) will be a key element in the long term deployment of H_1 improved varieties. With its capacity for long-term germplasm preservation and scale-up technologies, SE seems to be the preferred avenue to accelerate selection and operational deployment of value-added genotypes.The objectives of the study were to 1) examine the adaptation of seedlings produced by SE in various planting conditions, 2) to obtain preliminary estimates of variability and potential genetic gain of SE lines, 3) and finally to determine the best SE lines for various bioclimatic domains. From 2000 to 2002, seven clonal field tests were established in four contrasting bioclimatic domains. Over 100 SE lines originating from three years independent SE batch productions are represented in those clonal tests. The SE lines originated from 26 families (1998-2000 controlled crosses, MRNF) and various controls like seeds from MRNF seed orchard and two European seed orchards (F. von Lochows-Petkus and FHNO 1002). According to controlled crosses and SE development, the number of SE lines and seedlings had varied from year to year. Each clonal field test was set up in a randomized complete block design, with one or two trees per plot and seven or ten blocks according to year of induction. Results after 5 years in plantation will be presented.

Notes

Interspecific fundamental study in hybrid larch breeding program in Québec

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For decade, breeding programs are using hybrids between different species. Indeed, H_1 hybrid's present a superiority relative to its parents mean value, a phenomenon called "heterosis". However, genetic mechanisms responsible for heterosis remain poorly understood (Swanson-Wagner 2006). Multi-species genetic improvement programmes seeking to achieve the full benefits of interspecific hybridisation are then complicated. To overcome this lack of information, four experiments will be integrated (2004-2020) within our second generation hybrid larch (*L.* × *marschlinsii* Coaz.) activities leading to recommendations regarding the best families for use in reforestation (cuttings). Experiment one: Using *L. decidua* or *L. kaempferi* as mother tree of H_1 (ExJ or JxE). Experiment two: detecting the origin of heterosis in *L.* × *marschlinsii* with an intra- and inter-specific factorial crosses (10 × 10); organised in such a manner as to create four 5 × 5 subgroups, two intra- and two inter-specific (LI and WU 1996). Experiment three: Using GCA or GHA-based selection. Experiment four: comparing synthetic species strategy (SYN; H_1 hybrids as genitors of H_2, H_3 etc. generations) with reciprocal recurrent selection with forward selection (RRS-FS). Our approach will increase the knowledge base required in choosing a breeding strategy better targeted at optimizing the use of heterosis. Our experiments can also serve as the basis for future studies of a better understanding of heterosis through molecular genetics.

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Notes

Larch in the genetic gains demonstration network: 10-year results
Les mélèzes dans le réseau de démonstration des gains génétiques : résultats à 10 ans

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In 1996, a network of plantations was established in Québec to demonstrate the genetic gains made over the previous 25 years through the genetic improvement program. Among the softwood species demonstrated the larch was planted over three sites, representing two Québec bioclimatic areas.

For all sites, the larch showed exceptional growth compared to that of other species. At Notre-Dame-du-Lac, the average height of the larch at 10 years was 7.4 m and the average height of the white and black spruces were only 2.5 m. At the other 2 sites, the height of the larch was just over double that of the other species (6 m vs. 3 m). The best performer was a source of Sudeten European larch from a German seed orchard called “Sudeten” (Grohnde) and composed of 28 original selected clones from Poland and the Czech Republic.

Given its high growth potential in short rotations (35–40 years), larch is an excellent choice to include in any intensive silviculture strategy. The quality of the wood and fibres offer exceptional possibilities for diversification and the development of many high-quality products. The demonstration network allows us to show the huge growth potential of genetically superior sources of hybrid and European larch and will continue to be a regional window for promotion.

Web site: http://cfs.nrcan.gc.ca/subsite/demonstration

Notes

Genetic Improvement of Siberian Larch for Agroforestry

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Siberian larch, (*Larix sibirica* Ledeb.) was first introduced to the Canadian prairies in the early 1900's. It was first evaluated as a woodlot species for production of poles and lumber. One of the first plantations in the prairies was planted at the Indian Head Forest Nursery Station in 1908 using seed collected in the eastern Ural mountains of Russia. Siberian larch has been planted in field and farmstead shelterbelts in the Canadian prairies since the late 1970's. For the past 25 years, Siberian larch has been a one of the main species targeted for genetic improvement. The larch breeding program at AAFC-PFRA has included international germplasm exchange, provenance testing, breeding and seed orchard development. The major outcome of this program to date has been the development of the genetically superior seed strain known as 'Lindquist' Siberian larch. This poster describes the Siberian larch genetic improvement at the AAFC-PFRA Shelterbelt Centre, its activities, accomplishments and impacts.

Notes

Development and structure of bark in the stem of *Larix*

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Samples including bark and adjacent xylem were cut from *Larix kaempferi* trees cultured at the Forest Tree Breeding Centre, Tohoku Regional Breeding Office, Japan. In addition, bark samples from three species *(L. kaempferii, L. gmelinii, L. principis-rupprechtii)* were taken from the xylarium of the Research Institute for Sustainable Humanosphere (RISH), Kyoto University. The samples contained barks of different ages, ranging from one to more than 60-year-old. They were embedded in PEG 1500, cut on a sliding microtome, and observed by conventional, polarized-light, fluorescence and confocal microscopy. The structure of bark, with particular reference to rhytidome, schlerenchyma, crystals, and resin cavities, was compared between the species. The variation in the structure of bark elements is discussed in view of the taxonomy of the genus *Larix*.

Key words: bark development and structure, *Larix*, calcium-oxalate crystals, chlorenchyma, collenchyma, phelloderm, sclerenchyma, rhytidome.

Notes

Larch seed extraction improvement, a finalized project from preliminary studies to operational transfer in French forest seed industry

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Cemagref demonstrated in cooperation with French seed dealers that: i) up to 50% of the seed potential remained in the larch cones after processing; ii) remaining seeds had same germination potential as seeds extracted first. Consequently, it appeared necessary to improve seed extraction techniques in order to optimize economic and genetic valorisation of valuable seed sources. The seed release is incomplete due to an insufficient scales opening angle despite artificial drying; this is particularly the case with European larch, *Larix decidua* Mill. Therefore, it was decided to explore mechanical solutions to disintegrate the cone structure. A survey of extraction techniques in European countries concerned with recalcitrant larch cones put in evidence two main mechanical ways to improve seed release, the crushing of cones and the grinding of scales. Firstly, cone crushing was evaluated as a fast and relatively efficient technique despite a high percentage of damaged seeds. Secondly, different rotating grinding drums were designed and evaluated. The most efficient prototype was circular and made out of a strong abrasive grid favouring attrition and limiting shocks encountered in angular drums. In order to hasten the scale grinding process an inside retaining blade was adopted to increase the scraping energy. A specific design including a balancing load maintains the blade in the most convenient working angle. The grinding drum and retaining blade allow an average seed yield of 0.9 to 1.8 kg per hectolitre of European larch cones compared to 0.45 to 1 kg with the standard drying and tumbling extraction technique. This method has been transferred to the National Forest Office seed centre. Industrial scaled up equipment with two hectolitres capacity was designed and built on the basis of the prototype; it is used satisfactorily in routine for larch seed extraction.

Notes

The genetic-silvicultural value and capability of adaptation of polish provenances of larch under conditions of the beskid sadecki mountains (Southern Poland).

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Abstract

The paper presents results of a 20-year study on adaptation and genetic-silvicultural value of 20 Polish provenances of larch. The study object consisted of 40-year-old stands (partial populations) of larch tested under mountain conditions of Poland (Forest Experimental Station in Krynica – the Beskid Sądecki mountain range in the Carpathians) as a part of the Polish Provenance Experiment of Larch 1967 (Fig. 1). A considerable stability of adaptation of analyzed larch provenances was found with a certain tendency of its increase with increase of average height of trees (Table 1, Fig. 2).

At age of 8 – 11 years it is possible to predict with a high probability the growth of larch trees of the investigated provenances during subsequent years. This was shown by values of autocorrelation coefficients of height, which were statistically significant up to the 40th year of tree life (Table 2). Thus, they may play a significant role in so called indirect selection of larch.

A complex estimation of the genetic-silvicultural value, including height, d.b.h., and quality of the stem, as well the resistance of trees to larch canker showed that larch from Kłodzko (the Sudetes) was of a very good quality (Fig. 3). The group of provenances of good silvicultural quality included larches from the Świętokrzyskie Mountains (Bliżyn, Świętokrzyski National Park, and Moskorzew) and larches growing outside the natural range of larch in Poland (Myślibórz Północ and Konstancjewo–the Płonne reserve). A geographical variation (Fig. 4) of the analyzed trait indicated a considerable mixing of larch populations of different quality not only on the level of the country but also within individual forest management districts or even smaller areas. This creates the possibility to widen the existing base of larch seed in Poland by including new populations hitherto little known and untested.

Keywords: larch, provenance, adaptation, height, autocorrelation, indirect selection, silvicultural value.

Introduction and aim

The distribution of larch in Poland is scattered. It grows in greater or smaller isolated localities (Boratyński 1986). The greater areas occur only in mountain regions: the Świętokrzyskie Mountains, the Sudetes and the Carpathians. This island occurrence of larch resulted in distinguishing two subspecies within the species *Larix decidua* (Mill), i.e. European larch (*Larix decidua* subsp. *decidua*), occurring only in the Alps and the Tatra (mainly at the upper forest limit and in the upper part of the upper mountain zone), including the Sudetic variety (*Larix decidua* var. *sudetica* (Cieś. Domin.)), occurring in the lower mountain zone of the eastern Sudetes, and Polish larch (*L. decidua* subsp. *polonica* Domin, *Larix polonica* Racib.). The range of Polish larch occurs in the central part of Polish uplands (Małopolska Region and the Świętokrzyskie Mountains), while in Polish lowlands (Mazowsze-Podlasie Region and Silesian Region) it occurs in scattered localities. In this scattered form of occurrence it may be found in almost the entire area of submontane and lower mountain zones of the Carpathians.

The knowledge on gene resources of larch, a pioneer and fast growing species of potential economic importance in Polish forestry, is still fragmentary and unsatisfactory. To widen this knowledge, studies on intra-specific variation of larch were undertaken in 1967 including the entire territory of Poland. The results of this Polish Provenance Experiment of Larch 1967, obtained so far on five experimental plots (Sękocin, Bliżyn, Rogów, Siemianice, and Krynica), and concerning such traits as growth dynamics, stem quality, resistance to canker, morphological characteristics of needles, and also silvicultural usefulness of Polish provenances of larch, showed a high diversification of partial populations within a single forest region or sub-region, but also within a single forest management district (Andrzejczyk and Bellon 1999, Kulej 1995, 2001, 2002, 2004, 2006, Rzeźnik 1992).

The purpose of this study was to determine adaptive properties as well as a genetic-silvicultural value of Polish provenances of larch, 40 years of age, tested under conditions of the Beskid Sądecki mountain range.

Material and study area

The study included 20 provenances of larch originating from the entire Polish territory tested in the experimental area of the Forest Experimental Station in Krynica. Location and distribution of larch of tested partial populations in Poland are shown in Fig. 1. Each provenance was planted in five replications according to the "Latin rectangle". The experimental area is situated in the Carpathian Forest Region, sub-region of Gorce and Beskid Sądecki mountain ranges, at altitude of 785 m, longitude 20°58' E, latitude 49°21' N. A detailed climatic and soil description may be found in earlier paper of the author (Kulej 2001).

Methods

The results of height measurements carried out during 1969–2004, i.e. when trees were 5, 8, 11, 15, 20, 25, 30, 35, and 40 years old, were analyzed in detail. The effect of the genotype (provenance) on formation of the investigated trait of larches of individual provenances during the analyzed period of time was determined on the basis of the analysis of variance and the Snedecor's F test. While the mutual relationships of the mean height of tested larches during consecutive years of the measurement were determined using coefficients of autocorrelation (Krysicki *et al.* 1995).

The estimation of adaptive properties of larches of investigated partial populations was accomplished with the Finlay-Wilkinson (1963) method. It was based on the interaction effect “provenance x years of observation” (G x E_{age}) in relation to the mean height of trees of a given provenance at the age of 40 years. A linear regression ($H_{prov.}$ x $H_{loc.}$), which determines the range of influence of a complex of environmental factors on the effect of the interaction “provenance x years”, was computed for larches of respective provenances. Also the value of this effect and changes caused by seasonal factors were computed. The partial populations reaching the value of the coefficient of regression $b = 1$ are characterized by the average stability in a given environment. When $b > 1$ or $b < 1$ it may be supposed that stability of tested larches is below or above the mean, and the effect of the interaction “genotype x years” in relation to height of trees is high. Furthermore, when $b > 1$, a given population is characterized by a high reactivity to environment (worsening or improvement), and when $b < 1$, it is characterized by a high stability.

In a complex estimation of the genetic-silvicultural value of tested larch provenances, when trees were 40 years old, mean values of height (x_1), d.b.h. (h_2), stem quality (x_3), and resistance to canker (x_4) were taken into account. The so called index of silvicultural value W_h was assumed to be a measure of this value. It was computed using the following formula:

$$W_h = ¼ (x_1 + x_2 + x_3 + x_4)$$

where x_1, x_2, x_3, and x_4 are values of these traits expressed in standardized units (Krysicki *et al.* 1995). In assumed convention, equal economic weights ($E = 1$) were attributed to all traits. On the basis of values of the index Wh the classification of larches of tested partial populations was made using five classes of the silvicultural value: very good, good, average, poor, and bad. One standard deviation was assumed to be the class boundary.

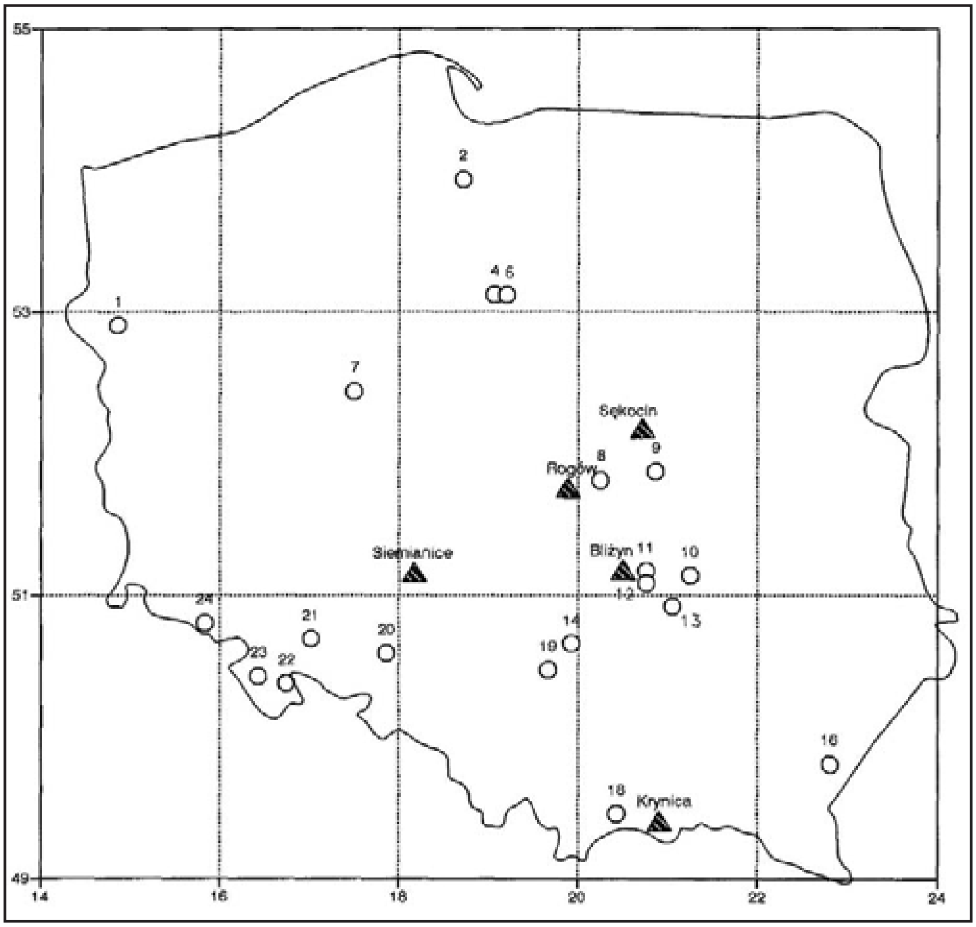

Fig. 1. Location of parental stands of larches of provenances investigated on test site at Krynica Experimental Forest Station; O – location of provenances, ▲ – location of test sites, 1–24 – provenance number and name: 1 – Myślibórz Północ, 2 – Pelplin, 4 – Konstancjewo rezerwat „Płonne”, 6 – Konstancjewo rezerwat „Tomkowo”, 7 – Czerniejewo, 8 – Rawa Mazowiecka, 9 – Grójec, 10 – Marcule, 11 – Skarżysko, 12 – Bliżyn, 13 – Świętokrzyski Park Narodowy, 14 – Moskorzew, 16 – Hołubla, 18 – Krościenko, 19 – Pilica, 20 – Prószków, 21 – Henryków, 22 – Kłodzko, 23 – Szczytna Śląska, 24 – Kowary

Results

Larch partial populations, investigated when trees were 5, 8, 11, 15, 20, 25, 30, 35, and 40 years old, made a heterogeneous statistically diversified material in respect to height growth. This diversification was already observed when trees were 5 years old. During the entire study period the level of significance (α) of the effect of the genotype (provenance) on variation of this trait was 0.05 and 0.01. Also there was a considerable variation of height of individual trees within a single provenance during a 40-year period. The mean height of trees, 5 to 40 years old (1969–2004), expressed as the percentage in relation to annual experimental mean (Table 1), varied from 83 to 116.

The following provenances were characterized by the percentage considerably above the annual mean (over 100): Świętokrzyski National Park, Moskorzew, Prószków, and Kłodzko. Actually, the provenances from Skarżysko, Hołubla, Szczytna Śląska, and Kowary could also be included in this group. While during the entire study period larches from Rawa Mazowiecka, Grójec, and Marcula were characterized by the percentage below the annual mean (below 100).

Larches from Pelplin, Skarżysko, Bliżyn, Świętokrzyski National Park, Hołubla, Pilica, Henryków, and Kłodzko showed an average adaptive stability ($b \cong 1$) in relation to height. Provenances from Myślibórz Północ, Konstancjewo-the Płonne reserve, Czerniejewo, Moskorzew, Prószków, Szczytna Sląska, and Kowary were characterized by reactivity to change of site ($b > 1$). While a high stability ($b < 1$) was shown by larches from Konstancjewo-the Tomkowo reserve, Rawa Mazowiecka, Grójec, Marcule, and Krościenko (Table 1).

The estimation of adaptive properties of larches of individual provenances under mountain conditions of the Beskid Sądecki based on the index of adaptation *“b”* and mean heights of 40-year-old trees are presented in Fig. 2. Generally, two groups of larches may be distinguished on the basis of location of points determining functional relationships between values of traits under discussion in the coordinate system (*b* and *H*) for the investigated 40-year-old partial populations. Populations from Myślibórz Północ, Pelpin, Konstancjewo-the Płonne reserve, Czerniejewo, Bliżyn, Świętokrzyski National Park, Moskorzew, Pilica, and larches from the Sudetes (Prószków, Henryków, Kłodzko, Szczytna Śląska, and Kowary) were the populations of a high reactivity and good height growth ($b > 1$ and *H* above the mean). While populations from Konstancjewo-the Tomkowo reserve, Rawa Mazowiecka, Grójec, Marcule, Skarżysko, and Krościenko were characterized by stability above the mean ($b < 1$) and height below the mean. Larch from Hołubla ($b \cong 1$, $H \cong$ mean) assumed the position between these two groups.

Table 1. Evaluation of adaptation traits of larches of investigated provenances according to Finlay- Wilkinson’s method (1963). Mean height of provenances in % of annual means; H_{2004} – mean height at age 40, *b* – regression coefficient

Prov. No.	Provenance name	Years									H_{2004} (m)	*b*
		1969	1972	1975	1979	1984	1989	1994	1999	2004		
1	Myślibórz Płn.	92	99	103	104	100	103	104	106	103	20,87	1,049
2	Pelplin	90	91	93	97	97	100	100	100	100	20,21	1,015
4	Konstancjewo – Płonne	97	96	94	102	100	104	106	101	102	20,59	1,036
6	Konstancjewo – Tomkowo	96	96	96	96	103	103	97	95	99	19,90	0,981
7	Czerniejewo	99	95	96	98	105	103	99	102	104	21,01	1,036
8	Rawa Mazowiecka	97	93	94	96	95	95	95	95	95	19,27	0,955
9	Grójec	97	97	94	94	93	91	94	92	93	18,76	0,922
10	Marcule	96	85	83	85	94	84	87	88	88	17,79	0,880
11	Skarżysko	107	103	102	103	99	104	101	100	99	20,07	0,995
12	Bliżyn	98	96	96	97	97	101	99	99	101	20,34	1,007
13	Świętokrzyski PN	105	105	105	102	102	101	100	102	102	20,59	1,010
14	Moskorzew	103	104	104	101	104	103	102	102	102	20,63	1,017
16	Hołubla	102	105	103	100	104	96	101	101	100	20,27	0,999
18	Krościenko	95	104	100	100	98	97	98	94	96	19,41	0,948
19	Pilica	96	98	98	97	101	99	98	100	101	20,49	1,006
20	Prószków	111	110	111	109	104	110	107	105	103	20,87	1,040
21	Henryków	101	97	98	97	100	95	98	102	101	20,34	1,007
22	Kłodzko	111	116	115	107	110	104	104	104	101	20,46	1,009
23	Szczytna Śląska	109	104	107	106	98	105	107	109	105	21,17	1,053
24	Kowary	101	105	107	107	98	101	104	107	104	20,91	1,029
Mean height (m)		1,08	2,71	4,77	7,93	10,73	13,18	15,57	17,50	20,20	20,20	–
%		100	100	100	100	100	100	100	100	100	–	–

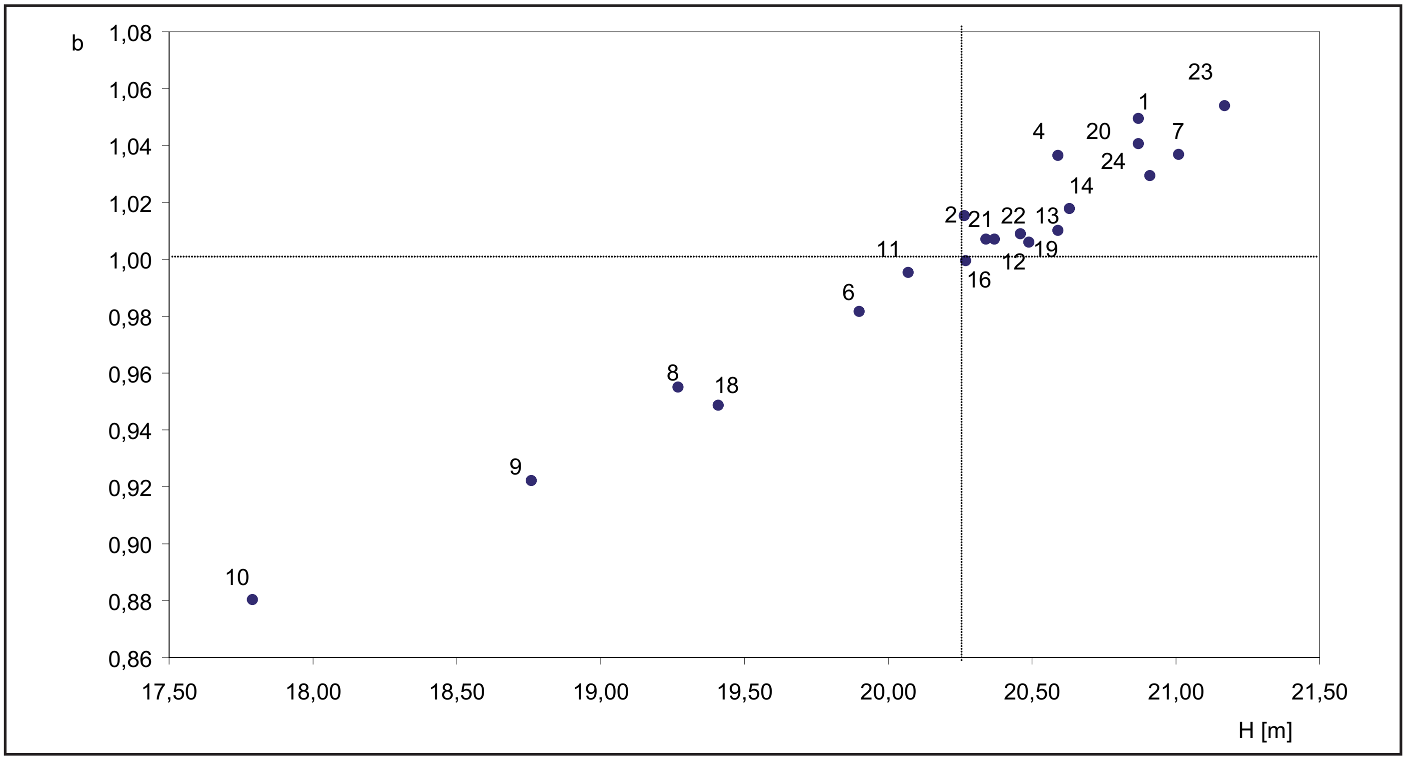

Fig. 2. Evaluation of adaptation traits of larches of investigated provenances on the basis of adaptation index *(b)* and mean height of provenances (H) at age 40. The Finaly- Wilkinson's method (1963); 24 provenances number.

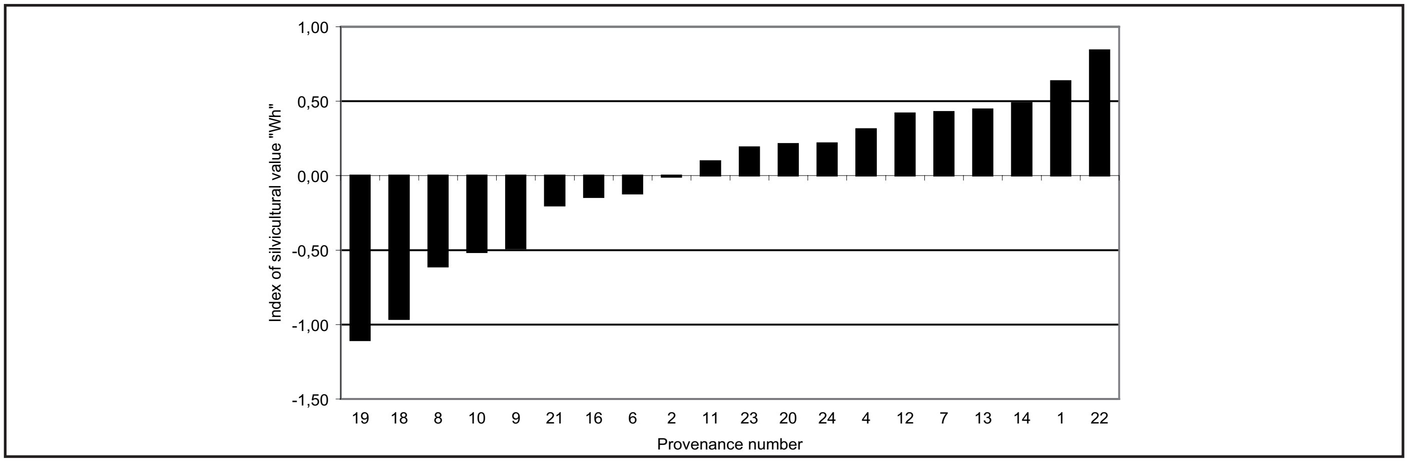

Fig. 3. Mean index of silvicultural value of tested provenances 40 years of age; 1 – 24 - provenance numbers.

Table 2. Matrix of auto-correlation coefficients for mean height of larches of investigated provenances during 1969–2004

Years	1969	1972	1975	1979	1984	1989	1994	1999
1972	0,7693 **	_						
1975	0,7177 **	0,9527 **	_					
1979	0,6115 **	0,8343 **	0,9490 **	_				
1984	0,5555 **	0,6765 **	0,6655 **	0,5700 **	_			
1989	0,4525 *	0,5938 **	0,7376 **	0,8556 **	0,6109 **	_		
1994	0,5018 *	0,6970 **	0,8626 **	0,9478 **	0,5490 **	0,8797 **	_	
1999	0,4753 *	0,6074 **	0,7648 **	0,8542 **	0,5744**	0,7856 **	0,8996 **	_
2004	0,3659	0,5318 *	0,6769 **	0,8004 **	0,5867**	0,8469 **	0,8732 **	0,9400 **

** significant for α = 0,01 * significant for α = 0,05

Mutual relationships in respect of the mean height of trees of individual provenances are presented in Table 2. These data indicate a significant correlation dependence of this trait. The autocorrelation coefficients, up to age of 40, were statistically significant, or nearly so, for α = 0.05 and 0.01. This means that they may play a considerable role in so called indirect selection of larch.

The highest value of the "assumed index of silvicultural value" – W_h (including four traits, i.e. height, d.b.h., stem quality, and resistance to canker) (Fig. 3) was reached by larch of Kłodzko provenance (0.843), and the lowest one by larch from Pilica (–1.105). On the basis of this value (expressed in standardized units) five groups of provenances of different silvicultural value were distinguished:

- silvicultural value very good – Kłodzko;
- silvicultural value good – Myślibórz Północ, Konstancjewo-the Płonne reserve, Czerniejewo, Bliżyn, Świętokrzyski National Park, Moskorzew;
- silvicultural value average – Pelpin, Konstancjewo-the Tomkowo reserve, Skarżysko, Hołubla, Prószków, Henryków, Szczytna Śląska, Kowary;
- silvicultural value poor – Rawa Mazowiecka, Grójec, Marcule;
- silvicultural value bad – Krościenko, Pilica.

The graphical presentation (Fig. 4) showed that the greatest diversification of the trait under analysis was found in the case of larches from the Świętokrzyskie Mountains and their southern fringes characterized by good, average, and poor qualities. Northern provenances from Myślibórz and Konstancjewo-the Płonne reserve, i.e. populations from little known hitherto untested localities in Poland, were characterized by a good silvicultural quality. From among provenances of the Sudetes larch from Kłodzko reached a very good silvicultural value. Decidedly bad was the silvicultural value of larch from Pilica and Krościenko. The fact that two neighboring populations from Konstancjewo-the Płonne reserve (good silvicultural quality) and Konstancjewo-the Tomkowo reserve (average silvicultural quality) differed in silvicultural quality is the basis for conclusion that these are partial populations of different origin.

Discussion

Long-term studies on genetic-silvicultural variation of different larch provenances from Poland yielded results which permitted to determine similarities and differences between individual partial populations, and also to determine their silvicultural usefulness in forestry under mountain conditions. These studies concerned tree survival, growth, selected morphological characteristics, wood quality, resistance to canker, and nutritional metabolism (Kulej 1995, 2001, 2002, 2004, 2006). In respect of height growth the partial larch populations tested under mountain conditions of the Beskid Sądecki are greatly variable. This variation was already observed when trees were 5 years old, and the effect of the genotype (provenance) on height growth was evident during the entire study period 1969 – 2004. The provenance variation of height growth decreased as the age of trees increased, although a considerable within provenance diversification among individual trees has been retained. Larches from

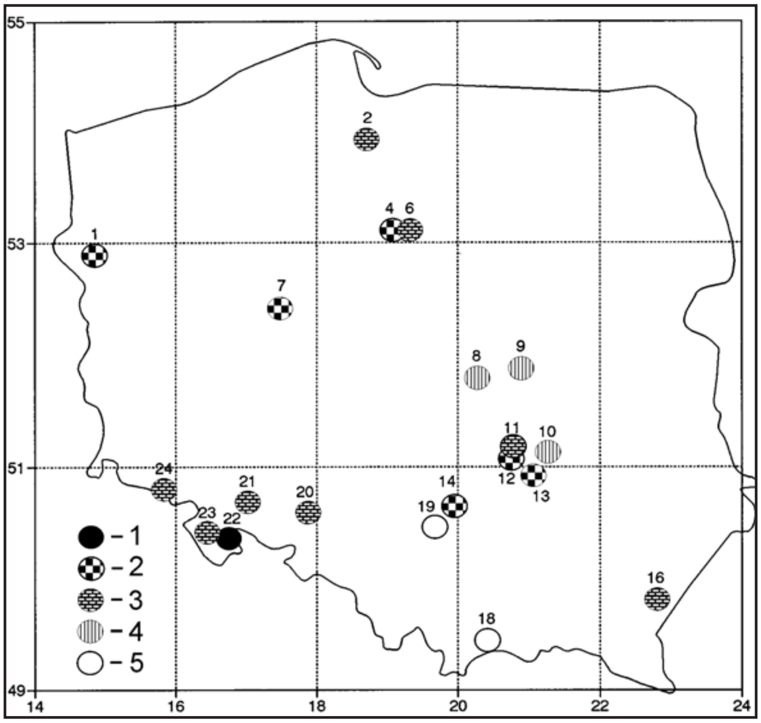

Fig. 4. Geographical variability of larch breeding value of investigated provenances at age 40; breeding value: 1 – very good, 2 – good, 3 – average, 4 – low, 5 – bad; 1–24 – provenance number.

Świętokrzyski National Park, Moskorzew, Prószków and Kłodzko belonged to leading provenances in respect of height growth during the entire 40-year period. A poor height growth was observed in the case of larches from Rawa Mazowiecka, Grójec, and Marcula.

The estimation of larch traits carried out using the Finlay-Wilkinson (1963) method showed a great adaptive stability of analyzed provenances under mountain conditions of the Beskid Sądecki, and certain tendency to decrease this trait with increase of the mean height of trees. This high adaptive ability of partial populations from Poland was especially evident in the case of larch from some localities of the Świętokrzyskie Mountains and the Sudetes. Provenances from these regions, tested in international experiments, have been called the plastic populations (Štástný 1971, Giertych 1979, Weisgerber and Šindelář 1996). The earlier studies of the author (Kulej 2001) showed that in selection of larch it is not possible to use the "early tests" based on weight of 1000 seeds and age of parent trees. This was proved by a lack of significant correlation between majority of analyzed adaptive, qualitative, and morphological traits. On the other hand, according to the author, the statistically significant autocorrelation of tree height during the entire study period found in provenances tested in Krynica, may be valuable for purposes of an indirect larch selection. On this basis when trees are 8 – 11 years old it is possible to predict with a high level of probability the growth of larch trees during subsequent years. Such authors as Štástny (1971), Dietze (1976), and Schober (1976) are of the same opinion. Making such a forecasting when trees are 5 years old or younger as suggested by Shier (1963) and Paques (1996) seem to be, however, risky and it may be burdened with a large error.

A complex estimation of silvicultural value of larch, where growth, stem quality and resistance to larch canker were the basic criteria to evaluate 40-year-old trees, showed that larch of the provenance from Kłodzko in the Sudetes was the best in this respect. Larches from the Świętokrzyskie Mountains (Bliżyn, Świętokrzyski National Park, and Moskorzew) as well as northern provenances from outside the natural range of this tree species in Poland (Myślibórz Północ and Konstancjewo-the Płonne reserve) formed a group of provenances of good silvicultural value. A good silvicultural quality of this group indicates that there is a possibility to widen an existing seed base of larch in Poland by including new hitherto untested populations. On the other hand, a geographical variation in respect of the analyzed trait showed a great mixing of larch populations, not only on the level of the country, but also in individual forest management districts or even among neighboring stands (e.g. Płonne and Tomkowo reserves of Konstancjewo). At the present stage of research the larch provenance from Kłodzko in the Sudetes should be accepted as the best one. It is suggested by its stand volume (451 m^3 per hectare), the highest among tested provenances, and also by its highest index of silvicultural value for the period 1969 – 2004 (Kulej 2001, 2004). While, larches from Pilica and Krościenko turned out to be useless for cultivation under site conditions of the Beskid Sądecki mountain range.

Finally it should be pointed out that author's long-term studies on genetic variation of larch of various provenances showed their great inter-population and intra-population diversification in respect of adaptive, qualitative and morphological traits (Kulej 2001, 2004, 2006). This diversification, however, has not been so far confirmed by iso-enzymatic investigations. The partial populations from central and southern Poland are characterized by an exceptionally small variation of the occurrence of certain genes as well as by a small heterozygosis (Lewandowski and Mejnartowicz 1986). This could possibly be explained by the occurrence of larch in Poland in isolated island sites hindering the gene migration. A similarly small diversification was found during estimation of larch from a wider European range (Lewandowski and Mejnartowicz 1991), which confirmed the theory about artificial distribution of natural populations of this tree species in Europe.

Conclusions

1. Larches of provenances tested under site conditions of the Beskid Sądecki mountain range showed a considerable diversification during the analyzed period of time in respect of adaptive, morphological, resistance, biochemical, and metabolic traits. This concerns larches from two main regions in Poland, i.e. the Świętokrzyskie Mountains and the Sudetes, as well as northern provenances outside a natural range of larch in this country.

2. A statistically significant autocorrelation of the mean height of trees turned out to be an important prognostic trait in an indirect selection of larch of partial populations tested in Krynica. On the basis of this trait it is possible in the early phase of growth (8-11-year-old trees) to predict the future growth of larch.

3. Results of long-term studies on genetic-silvicultural variation of larch have confirmed a great adaptive ability of its partial populations from Poland, especially from some localities in the Świętokrzyskie Mountains and in the Sudetes.

4. In Poland, there is a possibility to increase the existing seed base of larch by including little known and hitherto untested partial populations.

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Notes

Temperature related to phenological and growth traits in interspecific hybrids of larch in France

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The temperature effect on a number of phenological events is known and useful to predict them in crops. For this reason, several models describing phenological developments include air temperature like an elementary factor, mostly in agricultural models. The number of days needed for phenological developments or growth is linked to threshold temperature, which is basic to determine the necessary heat unit accumulation for a given progress of an event. In addition, some phenological developments, like flushing, are obviously related to starting growth.

The threshold temperature over which the temperatures have an accumulative effect is not well known for many forest tree species. In this study, we want to study

i) variability for this characteristics as well as heat-sum among larch species and hybrids progenies,

ii) their stability over years,

iii) their relationship with phenology parameters such as flushing and terminal bud flush,

iv) their predictive use to retrospectively date past flushing dates.

To carry out this study, we used progenies of European, Japanese and hybrid (F_1 & F_2) larch from two field trials installed at Peyrat-le Chateau and Orléans in France. Phenology (*e.g.* greening, bud burst, yellowing, bud-set) and growth (*e.g.* diameter, elongation from terminal bud, lignification) parameters were recorded every 10 days during the 2006 growing season and some observations were confirmed in 2007. Through growth chamber experiences, the threshold temperatures and heat-sums were calculated for bud flushing. The objectives of this study are: 1- to present result about the variability for threshold temperatures and heat-sums among parental species of larch and their interspecific hybrids and 2- to show the relationship between these heat-sums values and the phenological events related to them. It is well known that for different traits hybrids assume intermediate behaviour between parental species. We have found the same trends for analysed traits. For example, the threshold values from different types of hybrids were found to be intermediate between parental values. When associations between threshold values and degree days temperatures for different events were studied, we found different situations.

Notes

Phenology and growth variation among Larch species, intraspecific and interspecific families

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A 3 yr phenological observation after afforestation, growth rhythm and annual growth study of 7 larch species, 7 intra-species and inter-species families were carried out in Dagujia Forestry Farm, Liaoning Province. Variance analysis, principal components analysis and canonical correlation analysis were used for phenological variation and growth variation, phonological types classifying and relationship of growth and phenology. The result shown that all the phonological characters and growth traits (excluding branch bud bursting phase) were significantly differed among species and families, and so were among individuals within-family, which mean that there was big potential either for individual tree selection or family selection. *Larix gmelini* generally dehardened, Leafed and sprouted earliest in spring, and also had earliest cessation of shoot elongation in late summer among experimental materials. Then was *L. olgensis*, *L. sibrica* and *L. principis-rupprechtii*. The dormant stage of *L. olgensis* var. *L. koreana* and *L. deciduas* was 15 days later than *L. olgensis*. And *L. kaempferi* had latest bud bursting,leafing and dormant. All the inter-species hybrids, with better disease-resistance than male trees and better cold acclimation than female trees, behaved matroclinous inheritance from the point view of phenology. 14 genetic material were divided into four phenological types according to the result of PCA. Except branch leafing and main leader sprouting, all phonological characters had significant correlation with growth and had good prediction for them. *L. kaempferi*, as one of successfully exotic species in northern China, had best performance on fast-growing, followed by *L. olgensis* and *L. olgensis* var. *L. koreana*. Interspecific hybrids between *L. kaempferi* and *L. olgensis*, *L. gmelini* and *L. principis-rupprechtii* took great advantage of heterosis on growth, even better than both parent species. Therefore, the potential hybrid utilization of these species in the region was great.

Key words: *Larix* species; open-pollinated families; hybrid families; phenology; growth rhythm; early selection

Notes

The Study of Larch Hybrid Complex in Primorski Krai (Russian Federation) by RAPD Markers

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Abstract

The southern part of Russian Far East (Primorski Krai) is the convenient region to research the hybridization processes inherent in larches of this region in high degree. Twenty one populations that presented the morphologically distinct species *(L. sibirica, L. gmelinii, L. cajanderi, L. kamtschatica, L. kaempferi, L. olgensis)*, the hybrid species *(L. amurensis, L. komarovii, L. ochotensis)*, and larch samples from *L. olgensis* areal in Primorski Krai were studied using RAPD markers. As it follows from UPGMA analysis the populations from *L. olgensis* areal were clustered together with hybrid species populations. The morphologically distinct species were sequentially associated with mentioned above cluster. Surprisingly, *L. olgensis* that is considered as one of the parents of the hybrid species under study was placed far from them. *L. kaempferi* was distanced from all studied taxons. Our results shows that presumably all studied *L. olgensis* areal populations and the hybrid species populations represent together the multiple species complex formed in the result of long-term succession of introgressive hybridization.

Introduction

According to different literature sources the several larch species exist on the Russian Far East (Kolesnikov 1946, Dylis 1961, Bobrov 1972, Gukov 1976, Kabanov 1977). Some of them are morphologically distinct species (*L. gmelinii* (Rupr.) Rupr., *L. cajanderi* Mayr, *L. kamtschatica* (Rupr.) Carr., *L. olgensis* A. Henry), the others represent hybrid species (*L. amurensis* Kolesn., *L. maritime* Sukacz., *L. ochotensis* Kolesn., *L. komarovii* Kolesn., *L. lubarskii* Sukacz.). On the territory of Primorski Krai that locates in the southern part of Russian Far East, only *L. olgensis* has no hybrid origin, whereas the other forms may be considered as the members of complex originated in the result of introgressive hybridization (Bobrov 1972, Koropachinski 1989). The first time *L. olgensis* was described from Olga Bay by A. Henry in the 1915 (Bobrov 1972). The areal of *L. olgensis* in Primorski Krai was outlined by Gukov (1976) and it was located on the small-scale territory restricted by eastern macroslope of the southern Sikhote-Alin and Japan Sea coast. However, we are in some doubt about taxonomic status of some larch populations from this areal because of they have high variability of the morphological characters. Anyway this problem can be resolved using other markers.

Recently the population genetic investigations of Pinaceae representatives were carried out using the molecular genetic markers, namely the allozymic (Potenko *et al.* 1996, Goncharenko *et al.* 1997, Goncharenko 1999, Semerikov *et al.* 1999a, Semerikov *et al.* 1999b), randomly amplified polymorphic DNA (RAPDs) (Isabel *et al.* 1995, Lee *et al.* 2002, Nkongolo *et al.* 2002), microsatellite (SSRs) (Karhu *et al.* 2000, Khasa *et al.* 2000, Isoda *et al.* 2006) and etc. RAPDs are the very polymorphic markers (Williams *et al.* 1991), which are considered to be useful for determining of genetic variability parameters and comparing closely related species and genera (Weising *et al.* 1995).

In this study, we analyzed genetic differentiation of the larch populations from *L. olgensis* areal in Primorski Krai outlined by Gukov (1976) using RAPD markers to reveal the relationship these populations with the morphologically distinct and the hybrid larch species of Siberia and Far East.

Materials and methods

Plant samples consisted of young needles were sampled from twenty natural populations of Siberia and Russian Far East. The samples of *L. kaempferi* were collected in the Botanical Gardens of Moscow (Table 1).

The genome DNA was isolated using the technique of Isabel *et al.* (1995). The polymerase chain reaction (PCR) was conducted in the thermal cycler UNO II 48 (“Biometra”, Germany) with the decamer arbitrary primers (“Operon Technologies Inc.”, USA) with applying the reaction mixture and temperature regime described previously (Kozyrenko *et al.* 2001). The amplification products were separated by electrophoresis in 1.4% agarose, at the presence of ethidium bromide and examined under UV light. Fragment size of each RAPD marker was determined by comparison with the standard molecular weight marker, λ DNA double digested with HindIII and EcoRI («Fermentas» Lithuania). RAPD marker bands within the range of 500 –1700 bp were scored for the presence (1) or for absence (0). All RAPD assays were repeated at least twice and only the reproducible bands were scored. The same size between two fragments revealed in two different individuals was assimilated to a sequence homology.

Nei’s genetic distances (D_N) (Nei, 1978) were estimated using TFPGA package software (Miller 1997). The dendrogram was constructed based on the D_N values matrices, with applying unweighted pair group method of analysis (UPGMA) with the bootstrap values of the branching order reliability (1000 replications). A comparison pairwase differences (F_{ST}) among populations was performed using AMOVA in Arlequin (v.2.000; Schneider *et al.* 2000).

Table 1. Location of larch populations under study

Species	Provenance	Code	Latitude / Longitude	Sample size
Morphologically distinct species				
L.sibirica Ledeb.	Tomsk region (Western Siberia)	sib	56°30′39″ N / 84°49′45″ E	11
L. gmelinii (Rupr.) Rupr.	Evenkia, Talnah (Yakutia, Eastern Siberia)	gme	69°19′50″ N / 87°59′39″ E	13
L. cajanderi Mayr	Magadan region, outskert of Orotuk (Far East)	caj		11
L. kamtschatica (Rupr.) Carr.	Sakhalin Island, Anivskii region	kam	46°43′54″ N / 142°36′02″ E	7
L. kaempferi Lamb.	Botanical Gardens of Moscow	kae		4
L. olgensis A. Henry (*locus lassicus*)	Coast of Olga bay, Primorski Krai	olg	43°44′44″ N / 135°13′45″ E	18
Hybrid species from Primorski Krai				
L. ochotensis Kolesn.	Veselyi pass	OCH	45°48′ N / 137° E	6
L. amurensis Kolesn.	Khreimanov brook	AMUR	45°46′ N / 134°14′ E	9
L. komarovii Kolesn.	Upper Lagernaya river	KOM1	45°70′ N / 136°35′ E	14
	Verkhniy Irtysh river	KOM2	45°05′ N / 135°80′ E	9
Populations from Primorski Krai (obscure populations)				
L. olgensis A. Henry from areal by Gukov (1976)	Middle stream Arzamazovka river	ARZ-1	43°59’81”N / 135°10’56” E	9
	Arzamazovka river head	ARZ-2	44°02’97” N / 135°11’65” E	11
	Middle stream Margaritovka river	MAR	43°35’90” N / 134°35’99” E	10
	Zmeinyi kluch (confluent Bolshaya Ussurka river)	ZK	44°42’16” N / 135°39’08” E	11
	Zerkalnaya river head	ZER	44°16’93” N / 134°53’59” E	10
	Vysokogorskaya river head	VIS	44°29’13” N / 135°23’51” E	11
	Upper Pavlovks river (confluent Ussuri river)	PAV	44°17’34” N / 134°52’05” E	10
	Middle stream Listvennaya river	LIST	43°26’45” N / 134°39’45” E	12
	Gorbusha river	GOR	44°39’51” N / 135°39’51” E	9
	Cheremukhovaya river head	CHER	44°41’69” N / 135°45’13” E	9
	Coast of Valentin Bay	VAL	43°07’34” N / 134°19’22” E	13

Results and discussion

A total of 189 scorable RAPD loci were generated by the 8 primers, constituting on average 23.6 loci per primer. Of the 189 RAPD fragments, 181 (95.7%) were found to be polymorphic, 8 (4%) were monomorphic for all samples. The molecular data show that 4 (2%) polymorphic loci occurred in hybrid species and in populations of *L. olgensis* areal including samples from *locus classicus* of this species. Two polymorphic loci (1%) presented only in the hybrid species and the obscure populations. The rest of polymorphic loci met in all populations with different frequency.

Thus presence particular loci attributed only for hybrid species and obscure populations allow suggesting that the populations from *L. olgensis* areal have hybrid nature and forming the complicated hybrid complex with neighbour hybrid species.

Genetic distances among the different larch species samples ranged from 0.092 to 0.225 (gme-kam and kae-caj, accordingly), D_N distances among hybrid species samples varied from 0.048 to 0.068 (KOM1-KOM2 and KOM1-OCH, accordingly) and D_N among populations from *L. olgensis* areal ranged from 0.018 to 0.154 (GOR-CHER and MAR-VAL, accordingly). The average value of D_N distances between obscure populations and populations of morphologically distinct larch species identical with this one between populations of hybrid species and morphologically distinct species (table 2). It is notable that average genetic distances among populations from *L. olgensis* areal higher than average D_N among populations of hybrid species. These results are the additional argument in favour of viewpoint about hybrid nature of these populations. Nevertheless obscure populations are genetically closer to *L. olgensis* than population of hybrid species according to average values of D_N (table 2).

Cluster analysis of RAPD data using UPGMA (Figure 1) revealed that most part of the obscure populations grouped together according to geographical distribution with the exception of samples GOR, CHER and VAL. Group GOR-CHER jointed to obscure population cluster after hybrid species cluster. The morphologically distinct species were sequentially associated with hybrid species and obscure populations cluster. *L. kaempferi* was distanced from all studied taxons.

To take note of the fact that *L. olgensis (locus classicus)* was far from both the hybrid species and all but one of obscure populations. It was interesting because the obscure populations were collected from *L. olgensis* areal outlined by Gukov (1976), but they were genetically closer to the hybrid species. At the same time VAL sample (the most southern point of collection) clustered together with *L. olgensis*. This may be explained by similar ecological growing conditions of these populations (rocky coast of Japan Sea), that are optimum for this species.

According to the Monte Carlo approximation of Fisher's exact test, the populations of distinct species differed significantly from each other, just as the most of obscure populations. While no significant differences were found between populations of hybrid species. Nevertheless all pairwise differences (F_{ST}) between populations obtained by AMOVA were significant. The average values of F_{ST} between the distinct species, the hybrid species and the populations from *L. olgensis* areal are presented in table 3.

Our investigation shows that all studied *L. olgensis* areal populations are genetically heterogenous. We suppose that only the sample from Valentin Bay is genetically close to *L. olgensis* and the rest of obscure populations form the multiple complexes with the adjacent hybrid species due to a string of fragmentations and connections of these populations in conditions of geological period changes and heterogeneity of Sikhote-Alin landscape.

Table 2. The average values of genetic distances (Dn) among populations

Larch populations	Species (except *L. olgensis*)	Hybrid species	Populations from *L. olgensis* areal
Species (except *L. olgensis*)	0.154		
Hybrid species	0.131	0.056	
Populations from *L. olgensis* areal	0.131	0.099	0.082
L. olgensis (*locus classicus*)	0.161	0.144	0.121

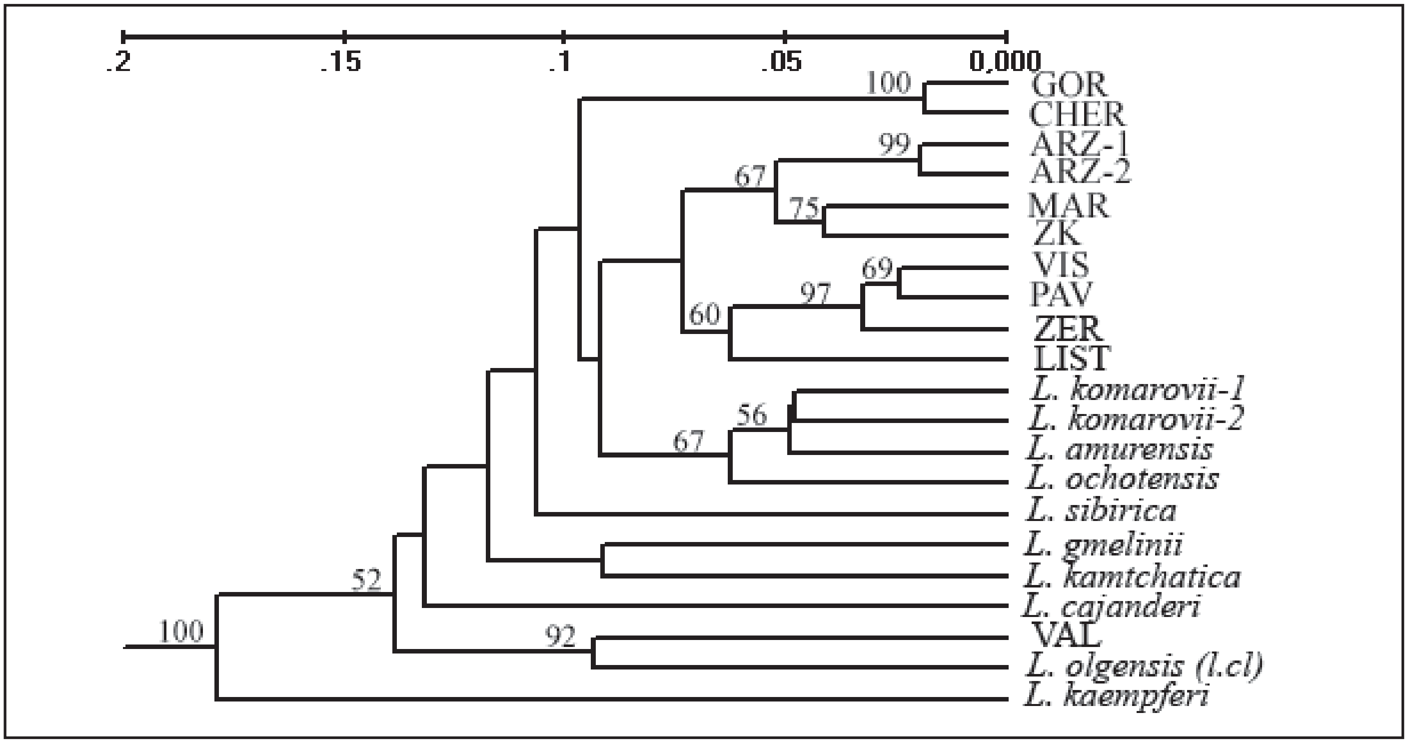

Fig. 1. UPGMA dendrogram of Nei's (1978) genetic distance between populations and species of *Larix*. Code of populations see Table 1. Numbers indicate clustering reliability values (bootstrap support).

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Notes

Notes

The Cell Wall Structure Formation of Earlywood and Latewood in Larch (*Larix sibirica* Ldb.)

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Abstract

The deposition of cellulose and polysaccharides in primary and secondary walls of tracheids in the course formation of earlywood and latewood in larch (*Larix sibirica* Ldb.) was studied. The active tissues at successive developmental stages of primary and secondary cell walls were sampled from the trunk of 25-year-old trees and characterized by the content of cellulose, hemicelluloses and pectin substances. The amounts of polymer carbohydrates, deposited at each of developmental stages, successfully isolated with cold water, ammonium oxalate, 4% and 24% KOH and were calculated per dry weight and per cell. Hemicelluloses were additionally divided according to the solubility in neutral medium. The deposition of arabinogalactan, arabinogalactan-proteins, pectin's, the fractions of A and B hemicelluloses and cellulose occurred with different rates at differentiation stages and corresponded to their participation in the cell wall structure formation of earlywood and latewood. During the period of primary wall development the polysaccharides, involved in cell wall mobility, were principally synthesized. The amounts of water soluble compounds (arabinogalactan, arabinogalactan-proteins), acid pectic substances and low molecular weight xyloglucan were different during the development of primary walls of early and late tracheids what coincided with distinctive radial sizes of these tracheids. The degree of accumulation of hemicelluloses, linked or nor with cellulose, has been found to be different at secondary wall thickening and lignification stages of early and late xylem. This shows the distinctions in the matrix before lignification. All together can be the reason for different physico-chemical properties of earlywood and latewood in larch.

Introduction

The tracheids of earlywood and latewood in larch, as well as in other conifers, are different by radial diameter and cell wall thickness while they develop by one program – cell production by cambium, growth of primary cell walls by expansion and deposition of secondary wall substances during the secondary wall thickening stage. Physiological and biochemical events in cells leading to such morphological differences resulted from the changes in cell metabolism under influence of environmental factors. The main of these factors is water stress, in particular, low water potential in tissues. It is water stress that is the principal reason of latewood formation (Zahner, 1963; Whitmore, Zahner, 1967). Low water potential in developing xylem (internal water stress) can arise because of the lack of rainfalls (external stress), high temperature and evapotranspiration or even large excess of water in soil (physiological drought) (Antonova, Stasova, 1997; Antonova, 1999). The changes along the chain of metabolic reactions because of internal water stress must lead to the differences in morphological parameters of two wood types in conifers. The water stress has been shown to decrease a growth changing of cell wall sizes, their mechanical properties and the cell wall composition (Morgan, 1984). Sukurai *et al.* (1987) showed the changes in pectic and hemicellulosic polysaccharides of squash hypocotyl walls under water stress conditions. Iraki *et al.* (1989 a, b) reported the alteration of the physical and chemical structure of primary cell wall in growth-limited tobacco cells adapted to osmotic stress. Studying of lignification in larch (*Larix sibirica* Ldb.) wood we found different dynamics of lignin deposition during the secondary wall development of earlywood and latewood tracheids (Antonova *et al.*, 2005). This means that differences can be not only in precursors, participating in synthesis of lignin, but in the secondary wall carbohydrates in those medium the polymerization of lignin precursors occurs. An important role of polysaccharide components of cell walls in organizing lignin precursors has been supposed (Houtman, Atala, 1995; Whetten, Sederoff, 1995). Grabner (2005) considered lignin-matrix interaction to influence the polymerization conditions of monolignols and to be under environmental factors.

The purpose of this work was to study the dynamics of deposition of cellulose, arabinogalactans, pectic substances and different hemicelluloses, linked or not with cellulose in cell walls at different steps of primary wall growth and secondary wall development of earlywood and latewood tracheids during annual ring wood formation in larch trees.

Materials and Methods

Forming xylem at different stages of tracheid differentiation was obtained from the stem cuttings of 20-year-old larch trees (height - 9-10 m, diameter at 1.3 m - 8-10 cm, two trees in each sampling). Xylem was collected tree times in a season: in early and late June and early in August. In each of these periods all differentiating tracheids along radial row as well as mature xylem cells developed as earlywood cells (June) or as latewood (August). The cells at developmental stages were collected by peeling one cell layer after another by the scalpel examining the accuracy of sampling at the cross-sections of strips under microscope after staining with cresyl-violet. Early in June the cells of cambium zone (Cam), the first and second part of radial cell expansion zone (G1 and G2 respectively) were isolated from trunk cuttings, while in late June the cells of Cam, G zones and cells with the secondary wall thickening but without lignification (D-1), the first (D-2a) and the second (D-2b) parts of lignification zone and mature xylem (M) were collected. In early August latewood cells of Cam, G, D-1, D-2a, D-2b and additionally the third cell layer (D-2c) were isolated. The sampled cell layers were immediately fixed with ethanol to the final concentration not exceeding 80%, weighed, and kept in a refrigerator until analyses. Simultaneously the tissues were sampled for maceration (0.05-0.1 g x 3) and moisture determination (0.5g x 2).

Tissue suspensions taken for analysis were filtered; dry residues were homogenized in liquid nitrogen with a mortar and pestle and extracted with 80% ethanol (1:10, v/v) at room temperature with periodic shaking. The solvent was changed every 12 h until the negative reaction of carbohydrates (Dubois *et al.* 1956). The extracts and solutions after sample fixation were combined, the volumes were measured and the aliquots were used to determine the content ethanol-soluble substances. The extracted tissues were air-dried and used to estimate the contents of arabinigalactans as cold water soluble component, pectic substances, soluble in oxalate ammonium, cellulose and polysaccharides. The latter's were divided according to their solubility in 4% and 24% KOH and in neutral medium. The content of cellulose, hemicelluloses, arabinogalactans and pectic substances was calculated per dry weight of the tissue and per cell. All procedures with ethanol-treated tissues were multiplied two times, all chemical analyses were repeated three times.

Results and Discussion

Primary wall development

The polysaccharides of forming xylem at primary cell wall developmental stage consists of cellulose, pectic substances and arabinogalactans (AGs), including arabinogalactan-proteins, and hemicelluloses. In cambium zone the content of cellulose forms 5.7% of primary cell wall (Fig. 1). The biosynthesis of cellulose increases at the first step of expansion growth zone and then decreases at the second part of the zone because of accumulation of other cell wall components. The amount of arabinogalactans increases 4.6 times during cell growth.

The important part of xylem is the pectic substances contained mainly in middle lamella and primary cell wall. The amount of them also enhances during growth stage according to enlargement of the space of primary cell walls (Fig. 2). But the increment of the amount at two steps of space development is different. With the growth of cell the content of these substances insignificantly changes from cambium zone to G1 step and sharply increases at the second part of expansion growth stage. The rate of cell growth has early been shown to be the highest at the beginning of this growth (Antonova, Shebeko, 1985). That is why the insignificant increase of pectic substances at the beginning of expansion growth zone means that the synthesis of the pectic substances is practically in on line with primary wall space. Then the content of substances continues to accumulate while the growth rate reduces. The substances, isolated with ammonium oxalate, consist of neutral and acid parts and low molecular weight xyloglucan. During the growth their ratio changes too (Fig. 3). Neutral pectins decreases at the first step and do not change at the next step while acid pectin (uronic acids) accumulates in the course of growth. Acid pectic substances are considered as a components taking part in strengthening of xylem tissue. Low molecular weight xyloglucan (XG) soluble in ammonium oxalate can be a precursor of high molecular polymer.

The other polysaccharides taking part in cell wall structure are hemicelluloses A and B (HA and HB) which are divided into the fractions of HA-4, HB-4, HA-24 and HB-24 due to the solubility in neutral medium after acidifying of alkaline solutions (4% and 24% KOH). The fractions of HA-4 and HB-4 are considered to be carbohydrates weak-linked with cellulose, while HA-24 and HB-24 are polysaccharides tightly-linked to cellulose. Fig. 4 shows that starting with cambium zone the principal polysaccharide is HA-4 and its amount decreases towards the end of cell growth. On the contrary, the amount of HB-4 (xyloglucan) gradually increases as well as that of HB-24 to make cell wall stronger. The content of cellulose, AGs, pectic substances and its composition, the amounts of separate hemicellulose fractions in the cells of cambium and expansion growth zones are different during earlywood and latewood tracheids differentiation. The cells in cambium zone of early xylem contain higher AGs than that of late xylem. The amount of AGs enhances two times in expanding early cells and a few changes in that of latewood cells. AG is hydrophilic component and can promote a mobility of cells. The content of pectic substances increases 6.5 times in the course of the growth of earlywood cells while in latewood cells it doesn't change. The distinction in the content of

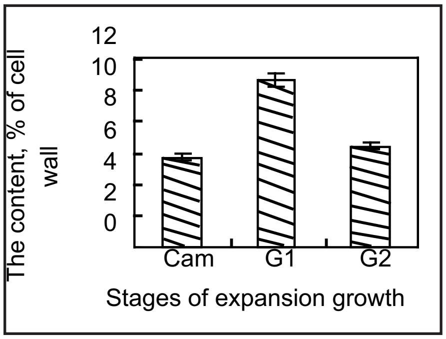

Fig. 1 The content of cellulose at the steps of cell expansion growth,

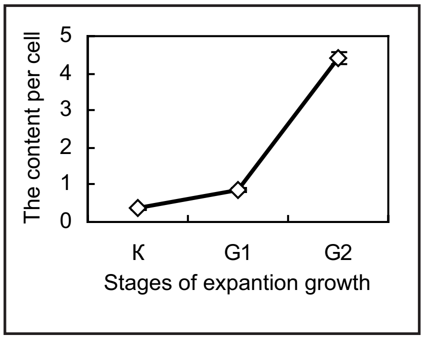

Fig. 2. The content of pectic substances at the steps of cell expansion growth, mg x 10^{-6}.

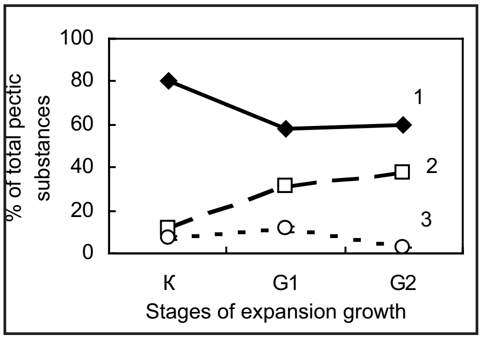

Fig. 3. The content of pectin (1) uronic acid (2) and xyologlucan (3) at the steps of cell expansion growth.

AGs and pectic substances at expansion stage of early and late tracheids can explains small radial diameter of the latter's. The composition of hemicelluloses in cambium zone and in G zone of developing early and late xylem is also different. The cambium cells in developing earlywood contain HA-24 and HB-24 two times less than those of developing latewood cells. In the course of expansion growth the amount of these polymers increases but the content of polysaccharides tightly-linked to cellulose is two times more in growing latewood cells than earlywood cells. This shows that the growth of radial diameter of latewood tracheids is restricted by the content of substances to make cell wall structure immobile.

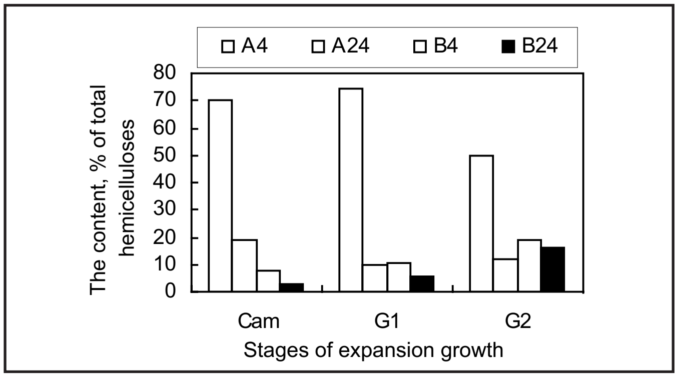

Fig. 4. The content of hemicelluloses at the stages of expansion growth

Secondary cell wall development

In the course of secondary cell wall development the principal biomass of annual increment is accumulated and lignification of tissue occurs. The last process follows the deposition of cellulose and depends on the availability of precursors and arrangement of matrix carbohydrates as mentioned above. The tracheids of differentiating early and late xylem at the beginning of the secondary wall development before lignification (D1) contain the distinct amount of cellulose, oxalate-extracted substances, hemicelluloses of A and B linked or not to cellulose. The amount of cellulose deposited in earlywood cell walls before lignification (D1 step) was less than that in latewood cell walls (9.9% and 12.8% respectively). The cellulose synthesis sharply increases at D-2a stage, where lignin deposition is observed, with the following decreasing towards mature xylem. Thus the deposition of cellulose being the core of cell walls, their fittings, occurs in early and late tracheids with similar dynamics. However, the cellulose, deposited at D2a step, is larger in latewood cells than in earlywood tracheids.

The principal part of the oxalate-extracted substances of early xylem is pectins, 40% of which is uronic acids (Fig. 5 a). Low molecular weight XG occupies not more than 1,7% of total substances. In the course of latewood development the cells at D1 stage contained pectins two times less but the content of uronic acids was 82% of that (Fig. 5 b.) This shows the differences in acid pectic substances before lignification of early and late xylem. With the start of earlywood lignification the content of overall pectins and their acid fraction changed a little at the first step and then increased up to 59% at the D2b stage, what coincides with the intensification of lignin deposition. At the beginning of latewood lignification (D2a stage) the content of pectin increased while uronic acid respectively reduced what is in on line with intensive lignin deposition. Such changes in the composition of pectins and their acid fractions may be the evidence of the distinctive fixation of mono- or oligolignols in middle lamella and cell walls on the one hand and of the ability of soluble pectic fragments to elicit the biosynthesis of lignin on the other hand. Bruce and West (1989) demonstrated such possibility in castor bean suspension culture. Tanabe and Kobayashi (1988) reported about aggregation of pectic substances and lignin-carbohydrate complex in bast fibers. The comparison of secondary wall development of two types of wood shows that earlywood cells contain much grater pectin and far less XG than latewood ones, especially at the stages of D2a and D2b. Xyloglucan fractions of oxalate-extracted substances can be probably considered also as the substances which may aggregate with forming lignin molecules. Xylose and glucose were observed in the composition of lignin-carbohydrate complex, for example in bast fibers (Tanabe, Kobayashi 1988). XG, isolated by oxalate ammonium because of its low-molecular weight may be precursors of higher molecular weight polysaccharides of HB extracted by 4% KOH, the amount of which increased towards M stage (Fig. 6) while the content of oxalate-isolated xyloglucan reduced.

At D1 stages of the secondary cell wall development of earlywood the hemicelluloses B (HB) are two times more than HA (Fig. 6 a). In the course of cell development the content of HB slightly increases while of HA, on the contrary, decreases. During latewood formation the cells at D1 stage contains HA significantly more and HB correspondingly less as compared with the same stage in the course of earlywood formation (Fig. 7 a). In the course of tracheid maturation the content of HA reduced and that of HB enhanced. There are proper peculiarities in the distribution of fractions of HA-4, HB-4, HA-24 and HB-24 which are characterized by different extent of linkage with cellulose in cell wall (Fig.6 b and Fig. 7 b).

In the course of earlywood cell wall development the HA-4 content fell just at the beginning of lignification (D2a stage), did not change at the next stage and again declined towards the end of maturation. At the same time the HA-24 content gradually increased (Fig. 5 b). The content of both HB-4 and HB-24 progressively enhanced starting with the secondary wall development but the former was always more than the latter. During latewood secondary wall development the relations between hemicelluloses are more complicated. Before lignification the content of HA-4 exceeded that of HA-24 more than 7 times and was more than other hemicelluloses (Fig. 6 b). Then its content dropped towards D2b stage and increased a little towards M. The content of HA-24 increased 3-fold just at D2a stage and gradually decreased after D2b stage. So, the secondary cell wall formation of early and late xylem is distinct by the content of HA and especially by the fraction of HA-4. The content of HA-4 before lignification was two times more in earlywood cells than in latewood, but that reduced almost 2-fold after the beginning of lignification. These results suggest that HA-4, presented evidently by arabinoglucurunoxylan (arabinoxylan), can play one of the principal roles in bounding of lignin precursors due to the side chains of this polysaccharide.

The content of HB-4 at all developmental stages was constantly more than that of HB-24 and both enhanced towards M stage. Three differentially extractible xyloglucan (XG) fractions being structurally distinct were isolated from the cell walls of etiolated pea and considered from the point of the metabolism of XG associated with cell expansion (Pauly *et al.* 1999). Our data showed that there are also three fractions of XG during the secondary cell wall formation in early- and latewood in larch. They differ by their solubility, molecular weight and linkages with cellulose and the content of these fractions is distinct in the course of secondary cell wall formation of earlywood and latewood.

So, it can also be suggested that the changes in HB be primarily related to assembling with cellulose microfibrills and creating of cellulose/xyloglucan network, while HA-4 involves in the fixation of lignin macromolecules into cell walls. Studying the lignin structure isolated from larch tissue at successive stages of secondary cell wall formation we found that carbohydrates polymers included in ether and ester linkages contain the residues of arabinose and glucose in the first case and that of xylose, arabinose and glucose in the other one (Antonova *et al.*, in press). In both cases the content of ether as well as ester-linked carbohydrates in latewood lignin preparations was more than that in lignins from earlywood. This coincided with the higher content of HA within the cell walls of differentiating latewood as compared with earlywood. But the participation of HB-4 in carbohydrate-lignin linkages can not be excluded especially before and at the beginning of lignin deposition.

As mentioned above the increase in HB-24, i.e. the polymers (mostly XG) which are tightly bound with cellulose promotes the strengthening of cell wall structure during the secondary cell wall development. Together with the other polysaccharides this must be resulted in different density of cellulose/hemicelluloses network, i.e. in the conditions for fixation of lignin precursors, their oxidative polymerization and the space between polysaccharides for lignin globule development. All this probably leads to the distinction in the sizes of these globules and the different rate of lignification of developing earlywood and latewood in larch.

The variation in the content and composition of carbohydrates polymers as well as in the lignin deposition rate during the secondary cell wall formation due to the differences in metabolism resulted from internal water stress is evidently the chemical base of distinctive physical properties of earlywood and latewood.

Acknowledgements

The authors are thankful to Ms. V.V. Stasova, this institute, for the technical help during the preparation of tissue samples.

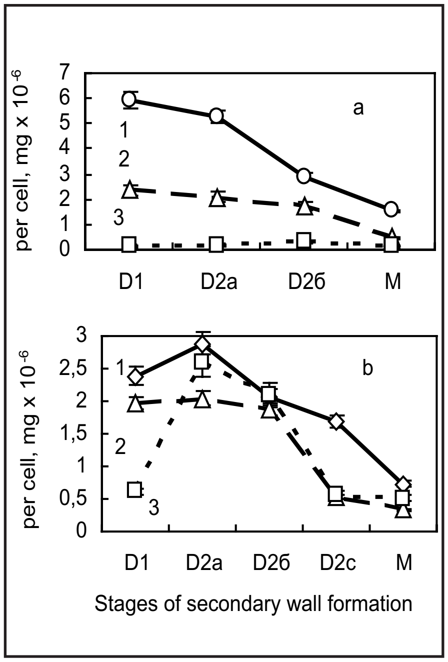

Fig. 5. Changes in the content of pectin substances (1), uronic acids (2) and xyloglucan (3) during secondary wall development of earlywood (a) and latewood (b) tracheids.

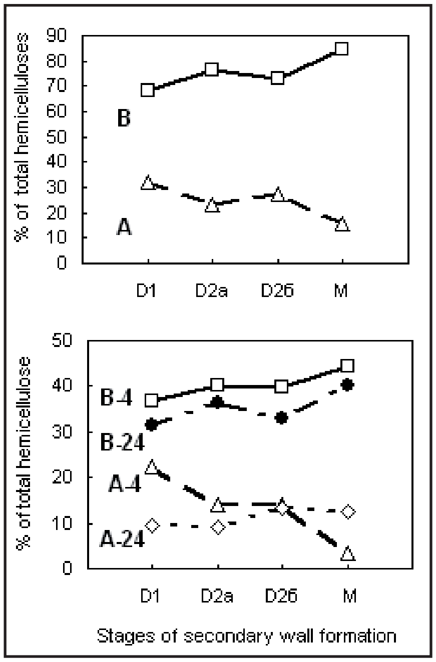

Fig. 6. The changes in the content of hemicelluloses A and B (a) and their fractions (b) at different stages of secondary cell wall formation during earlywood formation.

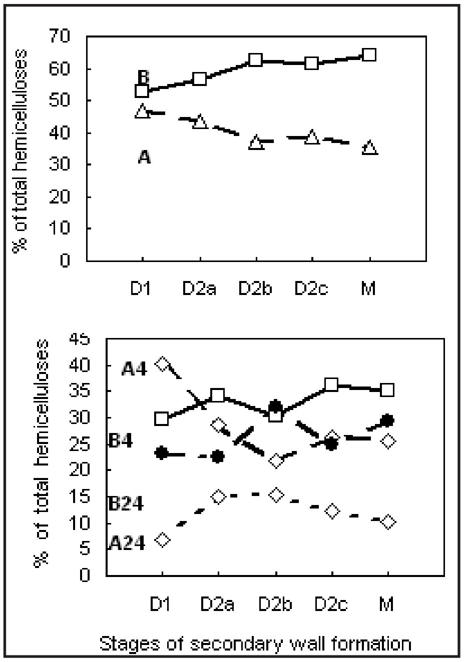

Fig. 7. The changes in the content of hemicelluloses A and B (a) and their fractions (b) at different stages of secondary cell wall formation during latewood formation.

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Notes

Decay resistance in Siberian larch, *Larix sibirica* Ldb, heartwood

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Abstract

The aim of this study was to determine the differences in durability between Siberian larch, *Larix sibirica*, European larch, *Larix decidua*, and Scots pine, *Pinus sylvestris* heartwood according to EN 252 and *in vitro* EN 113. Physical properties such as strength and water absorption were also determined. In the test performed according to EN 252 the samples originated from four different larch trees divided into yellow heartwood and red heartwood; fast grown, very heavy and 1st class wood quality. The decay resistance was expressed as remaining weight % and the strength in Newton. The sticks were placed in the ground for six years at three locations in Sweden: Småland, Värmland and Västerbotten, in three different soils; sand, cultivated ground and forest. The result shows that the mass loss is greatest for the samples located in Småland forest soil followed by forest soil in Västerbotten and cultivated ground in Småland. The strength was lower at these locations as well with a greater loss relative to the weight loss. In the study performed according to EN 113 the samples originated from yellow, green and red Siberian larch wood and Scots pine trees chosen randomly. The fungi used were *Coniophora puteana* and *Gloeophyllum trabeum*. The samples were also determined with regard to water absorption. Scots pine, yellow and green larch showed the greatest mass loss %, ca 20%, the red larch showing the least mass loss %, ca 13%, exposed to *C. puteana*. Exposed to *G. trabeum*, yellow larch shows a mass loss of approx. 16%, green 12%, red 8% and Scots pine only 3%, over all the red larch shows the least mass loss. The water absorption analysis was performed using contact angle measurements. The result shows that red and green larch had the greatest absorption and Scots pine the lowest value.

Keywords: Siberian larch, decay resistance, heartwood, strength, water absorption.

Introduction

In Sweden wood used for constructions mostly consists of treated wood to protect it from decay. The active substances used are mostly salts containing copper, chromium and arsenic or creosote; all substances that are hazardous to the environment not only in the production process of impregnation but also when used and when it is destroyed. With regard to that it would be preferable to exchange the treated wood into naturally decay resistant wood as much as possible.

Wood of a lot of tree species is more or less resistance to decay caused by different organisms and the heartwood is commonly more resistant than the sapwood (Hakkila & Winter, 1973). Tree species like larch *(Larix sp.)*, Western red cedar *(Thuja plicata)* and Douglas fir (*Psuedotsuga menziesii* Mirb.) are all examples of containing heartwood with relatively high natural decay resistance. Tree species like birch (*Betula* sp) and maple (*Acer* sp.) have a lower natural resistance to decay (Anon. 1990, Gorchin & Chernetsov 1966, Björkman 1944). The heartwood of Scots pine (*Pinus sylvestris* L.) is considered to be as decay resistant as heartwood of larch in Finland, Russia and Sweden (Nilsson 1997, Viitanen *et al.* 1997, Venäläinen *et al.* 2001). Though, in Siberian larch, decay tests *in vitro* have shown significant between-tree variation considering decay resistance (Venäläinen *et al.* 2001). An important difference from Scots pine timber is the volume percentage of heartwood in larch timber. The heartwood in larch is often twice as much as that in Scots pine of the same age and growing conditions.

Generally it is considered that the extractive content in the wood influences the decay resistance for most tree species. In Siberian larch (*Larix sibirica* Ldb.) the most important extractives are arabinogalactanes and the flavonoid dihydroquercitin also known as taxifolin. Especially taxifolin is considered to provide durability among others in *Larix decidua*, *L. leptolepis* and *Cedrus atlantica* (Scheffer and Cowling 1966, Hakkila and Winter 1973). Techniques to predict decay resistance of European larch (*Larix decidua* Mill.) and colour differences in larch wood has been studied by Gierlinger *et al.* 2003, 2004. They showed that Japanese larch (*Larix kaempferi* Lamb) heartwood is significantly more reddish (higher a*-values) compared to the European larch. The hybrids of *L. decidua* and *L. kaempferi* were intermediate. The red colour in Japanese larch was strongly correlated with the amount of phenols and decay resistance and therefore suitable for prediction of both parameters. The colour is an interesting factor considering Siberian larch heartwood since it present different colours; they can be reddish, greenish or yellowish.

Considering this background the aim of this study was to investigate whether there are differences in colour of the heartwood of Siberian larch with regard to decay resistance and water absorption. Furthermore, the aim was to study whether it is possible to manually i.e. by just using the human eye, choose the logs of different colour at the sawmill and get the logs with greatest resistance and good quality of the wood in a very easy and cheap procedure.

Material and Methods

Sample collection 1. Decay resistance test in field according to EN 252

In November 1999 a field trial to investigate the decay resistance of Siberian larch (*Larix sibirica* Ldb.), European larch (*Larix decidua* Mill.), and Scots pine (*Pinus sylvestris* L.), was started according to the European standard EN 252.

All logs of Siberian larch were imported from Siberia (Krasnoyarsk), Russia to Sweden. The old European larch

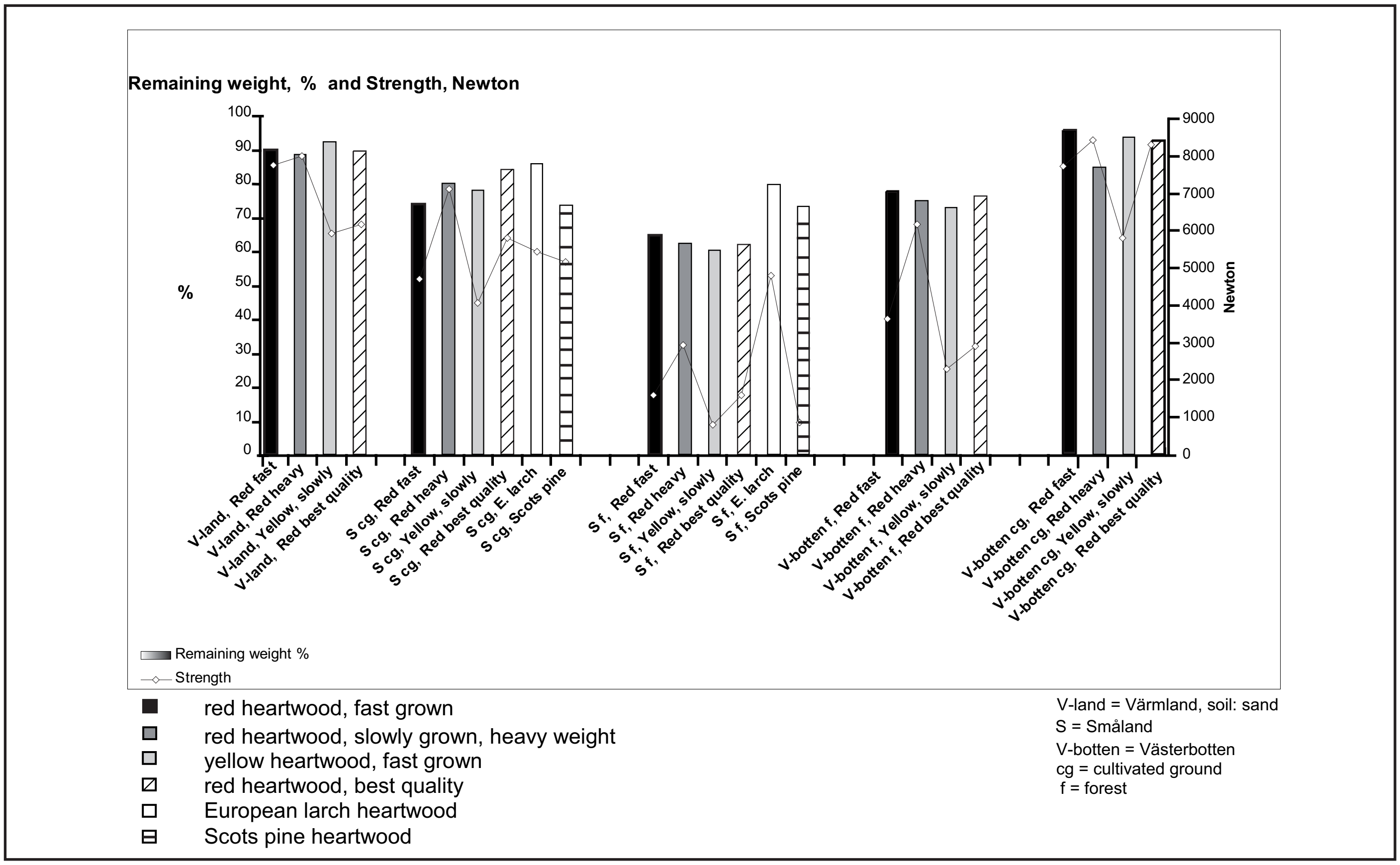

Fig. 1. The remaining weight loss in % and strength in Newton of the sample sticks after 6 years in the soil at three different places in Sweden, Värmland, V-land, Småland, S, and Västerbotten, V- botten. The different soils are sand (only in Värmland), cultivated ground, cg, and forest, f. The left axis and the bars show the remaining weight % and the right axis and the curves show the strength expressed in Newton.

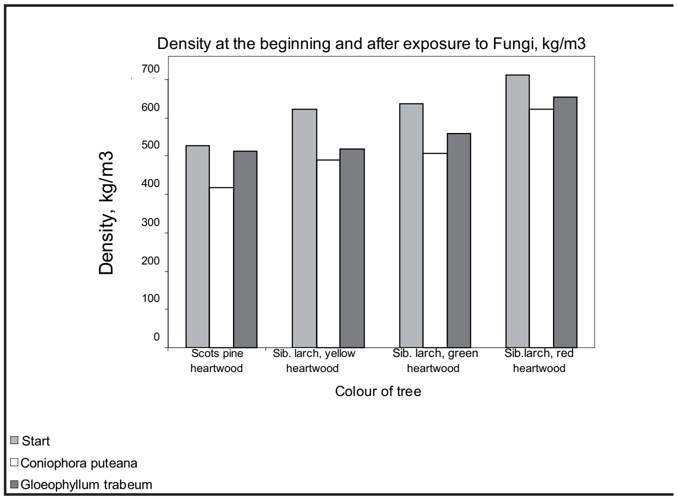

Fig. 2. The density of the four different samples; red, green and yellow heartwood of Siberian larch, *Larix sibirica* Ldb, and Scots pine heartwood, *Pinus sylvestris* L, before exposure to fungi and after exposure to ▩ *Coniophora puteana* ☐ or *Gloeophyllum trabeum* ■, the method according to EN 113.

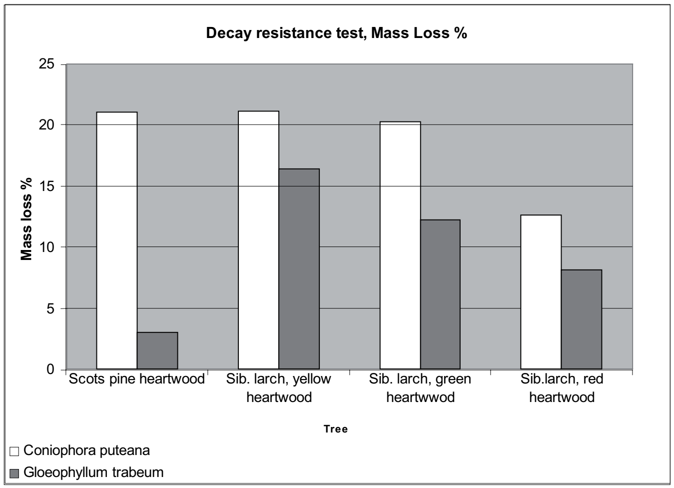

Fig. 3. The mass loss in percentage of the wood after exposure to *Coniophora puteana* ☐ or *Gloephyllum trabeum* ■ according to EN 113

log originated from Västergötland, Sweden and the old Scots pine log from Värmland, Sweden. The top diameter of the logs was 35-36cm and the length ca 6m. Six trees were used; Four Siberian larch trees, one European larch tree and one Scots pine tree originating from Sweden, age unknown for every tree.

The four Siberian larch logs were separated into different classes; red heartwood (fast growth), red heartwood with extremely high density (heavy wood), yellow heartwood (light and slowly growth), red heartwood (slow growth, 1st class wood quality).

The samples (sticks) with a dimension of 2, 5cm*5, 0 cm* 50cm were cut from the outer heartwood of the logs.

Eight sticks from each log were planted in three different places in Sweden; Småland, 57°45'N 13°50'E, south of Sweden, Värmland, 59°33'N 13°10'E, middle of Sweden and Västerbotten, 63°80'N 20°40'E, north of Sweden. There were altogether five different locations chosen according to type of soil; cultivated ground and forest in Småland and Västerbotten and sand in Värmland. Altogether 232 sticks were tested in the field trial.

The test was finished in September and October 2005, after six years, and evaluated according to EN 252, the wood was classified, weighed and strength measurements were done using a three-point pressure method.

Sample collection 2.
Laboratory tests including decay resistance test according to EN 113, water absorption tests

In May 2005 ten different logs of Siberian larch were chosen at Ansgarius Svensson Sawmill by eyesight in three different colours; red, green and yellow, ten different logs of Scots pine were also randomly chosen. The Siberian larch logs originated from Siberia (Krasnoyarsk), Russia and the Scots pine logs originated from Värmland, Sweden. The logs were cut at a height of approx. 6m in discs of approx. 30cm. The diameter varied between 25 and 36centimeters and the age at that height varied between 113 and 213 years in Siberian larch and the Scots pine diameter varied between 28 and 34cm and the age varied between 107 and 170 years.

The decay resistance test was performed according to EN 113, the fungi tested were *Coniophora puteana* (BAM Ebw. 15) and *Gloeophyllum trabeum* (BAM Ebw.109). Altogether 480 samples were tested.

The water absorption was analysed using a method measuring the contact angle using Fibro DAT 1100, during 10sec after a drop of water was applied to a piece of wood sample, 15*0,6*0,8cm. Altogether 120 samples were tested.

Results

Sample collection 1

The sticks located in Värmland, sand, and Västerbotten, cultivated ground, had the lowest mass loss i.e. the highest values for remaining weight, for all classes of wood differing from ca 88 remaining weight % to ca 96% (Fig.1). The sticks located in Småland forest ground showed the lowest values i.e. 60 % to 80 % where European larch showed the largest value and Scots pine the second largest. Comparing the results for the strength measurements the highest values are shown in Värmland located sticks and Västerbotten located sticks ca 6000 to 8000 Newton. The lowest values are again shown in the Småland forest ground located sticks i.e. from ca 1000 to 5000 Newton. Comparing the different classes of Siberian larch wood the log of yellow fast grown sticks has the lowest value in every location except for Småland forest ground where also Scots pine has the same low value i.e. ca 1000 Newton. The red heavy sample showed the highest strength at all locations except for Småland forest where European larch showed the largest value.

The sample were also classified according to EN 252; Class 0 = no attack, 1 = slight attack, 2 = moderate attack, 3 = severe attack and 4 = failure. In this classification the red heavy Siberian larch samples had the least attacks and were classified into class 1 for the sticks located in Värmland, Småland cultivated ground and Västerbotten cultivated ground while yellow Siberian larch, red best quality Siberian larch and Scots pine showed the greatest attacks and were classified into class 2 and 3 in all locations.

Sample collection 2

The density of the different samples were highest for red Siberian larch heartwood followed by green and yellow Siberian heartwood and Scots pine density had the lowest value from the beginning (Fig. 2) The density of the exposed wood samples was still greatest in red Siberian larch heartwood followed by green, yellow Siberian larch and Scots pine heartwood after exposure to Fungi (Fig. 2).

The decay resistance test showed the greatest mass loss altogether (considering both Fungi i.e. *Coniophora puteana* and *Gloeophyllum trabeum*) for yellow Siberian larch and then green Siberian larch. The smallest mass loss was found for red Siberian larch altogether, though the smallest mass loss was found for Scots pine heartwood exposed to *G. trabeum* (Fig. 3)

The wood samples tested are classified according to the EN 113 classification of the natural durability against fungal attacks tested in vitro (Table 1).

Table 1. Classification of natural durability against fungal attacks in vitro according to EN 113

Durability class	Description	In vitro test results expressed in DI* values
1	Very durable	DI≤0,15
2	Durable	0,15<DI≤0,30
3	Moderately durable	0,30<DI≤0,60
4	Not very durable	0,60<DI≤0,90
5	Not durabe	DI>0,90

**DI = durability index =*
mass loss % average of larch test pieces
mass loss % average of control test pieces

The red Siberian larch heartwood samples tested against C. puteana were classified into class 2 i.e. durable while the other Siberian larch samples were classified into class 3, i.e. moderately durable. The wood samples tested against G. trabeum were classified into class 1, *i.e.* very durable for Scots pine heartwood and class 2 for red Siberian larch heartwood and class 3 for the other two types of Siberian larch (Data not shown).

The results of the water absorption analyses shows that the water absorption for Scots pine heartwood is less compared to Siberian larch heartwood (Fig.4). Within the different colours of Siberian larch heartwood the yellow sample showed less water absorption than the two others which had the same pattern. The water absorption 10 seconds after application of the drop of water is 0, 20 µL for green, 0, 19 µL for red and 0, 12 µL for yellow Siberian larch heartwood. For Scots pine the water absorption has stopped i.e. the amount is 0 µL, though after ca 1 sec it was 0,031 µL.

Discussion

The results of the field test in this study reveal that the mass loss does not differ that much between the special kinds of wood though, the mass loss seems to be connected to the density from the beginning. However, there are great differences between the test site for the sticks, both in macro site and soil type. The greatest mass loss was shown for the sticks placed in forest soil in Småland and the least mass loss were shown for forest soil in Västerbotten and sand in Värmland suggesting that the mass loss might be prevented by the shorter growing season in these areas and that there are less micro organisms in sand to the other soils. Considering the strength of the sticks after exposure in the soil there are differences between the special kinds of wood. The yellow and the best quality Siberian larch wood had lower strength than the other Siberian larch wood after the period in soil. The highest values of strength were shown for red heavy Siberian larch heartwood at every location except in Småland forest where European larch had the highest strength which is hard to explain. In Småland forest the strength is much lower than at the other locations suggesting that the soil in the forest is very aggressive to the wood. Furthermore, the strength is also lower in the forest soil in Västerbotten as well compared to the other kinds of soil.

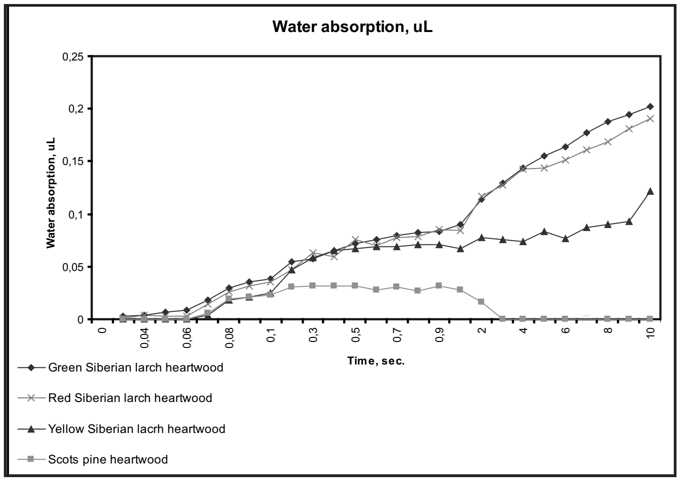

Fig. 4. Water absorption during 10 seconds after application of a 4 µL drop of water. Take notice to the scale of the x-axis.

When considering the classification of the wood again the red heavy Siberian larch heartwood had the best value which supports that the weight of the wood to a large extent decides the durability and strength of wood.

The laboratory test according to EN 113 also supports the results that show connection between weight and durability in larch species. The red Siberian larch heartwood had the highest density and showed the highest durability against the fungi compared to the other Siberian larch samples, though Scots pine had the highest durability against G. trabeum. This suggests a difference in how the fungi attack the wood.

When comparing the water absorption in this study it does not seem to be positively correlated to decay resistance since red and green Siberian larch heartwood showed the highest absorption. However it supports other studies suggesting that heavy wood contains a lot of arabinogalactanes which are water soluble, i.e. the arabinogalactanes absorbs the water quickly (Venäläinen *et al*. 2006, Hakkila and Winter 1973).

Finally, when considering both the field trial and the laboratory tests the red heavy Siberian larch seems to be the most decay resistant and strongest wood and so far it seems as if it is possible to use the human eye to choose the best quality wood. However, the number of samples tested may be too little so further investigation wood is recommended.

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Notes

Genetic parameters for traits related to carbon-sequestration from a full-diallel mating design in Japanese larch (*Larix kaempferi*)

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Abstract

For the development of a breeding program to improve plantation trees to enhance the capability of carbon sequestration, we estimated genetic parameters for growth traits and wood density in *Larix kaempferi*. The carbon content of a tree stem can be estimated from the stem volume and the wood density. Therefore, a progeny trial consisted of 56 full-sib families of *Larix kaempferi* was investigated in tree height, diameter at breast height, pilodyn penetrating depth at breast height over bark, and wood density at the stand age 30. The means (coefficient of variation) were 13.3 m (17.5%) for height, 14.9 cm (26.0%) for diameter, 17.9 mm (9.2%) for pilodyn, and 0.524g/cm^2 (13.1%) for wood density. The narrow sense heritabilities (h^2) of pilodyn and wood density were high (0.55 and 0.51, respectively). The variance components of general combining ability (GCA) and specific combining ability (SCA) were estimated. The ratios of SCA variance to the total variance were very low in all the traits, indicating that an efficient improvement is expected by open-pollinated seed production with seed orchards. The correlation coefficient in breeding value was remarkably high (r = -0.95) between Pilodyn and wood density, indicating the efficiency of non-destructive selection of wood density using Pilodyn. In addition, a strong correlation was found in wood density between each ring and average of all investigated rings. From these results, it is concluded that the investigated traits are highly heritable and the genetic improvement for capability of carbon sequestration is promising in *L. kaempferi*. Moreover, it was suggested that the wood density can be improved by non-destructive and early selection using Pilodyn in progeny trials.

Introduction

The role of forests and forestry is important in the carbon sequestration against the global warming issue. The ability of carbon sequestration capacity would be defined by stem volume and wood density. *Larix kaempferi* (Lambert) Carrière (Japanese larch) is one of the most important tree species in Japan, covering 10.2% of the plantation forest area. This species is good at juvenile growth in cool regions such as Hokkaido and north Tohoku, and at high altitude places in the central Japan. The timber shows good mechanical properties with favorable heartwood color. *L. kaempferi* is also known for its high wood density, therefore the larch forests are expected to be large sink for the increasing carbon dioxide in the atmospheric air. The genetic improvement of *L. kaempferi* was started in the 1950s, with the selection of plus trees for better growth and stem straightness. Until now, the genetic properties of several traits were studied. For wood density, Fujimoto *et al.* (2006) reported the narrow-sense heritability ranged 0.42 to 0.59 in hybrid larch *(Larix gmelinii x L. kaempferi)*. Oshima (1998) estimated the narrow-sense heritability of the wood density was 0.80 in *L. kaempferi*. Oshima and Kuromaru (1995) also reported that the narrow-sense heritability of wood density was 0.55 in hybrid larch *(L. gmelinii x L. kaempferi)*.

The non-destructive measurement of wood density is required in tree breeding because it provides lower-cost and rapid measurements than the destructive methods. Tamura *et al.* (2002) studied on the non-destructive measurement of wood density in *L. kaempferi*, showing that the correlation coefficient was -0.73 between pilodyn penetration value (Pilodyn) without bark and wood density of sapwood.

Early selection is important in tree breeding due to the long rotation age in forestry. Oshima (1998) showed a strong genetic correlation between juvenile and mature wood in wood density in *L. kaempferi*. Fujimoto *et al.* (2006) revealed that the genetic correlation of wood density was approximately 0.8 between age 10 and age 28.

The objectives of this study are to estimate the genetic parameters of several traits relating carbon sequestration, to assess the non-destructive measurement of wood density using Pilodyn, and to evaluate the efficiency of early selection by estimating the time-course variation of the breeding value of wood density, from a test site designed with a diallel mating.

Materials and Methods

The progeny trial sampled in this study was located at the southern foot of Mt. Asama, Nagano Prefecture, Japan (36º21’N, 138º31’E). The test stand consisted of 56 full-sib families crossed by controlled pollination under an 8 x 8 full-diallel mating design, precluding self-pollinated families. The parents for the crossing were plus trees for fast growth and stem straightness selected by the Japanese forest tree breeding program. The stand was established in 1977, under a randomized block design with five replications. Each quadratic plot consisted of 20 trees.

In 2006, the measurement and sampling in this study were done in only three replications out of five. The stand age at the measurement was 29. Tree height, diameters at breast height, and pilodyn penetrating depth (Pilodyn) were measured on all the living trees. The survival rate of the planted trees was 66% in average at that time. No thinning was operated in this trial so far. The number of measured trees was 2330 in total. Height was measured by height-measuring pole. Pilodyn were measured by Pilodyn 6J Forest (PROCEQ, Schwerzenbach, Switzerland), a steel rod of 2mm in diameter strikes stems and the penetrating depth is known to be correlated with wood density in many species. In this study, the Pilodyn measurement was performed at two positions over bark at breast height per sample tree, and averaged.

Twenty families were selected as a complete (but excluding self-pollinated) 5 x 5 full-diallel cross for a wood density measurement. The Increment cores of 5 mm in diameter were sampled at breast height from three trees in each plot for each selected family. The number of the sampled core was 180 in total. A section of 2.2 mm in thickness was cut from each core using a twin-blade saw (Dendrocut, WALESCH Electronic, Effretikon, Switzerland). The sample sections were air-dried in a room with constant temperature (20 °C) and humidity (60%) until the specimen weight became stable. The sections were placed on a film, beside a calibration wedge, and were irradiated by a soft X-ray apparatus (Softex Co., Ebina, Japan). Processed films were scanned by a film scanner (GT-X900, Seiko Epson Co., Suwa, Japan) and converted into digitized images. Density analysis was carried out using a software package (WinDENDRO, Regent Instruments Inc., Quebec City, Canada). The rings with cracks were excluded from the analysis. The wood density of a growth ring (RD) was calculated for each ring and each trees from the digitized image. The rings formed in the year 2006 (outermost ring) were excluded because it was too difficult to distinguish the outermost ring from the bark in the wood density measurement. Generally, we couldn't take incremental cores including pith because the deviation of borer at the sampling. The weighted average of wood density of the outermost 20 rings (ARD20) was calculated for the mean wood density of each sample tree because we could obtain almost all the ring density data during the years 1986-2005. ARD20 is defined as follows:

$$ARD20 = \sum_{i=2a05}^{1986} \left(RD_i \times RW_i\right) \Big/ \sum_{i=2005}^{1986} RW_i \qquad [1]$$

where *ARD20* is the weighted average wood density between the year 2005 to the year 1986, RD_i is the ring density of the year *i*, and RW_i is the ring width of the year *i*. As described in Result and Discussion, the weighted average wood density for the outermost 10 rings (1996-2005, ARD10) was also calculated for the correlation analysis with Pilodyn:

$$ARD10 = \sum_{i=2005}^{1996} \left(RD_i \times RW_i\right) \Big/ \sum_{i=2005}^{1996} RW_i \qquad [2]$$

The ring number of pilodyn penetrating was estimated from the length pilodyn penetrating and the ring widths measured from each increment core. The bark thickness was assumed to be 5mm in all trees because we did not measure the bark thickness. The number of rings pilodyn penetrating (RPP) were estimated from the number of ring between the year 2006 and the year Y, where Y was the maximum satisfying following condition:

$$\sum_{i=2006}^{Y} RW_i > P - 5 \qquad [3]$$

where *Y* is the year earlier than the year 2006, RW_i is the ring width (mm) in the year *i*, *P* is the depth of pilodyn penetrating over the bark. The specimens with some cracked rings were removed from this analysis.

We analyzed the dataset as a half-diallel mating design. The MIXED procedure in the SAS system (SAS Institute Inc, NC, USA) was used for the analysis according to Xiang and Li (2001). We estimated variance components and heritability; and calculated the best linear unbiased predictors of general combining ability (GCA), specific combining ability (SCA) and breeding value of the parents used in the mating, based on the linear mixed model:

$$Y_{ijkl} = \mu + B_i + G_j + G_k + S_{jk} + P_{ijk} + E_{ijk}l \qquad [4]$$

where Y_{ijkl} is the *l*-th observation of *j*-th block for *kl*-th cross; μ is the grand mean; B_i is the fixed effect of *i*-th block, G_j and G_k are the GCA effect of *j*-th parent and *l*-th parent, respectively, NID(0, σ^2_{GCA}); S_{jk} is the SCA effect of *j*-th and *k*-th parents, NID(0, σ^2_{SCA}); P_{ijk} is the effect of *jk*-th cross in *i* th block, NID(0, σ^2_{plot}); E_{ijkl} is within plot error, NID(0, $\sigma^2_{\mathrm{error}}$); where NID(mean, variance) means normally independently distributed (NID) with a mean and a variance. The breeding values of the parent were estimated as deviation from the grand mean, as 2 x GCA.

The individual tree heritability was estimated:

$$h^2 = 4\sigma^2_{GCA} / (2\sigma^2_{GCA} + \sigma^2_{SCA} + \sigma^2_{plot} + \sigma^2_{error}) \qquad [5]$$

where h^2 is the individual tree heritability. The variance of the heritability was estimated using Dickerson's approximation (Dieters *et al.* 1995) as following:

$$Var(h^2) = 16Var(\sigma 2_{GCA}) / (2\sigma^2_{GCA} + \sigma^2_{SCA} + \sigma^2_{plot} + \sigma^2_{error})^2 \qquad [6]$$

where $Var(h^2)$ is the variance of the heritability, $Var(\sigma^2_{\mathrm{GCA}})$ is the variance of the variance component of GCA.

Relations between Pilodyn and wood density were evaluated by the Pearson's product-moment correlation coefficient.

Results and Discussions

General results and genetic parameters

The diameter growth was the largest in the year 1983 (Fig. 1). The diameter growth dropped markedly from the year 1982 to 1990. After the year 1991, the diameter growth decreased slightly. Table 1 shows the grand means, variance components, and heritabilities of the investigated traits. Diameter showed the largest phenotypic variation but its heritability was not high. Height showed high heritabilities in the 8 x 8 set. In the 5 x 5 set, however, the heritability of height was the lowest. The heritability of the Pilodyn is high in both the two data sets. ARD20 also showed high heritability. SCAs were considerably lower than GCAs for all the traits investigated, especially in the 5 x 5 set.

The variance components of GCA were higher than SCA for all the traits investigated. This suggests that an efficient improvement is expected by open-pollinated seed production with seed orchards. The heritability of height was high in the 8 x 8 diallel set but decreased when the 5 x 5 diallel set, which was selected from the 8 x 8 set, were investigated. This was due to the exclusion of the several inferior parents from the first set. This result implies the risk of the inaccurate estimation of genetic parameters when a few parents are used. However, it was showed from the high heritability that the genetic improvement on tree height was possible when a large phenotypic variation exists in the population. The high heritability of the wood density is consistent with other reports (Fujimoto *et al.* 2006, Venäläinen *et al.* 2001). The heritability of Pilodyn is similar to the heritability of wood density, implying strong genetic control over the traits related to Pilodyn.

Non-destructive measurement of wood density using Pilodyn

The number of rings pilodyn penetrating (RPP) was 10.0 on average and ranged from 4 to 19 rings under an assumption that the thickness of bark was 5 mm. We compared Pilodyn with wood density in the range of pilodyn penetrating by using ARD10. Relations between Pilodyn and ARD10 were shown in Fig. 2. At the level of individual trees (Fig. 2a), the correlation between Pilodyn and ARD10 is not high (*r* = -0.42). The correlation increased in the plot mean (Fig. 2b, *r* = -0.59) and the family mean (Fig. 2b, *r* = -0.81). The correlation in the breeding values of the parents between Pilodyn and ARD10 (Fig. 2d) was remarkably high (*r* = -0.95). Subsequently, we compared Pilodyn with ARD20 to evaluate the opportunity for the estimation of whole wood density at breast height by Pilodyn. The correlation coefficients were -0.36 in the individual tree level, -0.55 in the plot mean, -0.78 in the family mean and -0.93 in the breeding values. The correlation coefficients were slightly lower with ARD20 than with ARD10.

The correlation between Pilodyn and ARD10 was not so high at individual tree level (Fig. 1). Pilodyn was affected not only the wood density but also other factors, such as bark thickness. From our results, it was suggested that the selection of individuals in wood density using only pilodyn was not suitable. Tamura *et al.* (2002) showed that Pilodyn was highly correlated with wood density when Pilodyn was applied without bark in *L. kaempferi*. Therefore, removing bark would be necessary for the evaluation of individual trees in pilodyn measurement. However, the correlation coefficients in the breeding values between Pilodyn and ARD10 was sufficiently high (*r* = -0.95), indicating the efficiency of Pilodyn in the selection of parents in progeny tests.

Pilodyn only penetrates only outer parts of wood, suggesting that the factors affecting Pilodyn are limited to the characteristics of the outer part of wood. In addition,

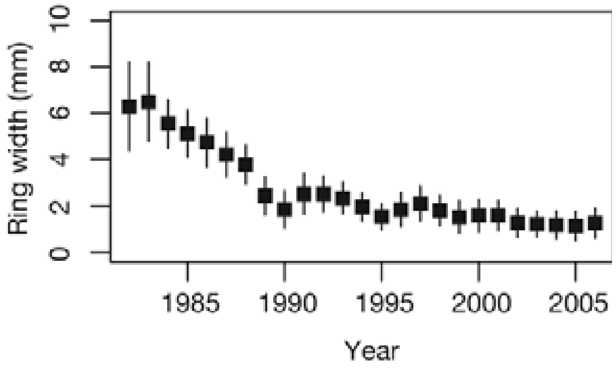

Fig. 1. The time-course variation of ring width from year 1982 to 2006. The ring width of each tree in each year is shown. Filled squates and vertical lines indicate the grand means and standard deviations of ring width. Sample numbers vary in every year, from 150 to 180.

Table 1. Means, variance components and heritabilities of investigated traits. ARD20 is the average wood density of outermost 20 rings. CV means the coefficient of variation (%) of all investigated trees.

		Height (m)	Diameter (cm)	Pilodyn (mm)	ARD20 (g/cm³)
8 x 8 diallel	Mean (CV)	13.3 (17.5)	14.9 (26.0)	17.9 (9.2)	
(2330 trees)	σ^2_{GCA}	0.737	0.689	0.336	
	σ^2_{SCA}	0.152	0.178	0.026	
	σ^2_{plot}	0.206	0.312	0.146	
	σ^2_{error}	4.051	12.817	1.759	
	h^2 (variance)	0.50 (0.08)	0.19 (0.01)	0.52 (0.08)	
5 x 5 diallel	Mean (CV)	14.2 (11.5)	16.1 (18.5)	17.4 (8.7)	0.524 (13.1)
(180 trees)	σ^2_{GCA}	0.102	0.502	0.311	0.000522
	σ^2_{SCA}	0.005	0.000	0.000	0.000000
	σ^2_{plot}	0.000	0.292	0.102	0.000000
	σ^2_{error}	2.476	7.729	1.526	0.003012
	h^2 (variance)	0.15 (0.02)	0.22 (0.04)	0.55 (0.19)	0.51 (0.16)

RPP varied between trees. These results indicate Pilodyn cannot estimate directory the entire wood density or use for the even-aged woods. Nevertheless, the correlation between Pilodyn and ARD20 were only slightly lower than that of Pilodyn and ARD10. The strong correlation between RDs and ARD20 mentioned below would affect this result. These results indicated the efficiency of Pilodyn as a tool for non-destructive selection of the parents in progeny tests.

Early selection of wood density

The time-course variation of the grand mean of RD was shown in Fig. 4a. The grand mean of RD increased from 1983 to around 1990. Any increasing- or decreasing-trend was not observed from 1990 to 2005. The breeding value of each parent was fluctuated between years, keeping the ranking between parents (Fig. 4a). The high correlation was observed in the breeding value between ARD20 and RD at younger age (Fig. 4b).

The rank of the parents in the breeding value of wood density was considerably constant with the increase of the age (Fig. 4a). Early selection is important for forest tree breeding because of the long rotation age of forestry. Our results showed that the rank of the breeding values of ARD20 could be predicted by RDs at younger stage. Because the ring width decreased (Fig. 1) and wood density increased (Fig. 4a) until the year 1990, the wood produced before 1990 is considered to be juvenile wood. These results indicate that the early selection is applicable for wood density in *L. kaempferi* by measuring the juvenile wood in progeny tests. Fujimoto *et al.* (2006) described the optimum selection ages for wood density ranged from age 8 to 14 in hybrid larch *(L. gmelinii x L. kaempferi)*. Our result is consistent with their findings.

In conclusion, the investigated traits are highly heritable and the genetic improvement for capability of carbon sequestration is promising in *L. kaempferi*. In addition, an efficient improvement is expected by open-pollinated seed production with seed orchards because of the high GCA and low SCA. Better parents can be selected by non-destructive measurement with Pilodyn from progeny tests. Early selection is applicable for the whole wood density of 30 year-old trees by measuring juvenile wood. The combination of non-destructive measurement and early selection is a promising approach for the genetic improvement on the wood density and the consequent carbon sequestration capability in *L. kaempferi*.

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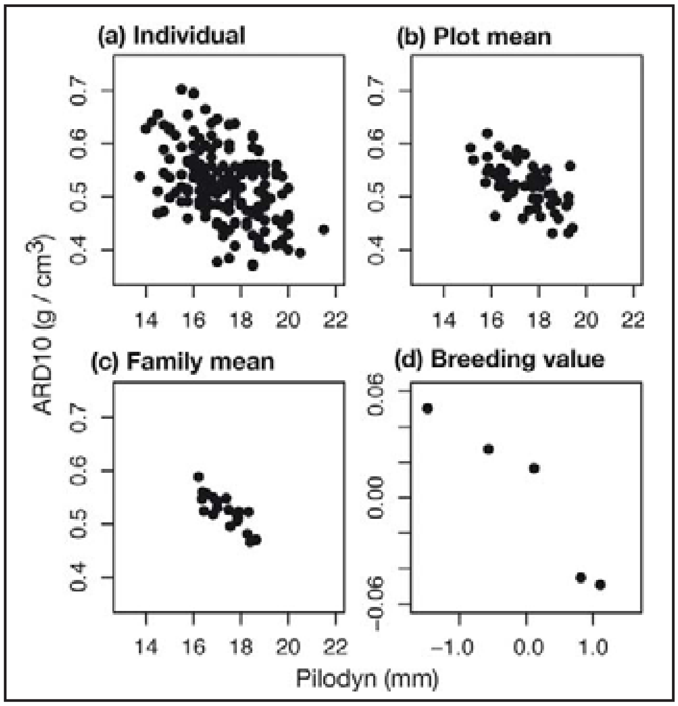

Fig. 2. Relations between Pilodyn and the average wood density of outermost 10 rings (ARD10). (a) individual trees, n = 180, (b) plot means, n = 60, (c) family means, n = 18, (d) breeding values, n = 5.

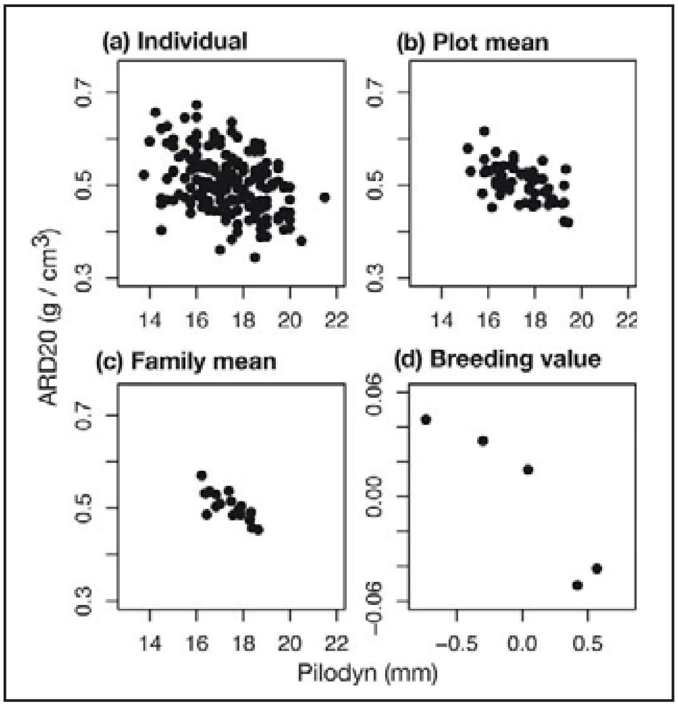

Fig. 3. Relations between Pilodyn and the average wood density of outermost 20 rings (ARD20). (a) individual trees, n = 180, (b) plot means, n = 60, (c) family means, n = 38, (d) breeding values, n = 5.

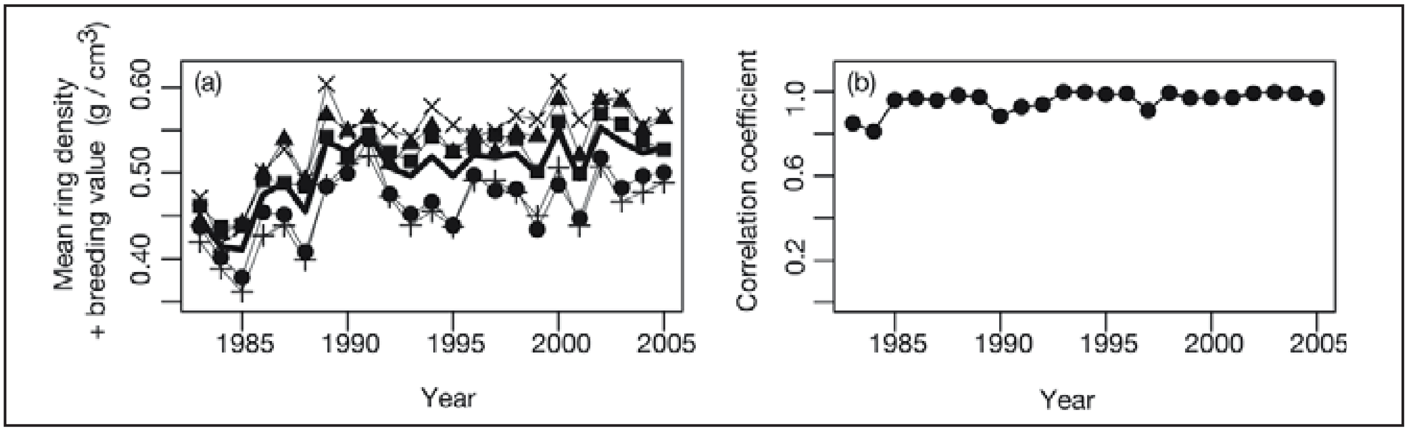

Fig. 4. The time-course variation of RD and correlation in the wood density between RD and ARD20 from the year 1983 to year 2005. (a) the time-course trend of breeding value and the grand mean of RD. Each marker shows the breeding value of different parents + grand mean of RD. Thick line shows grand mean of RD of each year. (b) the correlation coefficients between the breeding values of parents between RD and ARD20. n = 5 for each year.

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Notes

Genetic variation and breeding value of European larch (*Larix decidua* Mill) in Poland

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European larch (*Larix decidua* Mill.) occurs at the entire area of Poland, but its proportion in stands is relatively small (less than 1% of forest area). Nevertheless, the interest in the species is significant because of its growth property as the fastest growing native tree species in Poland.

Long term studies on genetic variability of quantitative and qualitative characters of larch on individual and population level from central European subrange of this species have been done in Department of Genetics and Forest Tree Physiology of Forest Research Institute. Studies were curried out on plots established in different time. The oldest one was established in 1933 in Sudety region in the series of Schwappach experiments. Totally more then 40 populations, mainly from Poland, but also some from Austria, and Switzerland and more the 300 progenies of the single trees growth at experimental plots. Larch provenance tests, conducted over almost 70 years, show large variability of the growing features between populations as well as within them. In comparison with other main Polish forest tree species (pine, spruce and fir) these variability is two to four time bigger.

The volume production of the best populations in 50 year period is about 600 m^3 per hectare, mean year increment 12 - 14 m^3/ha/year. Volume production of half sib families and single trees in progeny experiments is also strongly varied.

Nearly the same level of variation is observed in qualitative characters of the trees (stem straightness, width of crown, thickness and angle of branches) and also in some adaptative traits (resistance to cancer).

This unusual variability with rather punctual then clinal character is probably done by long term discontinuity of the natural range of this species cause by specific ecological demands (specially for light) and long term influence by human activity. Lack of gene flow done by fragmentation of natural range of this species in long period also create new variation. The variation observed in breeding characters is partly confirmed also by molecular analyses (izoenzymes, DNA).

Notes

Testing of hybrid larch under different site conditions in Germany

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Abstract

A program of breeding hybrid larch (*Larix x eurolepis* Henry) started in the eastern part of Germany at Waldsieversdorf 40 years ago. Controlled crosses between different plus trees of European and Japanese larch were carried out in the frame of this program from 1968 to 1990. The parent plus trees of European larch were descended from stands selected in Sudeten mountains, Poland, and secondary stands in East Germany. Japanese larch trees were selected from natural stands in Japan, but from secondary German stands too.

Three different series of progeny tests with 10 (3, 2, 5) trials were established 1974, 1986/87, and 1992. Altogether 153 hybrid progenies derived from controlled crossings were included in these trials. The aim of the tests was the identification of progenies with an outstanding growth performance, good stem form and stability under different site conditions. Also the combination ability of the parent trees should be tested.

Depending on the trial and the progenies tested the growth of many of the hybrids was significantly better in comparison to the pure European larch. Large differences were detected between the hybrids concerning growth and stem form. The results will de discussed in view of the application in forestry and further breeding of hybrid larch.

Introduction

Outstanding growth performance is observed for hybrids between European and Japanese larch (*Larix decidua* and *L. kaempferi*) often. For this reason crossing experiments with these two species were carried out in different countries to produce hybrid larch (*Larix x eurolepis* Henry). In Germany the first crossings with larch were done by Dengler and Langner 70 years ago (Dengler 1941; Langner 1951/52). Programmes were initiated for the breeding of hybrid larch at different institutes in Germany between 1950 and 1970 (Hering *et al.* 1989, Weiser 1992, Franke 1995, Langner and Schneck 1998). These programmes resulted in a number of field trials and the approval of basic material for the production of tested reproductive material according the guidelines of EU.

Breeding of hybrid larch started at Waldsieversdorf about 40 years ago. Since this time three different series of crossings and progeny tests were carried out. Altogether ten field trials were established with 153 hybrid progenies (Table 1). The aims of this breeding programme are the improvement of growth, quality and stability. Furthermore parent trees with good combining ability should be selected for seed orchards. Results were published for the oldest series (1974) after 20 years (Weiser 1992) and for the series planted 1992 after 10 years respectively (Schneck *et al*. 2002). Here the results will be presented of the last measurements of the two older series (1974 and 1986).

Materials and trials

The crossings of the first series were carried out between 1968 and 1971 in two clonal archives. Seeds were sown in spring 1972. Tests were established at three sites in 1974. In the whole 81 different progenies were planted but only 32 of them are growing at all three sites. 65 progenies are interspecific hybrids (57 *L. decidua* x *L. kaempferi* and 8 *L. kaempferi* x *L. decidua*). Three progenies of seed stands of European larch were used as standards in the trials.

A second series of crossings were carried out between 1978 and 1982 in the same clone collection as before. This work resulted in a test with 35 progenies of *L. decidua* x *L. kaempferi*, five of *L. kaempferi* x *L. decidua* and one of *L. kaempferi* x *L. sukaszewii* at two sites.

Table 1. Summary of different series of progeny tests established in the frame of the hybrid larch breeding programme at Waldsieversdorf

Year of planting	Progenies*	Test sites	Year of measurement
1974	57 HLA(E); 8 HLA(J); 9 ELA x ELA; 2 JLA x JLA; 3 ELA (stands)	3	1983; 1988; 1991; 2005
1986	35 HLA(E); 5 HLA(J); 8 ELA (stands); 1 JLA x *L. sukaszewii*	2	1988, 1993, 2001
1992	36 HLA(E); 6 ELA (stands)	5	2001

*HLA(E) – hybrid larch with European larch as mother tree; HLA(J) – hybrid larch with Japanese larch as mother tree; ELA – European larch; JLA – Japanese larch

Table 2. Site characteristics of the trials

Location	Latitude	Longitude	Climate*			Soil	Site class type
			m.a.t.	a.p.	d.v.		
Series 1974							
Floessberg	51° 08’ N	12° 05’ E	8.9 °C	629 mm	169 d	clay	moderate eutrophic, medium fresh
Pfefferteich	53° 00’ N	12° 42’ E	8.5 °C	545 mm	164 d	loamy sand	moderate eutrophic, medium fresh
Luebz	53° 29’ N	12° 05’ E	8.2 °C	598 mm	158 d	loamy sand	eutrophic, medium fresh
Series 1986							
Thiessen	51° 56’ N	12° 40’ E	8.8 °C	554 mm	167 d	sand with little clay	moderate eutrophic, medium fresh
Vierraden	53° 08’ N	14° 18’ E	8.6 °C	520 mm	165 d	loamy sand	eutrophic, medium fresh

* m.a.t. – mean annual temperature, a.p. – annual precipitation, d.v. – duration of vegetation period (temp.>10 °C)

Plus trees of European larch for the crossings were selected in autochthonous stands in the Czech Republic and Slovakia (Sudeten and Tatra mountains), in secondary stands of unknown origin in eastern Germany and in an old provenance trial (Fig. 1). Japanese larch trees came from natural stands in Japan and planted stands in Germany.

The trials of both series are situated in the eastern part of Germany (Table 2). The annual precipitation varies from 520 to 629 mm. The half of this amount falls in the vegetation period. Sites vary in soil quality and water supply too.

All trials were measured several times. Last assessments took place for the trials planted 1974 in the year 2005 when the trees were 34 years old and for the trials planted 1986 in 2001 (age of trees 18 years) respectively. Height and diameter at breast height (dbh) were measured and stem form was assessed according to a three-step scale (1-straight, 2-slightly bent or curved, 3-clear bent or curved) for all trees. All data were analysed for every single trial separately and jointly for the trials belonging to the same series. Analysis of variance and Dunnett’s One-tailed test were used for comparison of the progenies with a standard (European larch stand “Hasselburg”).

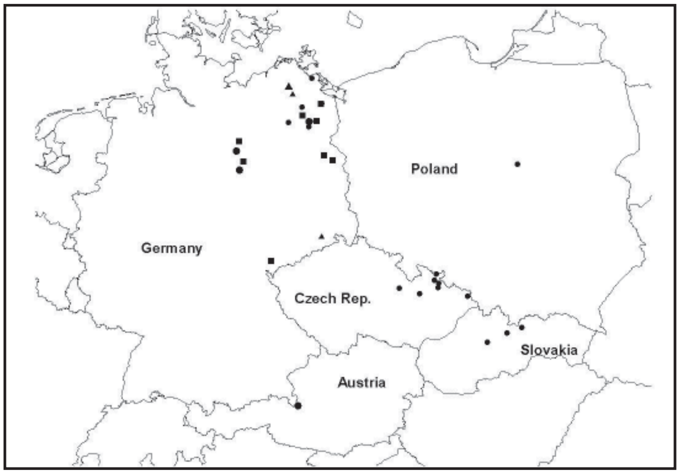

Fig. 1. Origin of parent trees of progenies and of European larch stands (dot = European larch, triangle = Japanese larch, square = European larch stand).

A selection index was calculated for complex evaluation of the progenies for every single trial according to the following formula:

$$I_W = \sum_{i=1}^{k} a_i \frac{v_i - \bar{v}_i}{s_{v_i}}$$

where I_w= selection index, k= number of traits, a_i= evaluation value, v_i= mean of the respective progeny, $\bar{v}_i$ = trial mean, s_{v_i}= standard deviation of the trial mean. The characteristics were weighted with the following values: *height* = *2, dbh* = *1, stem form* = *2.*

Results

Differences between progenies as well as between the trial sites were significantly for both series. The interaction between sites and progenies was significantly for dbh, single tree, and stand volume but not for height and stem form within the 1974 series and for height and stem form but not for dbh within the 1986 series. In the case of a significant interaction every single trial was analysed separately. At near all sites the average growth performance (height, dbh, volume) was better for the hybrids in comparison with pure European larch (Fig. 2 and 3). That was true for both kinds of hybrids (*L. decidua* x *L. kaempferi* and *L. kaempferi* x *L. decidua*). The trial at Thiessen is an exception. There the average of hybrids was lower than that of European larch. But also at this site some hybrids grew better than the standard. On average superiority of hybrids in height amounted to 6 to 10 % for series 1974 and 11 to 14 % at Vierraden for series 1986 respectively. That is corresponding up to one and a half yield class in comparison to the yield table for European larch. The superiority of hybrid progenies was very impressive in volume production per hectare at several sites (Luebz, Floessberg, Vierraden). The best hybrids outperformed the standard between two to three times there (Fig. 2). The largest number of progenies significantly better in height growth than the standard could be found at the site “Luebz”. In contrast no progeny with significantly better height growth was detected at site “Thiessen”. Similar results were observed for dbh and volume growth.

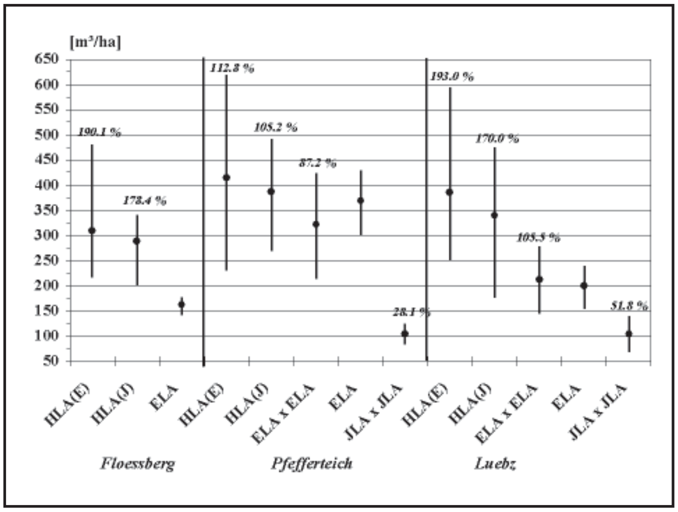

Fig. 2. Progeny test 1974 – mean and range for stand volume per ha 2005 of different groups of progenies (percentage quotation=relation between group means and mean of European larch stands (ELA))

The phenotypic correlation was weak and not significantly between height growth and stem form within the single trials (0.17 – 0.35). So there are well growing hybrid progenies with satisfactory stem form.

A selection index was calculated for the single trials of series 1974 to combine growth performance and quality (Fig. 4). Although the correlation between height growth and stem form was weak a rank shifting was visible in comparison to the ranking according height. An example is progeny CSSR 656 x W 2, which had a very good height growth but a poor stem form. Progenies e. g. like Gr 27 x M 1003, M 1001 x M 310 or Gr 90 x G 2, which have a positive selection index at all three sites are of special interest especially if they show a significant superiority compared to the standard in one or more traits.

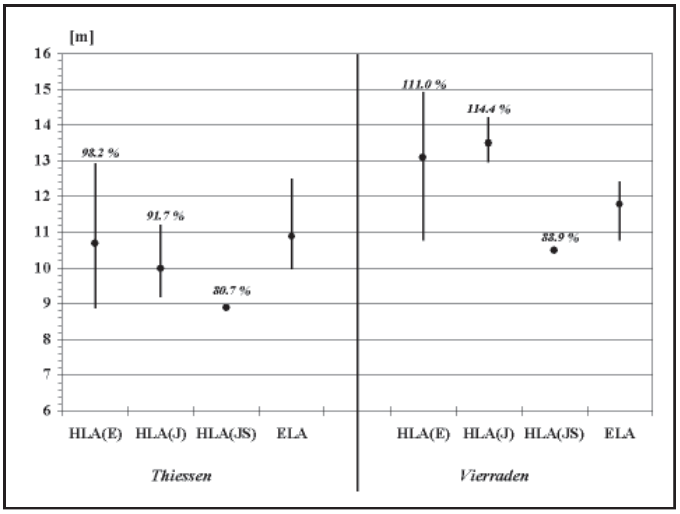

Fig. 3. Progeny test 1986 – mean and range for height 2001 of different groups of progenies (percentage quotation=relation between group means and mean of European larch stands (ELA))

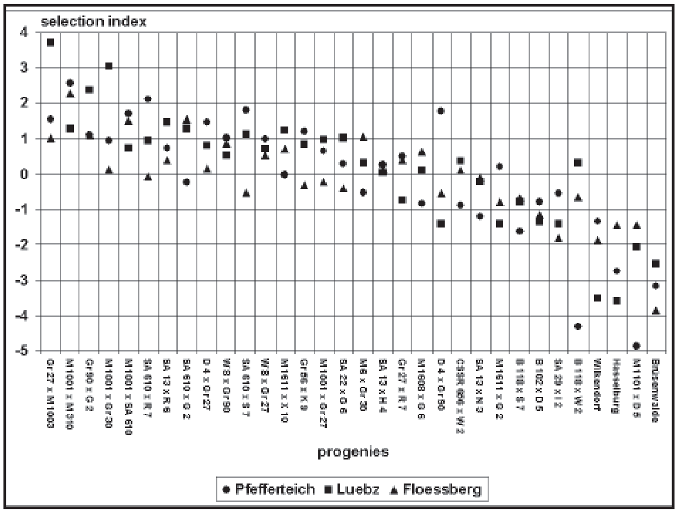

Fig. 4. Progeny test 1974 – selection index for progenies growing at all three sites ("Hasselburg", "Wilkendorf", and "Brüsenwalde" = European larch stands)

Discussion

The results of these progeny tests verify the superior growth performance of hybrids between European and Japanese larch in comparison to progenies of pure European larch. As mentioned before there were visible differences in growth performance of the progenies between the different sites. Surprisingly the progeny group of hybrids did not outperform the European larch at the poorest site "Thiessen". Besides the poor site conditions also an attack of weevils in the planting year may be a reason for it. In general the differences between hybrids and pure European larch became more evident with increasing site quality. The amount of superiority in volume growth of hybrid larch was much higher at the two better sites of the series 1974 in comparison to the third site. Different silvicultural treatment (earlier and more thinnings) had had a strong influence besides site quality.

Large differences existed within the hybrid group. There were hybrids growing not better as the standards at nearly each site. Therefore the performance of hybrid larch depends on the right and careful choice of the parent trees. The mating designs used in these crossing series is not suitable for the identification of parent trees with a good general combining ability since most of the trees were used no more than one or two times as female or male. Therefore combinations of parents could be selected with good specific combining ability only. The weak correlation between growth and stem form is remarkable and in contrast to other observations (Hering *et al*. 1989). One reason therefore may be that the parent trees for crossings were selected very carefully according to their stem form. The importance of the selection of mother trees for the stem form of hybrids was pointed out by Schneck and Langner (2000). In their breeding program hybrids with mother trees from the Alps showed best stem forms. In spite of the large differences between the single trials and some shifts in ranking progenies that combine outstanding growth performance and satisfactory stem form can be found in both series. Mostly those are progenies with a positive selection index at all sites.

Up to now 2 parents of families were approved as basic material for the production of tested propagation material according to the German regulations and one seed orchard was established as an outcome of this breeding programme. The results of the most recent evaluation presented here allow the selection and approval of further trees. Such trees can be used for the establishment of seed orchards for production of hybrid larch seeds.

Although the results of our and other trials with hybrid larch show the large potential for increasing wood production there is a very low interest in practical forestry in reproduction material of hybrid larch in Germany today. A reason for this is that silvicultural aims have been radically changed in direction to a more close to nature forestry and so the extent of artificial reproduction especially of conifers has been reduced considerably. Nevertheless, hybrid larch may be planted for establishing new forests at former farmlands and for completing regeneration areas (Kohlstock 1992). Hybrid larch is useful for afforestation because of its suitability as nurse crop. In comparison to other nurse crop species, it produces marketable wood assortments in shorter time. Also the increasing demand of wood for heating and power production may have a positive effect on the planting of hybrid larch in the future.

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Notes

Notes

Towards the integration of somatic embryogenesis in a breeding programme of *Larix* species

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Advances in plant biotechnology offer new opportunities in the field of plant propagation. Development of clonal propagation method, such as somatic embryogenesis has potentially numerous applications such as the production of a large number of genetically improved plants and the amenability of embryogenic cultures to cryogenic storage. Since the 90's, INRA engaged researches on somatic embryogenesis in *Larix* species. Several improvements and simplifications of somatic embryogenesis protocol were developed at all steps including initiation, cryopreservation, maturation and plant recovery. The improved procedure for somatic embryogenesis of *Larix* species leads to well-synchronised and controlled somatic embryo development. Among conifer species, somatic embryogenesis of *Larix* is becoming a model ; its integration in a breeding programme is now undertaken for clonal propagation of improved material of hybrid larch *Larix x eurolepis*. Indeed, we believe that somatic embryogenesis should influence breeding strategies by offering an alternative tool for accelerated production of plants for genotype evaluation through clonal testing; in addition, cryoconservation allows keeping juvenility of material and its production at any time.

Notes

Silviculture and prediction of mechanical properties for French larch (*Larix decidua*)

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Abstract

Enhancing the value of a specie for use in structural timber requires an accurate knowledge of its characteristics, especially the mechanical ones. Larch is mainly used in timber construction, especially in the South East of France. However, until recently, timber professionals had not benchmark data likely to direct the choice of architects and designers towards the use of this specie.

160 tested trees were taken from 18 stands exploited in the South East of France. The age of the trees was between 76 and 276 years old. 1800 timber pieces (50*150*3000 mm) were used in destructive tests. The three criteria to establish the strength classes (density, modulus of elasticity and stress) are high, which confer on this wood very good mean mechanical properties. FCBA thus proposed some elements of reference in order to use Larch in construction, including it in the new French construction standard NF B 52-001 (2007) in order to give visual grading rules for EC marking.

However, FCBA have used one macroscopic approach to determine as well the prediction of structural properties for larch. Data coming from structural properties have been sorted in sub sample for sylviculture parameters as fertility, DBH and position along the trees. Mathematical distribution was fitted between data to reach the best results. At the end, saw miller can predict the quality of wood according to their production and gain add values on final product. Therefore, those model did not prevent grading rules for EC marking.

Objectives

Enhancing the value of a species for use in structural timber requires an accurate knowledge of its characteristics, especially the mechanical ones. To suppress the lack of information, FCBA engaged in 1998 a study on the Larch at the national level. Only one area (Provence-Alpes-Côte-d'Azur) was retained for sampling: Indeed, this area concentrates with it only the three quarters of the volume of French larch and 82% of surfaces. Thus the sampling carried out by FCBA is limited to this area, but will have a national value in term of interpolation of the results. One of the questions is the possibility to improve the output of parts usable in housing by using grading logs or trees. The paper describes methodologies and results.

Sampling and mechanical behaviour

The national resource of Larch in France is estimated at more than 100000 ha for an approximate volume of 16,5M m^3, it is 2% of the coniferous trees. If Larch is represented in 49 French departments, only Provence-Alpes-Côte-d'Azur area concentrates 82% of total surface and the three quarters of the production (Reuling 2002).

18 stands were selected and 158 trees sampled in seven-forest subdivision defined by the French trees Inventories. To be representative with the Larch resource, the stands and the trees have been selected according to:

- The geographical distribution: taking away within seven forest subdivision,
- The altitude (from 1400 to 2100 m)
- The forest density
- The classes of growth: a high and a low classes of fertility,
- The DBH: from 35 to 50 cm,

The adult resource of Larch has low annual growth ring related to the mountain sylviculture. Its texture is strong compared to other resinous such as Spruce and European Silver Fir, which confers it very good mechanical properties (particularly in bending strength). Each tree has been transformed in one sawn cross section (50*150 mm^2) by 3000 mm long. The different lumbers (1820 results of edgewise four points bending test) have been identified to know their origin (forest, stand, log, etc.).The following table give some overview on properties.

The mean mechanical properties of the Larch are very interesting, only the modulus of elasticity can be a criterion discriminating in the choice of the timber pieces according to the various uses in the construction. The aesthetic of the Larch timber pieces is strongly knotty what implies a low correlation between the knots and the mechanical properties. After destruction, the mechanical potential of each piece is known. According to this potential, if the Larch timber pieces were classified according to the mechanical criteria defined in the standard EN 338, the outputs would be the following for optimal grading and visual grading.

If the timber pieces of Larch were graded in accordance with the new standard NF B 52-001 on the basis of

Table 1. Anatomical and morphological properties of the sample

158 trees	Average	Standard deviation	Coefficient of variation	Minimum	Maximum
Age (year)	160	48	30%	76	276
DBH (cm)	38	5	14%	28	53
Texture (%)	37	11	30%	7	77
Annual growth ring (mm)	1,4	0,5	36%	0,7	3,2

Table 2. Mechanical average values

	Average	Standard Deviation	Coefficient of variation	Minimum	Maximum
Density to 12% (kg/m3)	591	50	8%	449	765
Nodosity (projection in timber face) (%)	40	18	45%	0	97
Nodosity (projection in timber edge) (%)	39	28	72%	0	100
Knot Area Ratio KAR (%)	36	17	47%	0	95
Annual growth ring (mm)	1,3	0,6	46%	0,5	4,9
Modulus Of Elasticity at 12 % MOE (GPa)	10,9	2,3	21%	4,3	21,3
Modulus Of Rupture MOR (MPa)	50,0	17,5	35%	13,5	112,8

Table 3. Optimal grading

Grades	MOR (5th percentile)	MOEL (average)	MV (5th percentile)	Number of pieces	Outputs
C 27	27.1	13.5	518	1517	96%
C 18	*	*	*	*	*
Reject	14.7	9.3	500	59	4%
Total (*)				1576	100%

Table 4. Grading with visual grading rules (NFB 52-001)

Grades	MOR (MPa) (5th percentile)	MOEL (GPa) (mean values)	MV (kg/m³) (5th percentile)	Number of pieces	Outputs
ST-I – C27	30 (Admitted 27)	12.2 (Admitted 11.5)	517 (Admitted 370)	178	13%
ST-II – C24	26 (Admitted 24)	11.0 (Admitted 11)	521 (Admitted 350)	709	49%
ST-III – C18	24 (Admitted 18)	10.6 (Admitted 9)	502 (Admitted 320)	237	16%
Reject				315	22%
Total				1439	100%

Table 5. Number of specimen per forest parameters (NS nos significant)

Fertility	DBH Classes	H1 (3.6 m)	H2 (7.2 m)	H3 (10.8 m)	H4 (14.4 m)	H5 (18 m)	H6 (21.6 m)	H7 (> 25 m)
F1 (> 24 m)	< 45 cm	59	60	51	36	6 (NS)	*	*
	45 – 55 cm	134	136	113	89	44	8 (NS)	*
	> 55 cm	44	31	34	27	19	11 (NS)	4 (NS)
F2 (< 24m)	< 45 cm	41	33	27	4	*	*	*
	45 – 55 cm	128	124	103	59	10 (NS)	*	*
	> 55 cm	33	43	31	21	9 (NS)	2 (NS)	*

Table 6. Mean MOR modelled per forest parameters (NS not significant)

MOR Mean value	DBH 1 (< 45 cm)		DBH 2 [45-55 cm]		DBH 3 (>55 cm)	
	Fertility 1	Fertility 2	Fertility 1	Fertility 2	Fertility 1	Fertility 2
H1 (3.6 m)	50.5	57.3	62.3	59.6	68.2	50.8
H2 (7.2 m)	47.6	48.1	56.7	51.3	56.9	41.3
H3 (10.8 m)	43.4	44.3	48.1	43.9	53.9	34.2
H4 (14.4 m)	43.5	53.7 (NS)	43.2	42.0	46.3	35.1
H5 (18 m)	41.8 (NS)	*	37.6	43.2 (NS)	39.1	30.9 (NS)
H6 (21.6 m)	*	*	NS	*	35.5 (NS)	27.6 (NS)

visual criteria, outputs would be the following. The correspondence between mechanical and visual classes was defined into the EN 1912 A1 (2007).

The gap between both grading is useful for all species but could be reduce by mechanical machine.

Modelling

The previous table gives the yield of visual grading for the three allowed classes. However, sawn miller would like to know in amount the quality of product directly by the analysis of logs. A lot of machine like medical CT scanner (CT scan) are now able to check internal defect and knottiness with high speed [Longuetaud]. Therefore, the price of those equipments is too expensive for French small enterprises. FCBA have developed one macroscopic approach to determine as well the prediction of structural properties for maritime pine (Reuling 2005) and the modelling previously done was directly used for larch.

Firstly, data (structural properties) have been sorted as follows with three forest parameters:

- Fertility (2 parameters):
  - o Strong F1 > 24 m
  - o Low F2 < 24m
- DBH (3 parameters)
  - o DBH 1 < 45 cm
  - o DBH 2 45 – 55 cm
  - o DBH 3 > 55 cm
- Position of lumber along the tree by 3.6 m step (6 parameters).

The sampling was sorted as follows.

Secondly, the distribution was fitted with Weibull, normal or Log Normal law. The accuracy of each law for each sub-sample was established by the Anderson-Darling test. This procedure is used to test if a sample of data came from a population with a specific distribution. It is a modification of the Kolmogorov-Smirnov (K-S) test and gives more weight to the tails (than does the K-S test). The K-S test is a distribution free in the sense that the critical values do not depend on the specific distribution being tested. The Anderson-Darling test makes use of the specific distribution in calculating critical values. The advantage is to allow a more sensitive test but the critical values must be calculated for each distribution. Currently, tables of critical values are available for the normal, lognormal, Weibull and the best law were taking into account for the analysis. The mean values for MOR versus height for the two fertilities are summarised as follows for example.

The mean value and the range of fiability at 95% for MOE are plotted into the following curves (the MOR curves are similar).

Finally, as we can see in previous table and figures, mean MOR and MOE decrease along the length of the trees and increase with diameter excepted for the last DBH (fertility 2):

- Fertility 1 at DBH more than 55 cm provides best mechanical results
- Fertility 2 at DBH between 45-55 cm reaches one optimum. The median values (projection of knot in height) for DBH (> 55 cm) along the trees comes from fertility 2 are 40 % higher than fertility 1.

Conclusion

Larch is mainly used in timber construction, especially in the South East of France. However, until recently, timber professionals had not benchmark data likely to direct the choice of architects and designers towards the use of this species. By the way, 160 tested trees (76 and 276 years old) were taken from 18 stands exploited in the South East of France. 1800 timber pieces (50*150*3000 mm) were used in destructive tests. FCBA proposed some visual grading rules in order to use Larch in construction, including it in the French construction standard NF B 52-001 (2007)

However, sawn miller would like to know in amount the quality of product directly by the analysis of logs (the yield of visual grading for the three allowed classes gives only 13 % in C27). FCBA have developed one macroscopic approach to determine as well the prediction of structural properties for maritime pine and the modelling previously done was directly used for larch. Data (structural properties) have been sorted in sub sample (fertility, DBH, Position along the trees) in order to fit the best mathematical distribution against mechanical properties.

At the end, FCBA simulates the proportion of specimen, which could be graded according to EN 338 mechanical classes as follows.

Saw millers have now basic tool to determine easily the final quality of wooden construction product against forest parameters according to their production and gain add values on final product. Therefore, this model did not prevent the final grading for the EC marking (EN 14-081 2006) which be done by visual or mechanical grading.

Acknowledgements

Special thanks to FIBOIS 05 and the French Ministry of Agriculture for funding.

Table 7. Grade modelled per forest parameters (NS not significant)

DBH Classes	DBH 1 (< 45 cm)		DBH 2 [45-55 cm]		DBH 3 (>55 cm)	
	Fertility 1	Fertility 2	Fertility 1	Fertility 2	Fertility 1	Fertility 2
H1 (3.6 m)	C24	C24	C27	C30	C30	C24
H2 (7.2 m)	C24	C18	C27	C27	C30	C18
H3 (10.8 m)	C24	C18	C27	C24	C27	C18
H4 (14.4 m)	C24	(NS)	C24	C24	C24	C18
H5 (18 m)	C18 (NS)	*	C24 (NS)	C24 (NS)	C18	C18 (NS)
H6 (21.6 m)	*	*	NS	*	C18 (NS)	C18 (NS)

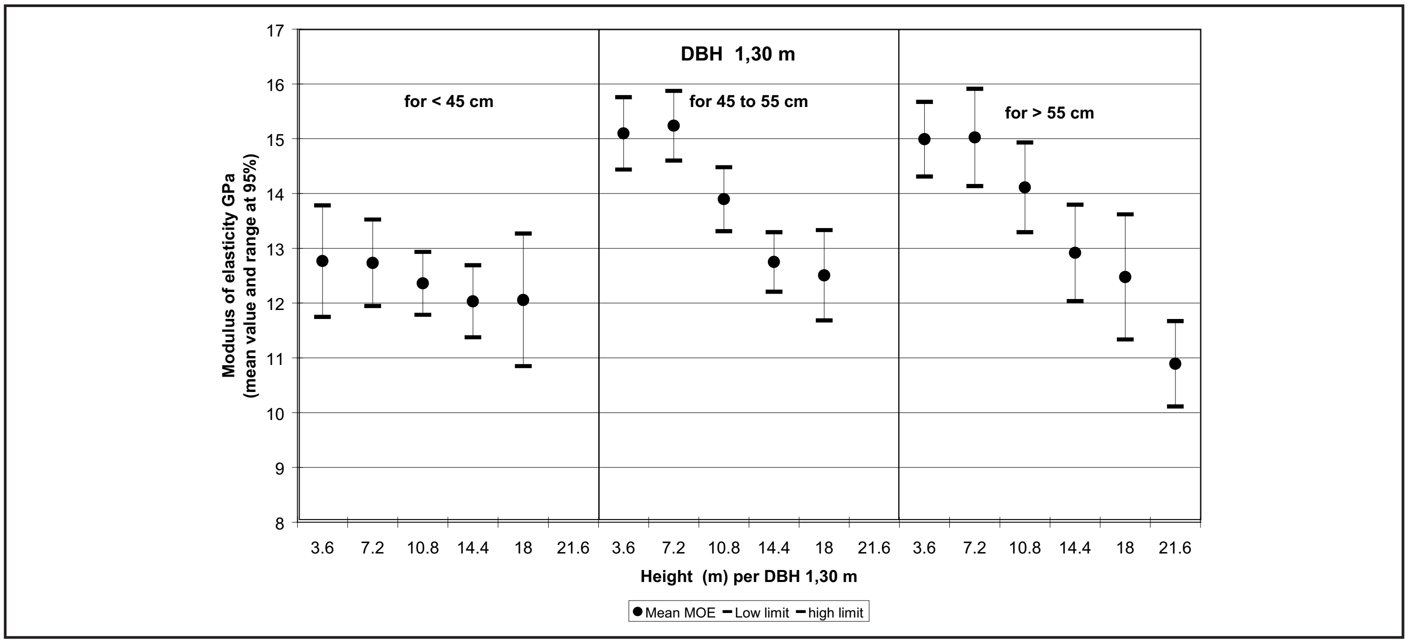

Fig. 1. Modulus of elasticity of Fertility1 versus DBH - Height.

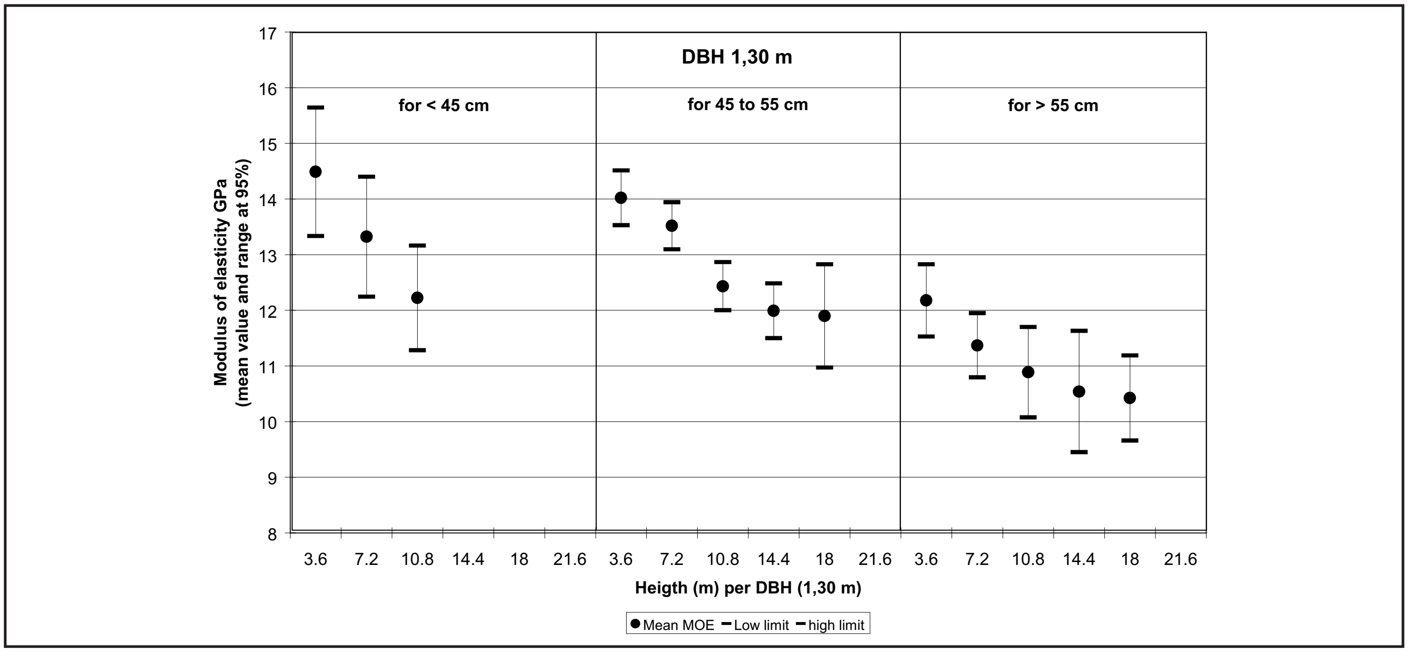

Fig. 2. Modulus of elasticity of Fertility 2 versus DBH - Height.

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Notes

Wood properties of native and exotic larches and their potential for appearance products

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Abstract

Larch wood has particular attributes compared to most native eastern softwoods. These attributes present important challenges for optimum processing. In the first part of this paper we present the particular wood attributes of tamarack and exotic larches. Solutions for efficient processing and optimum use, especially for high value applications such as appearance products are discussed. Hardness, abrasion resistance, weathering and color stability, machining and finishing properties of tamarack and exotic larches wood are discussed. These properties along with wood surface properties of larch are compared to that of woods conventionally used for appearance applications such as flooring.

Résumé

Le bois du mélèze possède des caractéristiques intrinsèques très particulières comparativement à la plupart des essences résineuses de l'Est du Canada. Ces caractéristiques présentent des défis importants pour une transformation optimale. En premier lieu, nous présentons les caractéristiques particulières des mélèzes indigènes et exotiques et les solutions envisageables pour une transformation efficace et une utilisation optimale. Nous traitons particulièrement des technologies disponibles pour assurer une transformation efficace et nous dressons le potentiel d'utilisation pour des applications lucratives, notamment les produits d'apparence. En deuxième lieu, nous présentons les propriétés d'apparence du bois des mélèzes exotiques et indigènes notamment, la dureté en surface, le vieillissement et la stabilité de la couleur, la résistance à l'abrasion, l'aptitude à l'usinage et à la finition. Finalement, nous présentons les propriétés des revêtements de planchers en bois de mélèze comparativement aux revêtements de planchers traditionnels.

Introduction

Tamarack (*Larix laricina* (Du Roi) K. Koch) has one of the widest ranges of all North American conifers. It grows in all Canadian provinces and territories. In the United States it extends from Maine to Minnesota (Little 1979). Exotic larches are introduced successfully to North America through several tree breeding programs and include mainly European larch (*Larix decidua* Mill.), Japanese larch (*Larix kaemferi* (Lambert) Carr.) and their hybrids (*Larix x eurolepis* Henry). Larches are among the Canadian eastern softwoods with the highest growth rates (Figure 1).

The use of larch wood for structural or appearance applications is less popular in North America compared to Europe due to several reasons including the availability of alternative species from the same group (SPF) and the difficulties in processing larch wood. These difficulties are due to many factors including particularities in stem and wood attributes of larch trees. Larch tends to develop stems with important taper and high frequency of knots which lower lumber recovery yields and grades. Larch wood has also the particularity of having high extractive content especially in the heartwood compared to spruces, pines and firs. This results in higher costs and difficulties in pulping and bleaching operations. This also might result in extensive color changes in solid wood products when exposed to UV and heat due to extractives bleeding.

Compared to other eastern softwoods (Figure 2), larch wood is characterised by a high differential between earlywood and latewood densities. This results in a contrast in the wood color, an interesting feature for some appearance applications. However, this differential leads to several difficulties in wood drying, machining and finishing operations.

The main objectives of this study are: to present an overall view of the properties of native and exotic larches growing in eastern Canada; to study the particular attributes of larch and their impact on processing and potential use; and to compare the properties of wood flooring made from larch to that made from conventional hardwood species.

Material and methods

Available information on mechanical and physical properties, of tamarack and exotic larches are taken from literature and summarized in the present paper. Wood density is measured on increment cores taken from four larch experimental plantations in Petawawa forest research station in Ontario Canada. From each experimental plantation, a total of 20 trees were randomly sampled. From a constant compass direction, increment cores 6 mm in diameter were taken at breast height from the sampled trees. Each increment core was wrapped in a plastic bag and kept frozen until preparation. After air drying, cores were sawn to 1.57 mm in thickness, extracted with a cyclohexane / ethanol solution 2:1 (v/v) for 24 h, and then in distilled water for another 24 h. Rings were scanned from pith to bark by X-ray densitometry in air-dry conditions. Earlywood, latewood and ring densities, and latewood proportion were computed from intra-ring microdensitometric profiles.

Properties of larch flooring were measured mainly on tamarack. The surface hardness was measured According To ASTM D1037 standard method. Abrasion properties were measured using Taber abrasion tester according to ASTM D 4060 standard method. In this test, wood samples were exposed to an abrasive wheel designated CS-10 at a fixed speed and weight (1 000 g). Wear resistance is expressed in terms of wear index, which is the weight loss per specified number of cycles under a specific load.

Wood flooring samples from larch and other conventional flooring species were exposed to an accelerated weathering cycle according to the ASTM Standard G155-OOa. The accelerated weathering cycle was conducted as follows: 3.8 hours light (35% relative humidity and 63 °C) 1 hour dark (90% RH and 43 °C). This cycle was repeated continuously for 30 days. Colour measurements were made before drying, after drying and after weathering with a spectrophotometer using the CIELAB colour system :L* is lightness; a* is red-green share; and b* is blue-yellow share. The total color difference, ΔE, integrates the difference between the three variables of the colorimetric system according to the following equation:

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$$

Results and Discussion

Wood physical and mechanical properties

Average wood density values for plantation grown tamarack and exotic larches are shown in Table 1. In Table 2, we summarize the available information for the physical and mechanical properties of plantation grown Tamarack and exotic larches. Data from Table 1 show

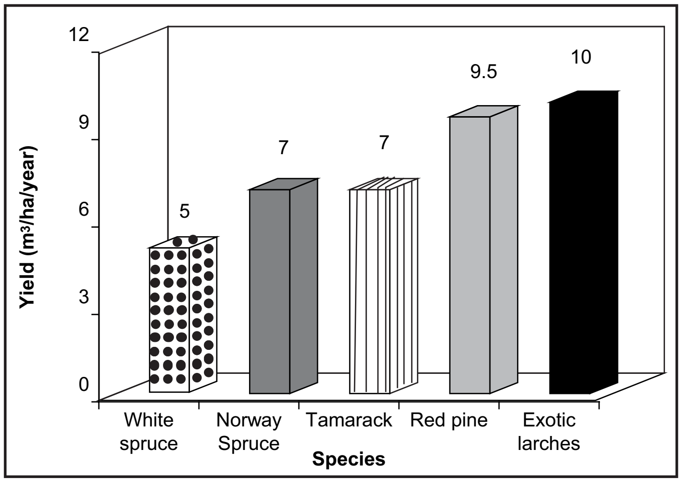

Fig. 1. Annual volume increment for softwoods grown in Eastern Canada (Ménétrier *et al.* 2005).

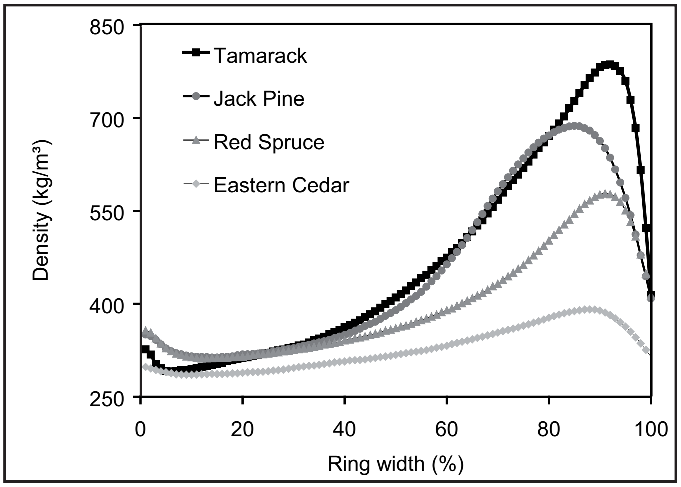

Fig. 2. Patterns of within ring density variation for four eastern softwoods.

Table 1. Ring density components and latewood proportion of plantation-grown tamarack and exotic larches (coefficients of variation % in parentheses)

Species	Ring density (kg/m³)	Earlywood density (kg/m³)	Latewood density (kg/m³)	Minimum density (kg/m³)	Maximum density (kg/m³)	Latewood proportion (%)
Tamarack	471 (13)	351 (10)	707 (10)	268 (13)	845 (10)	33 (40)
European larch	445 (11)	336 (10)	699 (20)	264 (14)	851 (13)	27 (41)
Japanese larch	435 (14)	337 (13)	693 (11)	268 (16)	812 (13)	27 (50)
Hybrid larch	382 (11)	330 (13)	630 (22)	258 (20)	744 (19)	16 (63)

Table 2. Physical and mechanical properties of plantation grown tamarack and exotic larches[1]

	Total Shrinkage (%)			Bending		Parallel Compression
	Radial	Tangential	Volumetric	MOE (GPa)	MOR (MPa)	Maximum Stress (MPa)
Tamarack- Stand	2.8-3.7	6.2-7.4	11.2-13.6	9.4-11.3	76-80	45-49
Tamarack- Plantation	-	-	13.2-14.0	5.9-7.2	35-71	34
European Larch	3.3-4.7	7.5-7.8	11.8-15.2	5.2-9.0	46-69	55
Japanese Larch	3.1	5.6	11.8	6.1-7.0	38-59	40
Hybrid Larch	3.4	6.3	13.4	6.2	55	40

[1] Data from Peck 1957; Jessome 1977; Isenberg 1980; Doucet *et al.* 1983; Beaudoin *et al.* 1989; Chui and MacKinnon-Peters 1995; Charron *et al.* 2003.

that tamarack has higher average density values than European, Japanese and hybrid larches. Earlywood density and latewood density followed the same trends as average density. Hybrid larch showed the lowest density mainly due to low latewood density compared to the other larches. These density averages are within the range of density reported earlier for larches grown in several north American locations (Isebrands et Hunt 1975; Doucet *et al.* 1983; ; Einspahr *et al.* 1983; Keith and Chauret 1988; Chui and MacKinnon-Peters 1995).

Patterns of radial variation of wood density for tamarack and exotic larches are shown in Figure 3. For all larches, the ring density of juvenile wood is high near the pith and decreases rapidly to reach a minimum in the transition zone leading into mature wood, where a slow but steady increase is observed. This pattern of variation is of type II (Panshin and de Zeeuw 1980) and is similar to those previously reported for plantation-grown European and Japanese larches (Doucet *et al.* 1983).

Within ring density profiles for tamarack and exotic larches are shown in Figure 4. These patterns show that tamarack has higher density profiles while the hybrid larch has the lowest density profiles. This illustration indicates a high differential between minimum density and maximum density for all larches. This differential is indicative of non uniformity of larch wood compared to other eastern softwoods (Figure 3). As discussed previously, the high differential in earlywood density and latewood density would be beneficial for appearance features. However, it results also in uneven moisture contents between earlywood and latewood for free and bounded water. Thus, drying between both woods is not uniform and more difficult and results in several defects mainly splits and checks. This differential leads also to machined defects, especially raised grain. When non-uniform wood is machined at moistures contents higher than 12%, the action of the knives forces the latewood bands into the softer earlywood bands on the flat grain surface. Subsequently, the compressed earlywood recovers and lifts the bands of latewood above the surface. This results in uneven surfaces which reduce the grade and usefulness of the finished product. Other impacts include non uniform finishing and gluing since the earlywood cells will absorb much more liquids than the latewood cells.

It is well known that plantation grown trees have lower mechanical properties than those grown in Natural stands. For Tamarack, Beaudoin *et al.* (1989) and Doucet *et al.* (1983) reached the same conclusion (Table 2). The Mechanical properties of exotic larches are lower than those of natural stand tamarack. However, from the available information, the wood mechanical properties of plantation grown larches are comparable since their ranges of variations overlap. Further studies and investigations are needed to confirm this trend.

The dimensional stability of the wood is among the most important properties for appearance applications such as wood flooring. The evolution of swelling as a function of wood moisture content is indicative to the dimensional stability of the wood in service. This relationship for Tamarack for tangential, radial and longitudinal directions is shown in Figure 5.

Processing properties

Logs from tamarack and exotic larches are used for lumber production and processed with other softwood species marketed under the S-P-F species grouping. Lumber production from larch wood is considered as relatively easy and could be processed at very high speeds (Benoît 1997). However, larch logs are more difficult to saw than most eastern softwoods (Balactinecz 1983) mainly because of the frequent presence of hard knots. The incidence of spiral grain in tamarack and exotic larches is very high. This results in strength reduction and an increase in the occurrence of twist in dried lumber. Compression wood in larch is present which could have a serious negative impact on lumber quality. Also, knots are frequent and contribute to strength reduction and lumber degrade. The wood density of tamarack is

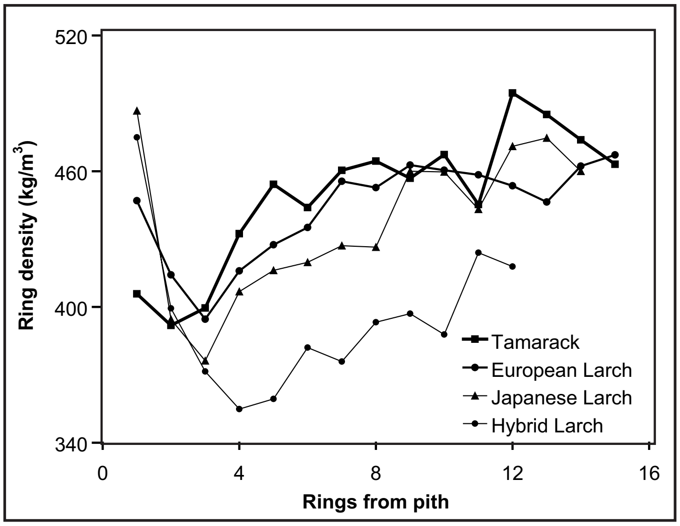

Fig. 3. Radial variation of wood density of tamarack and exotic larches.

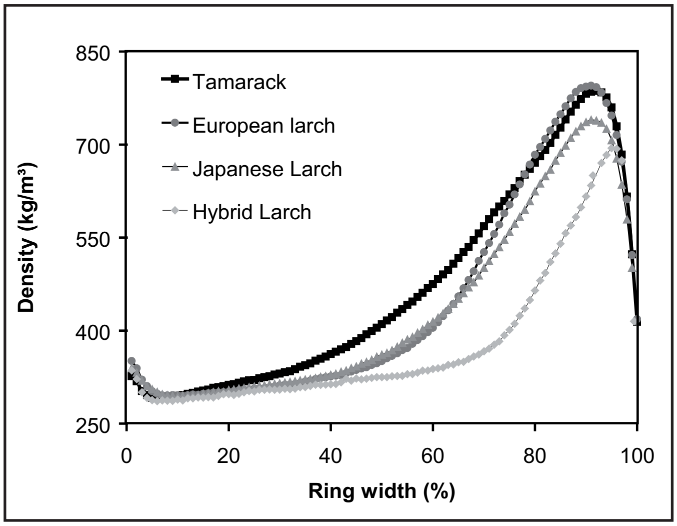

Fig. 4. Within ring density variations for tamarack and exotic larches.

higher than most of the other eastern Canadian softwood species and is not Uniform (Figure 1). The high variability in wood density along the variability in moisture content (Balactinecz 1983) contributes to variations in drying rate and hence, variability in final moisture content. The occurrence of compression wood and spiral grain in larch lead to excessive shrinkage compared to normal wood in the longitudinal direction and result in increased warping in the form of twist, bow, and crook. To prevent this kind of drying defect, proper stacking practices are recommended (Cech and Pfaff 1980). Some novel approaches are developed to dry larch wood including Vacuum drying, high temperature drying and drying under mechanical pressure. These approaches improved the drying quality and led to significant decreases in drying defects.

Larch is among the softwoods that are highly valued for appearance products due to its high density, good wearing properties, and colourful grain patterns. Larch lumber is used for furniture, flooring, paneling and wall coverings. Larch wood has better machining properties than eastern spruces and balsam fir (Lihra and Ganev 1999). However, the wood works with difficulty and requires considerable care in most operations for good results (Woods of the World 1996). Finishing properties are fair since the wood accepts a variety of finishes including paint, varnish, stain and finishing oils (Balactinecz 1983). Gluing larch wood with common adhesives gave good results. However, some difficulties are encountered due to larch's high extractive content. To avoid problems related to the presence of extractives in European larch, Benoît (1997) suggested drying the wood at temperatures in excess of 70 °C, then plan and sand the wood surfaces, glue immediately after machining and increase clamping pressure.

Surface and appearance properties

For appearance applications, dimensional stability, surface resistance and color stability are more important than structural properties. Hardness, a closely related property to wood density (Panshin and de Zeeuw 1980), is considered among the most important properties for

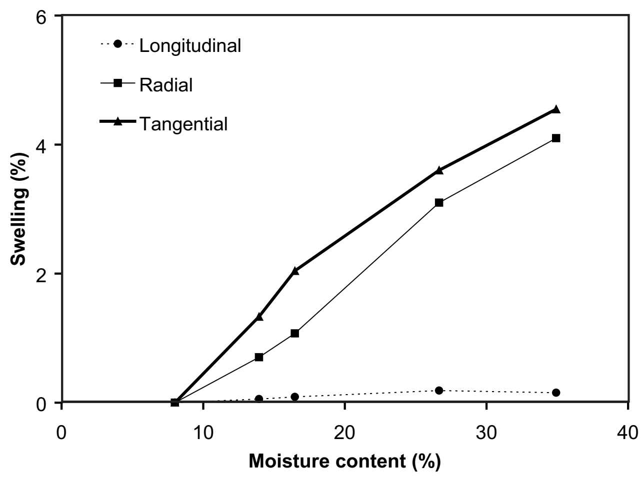

Fig. 5. Variation of swelling as a function of wood moisture content.

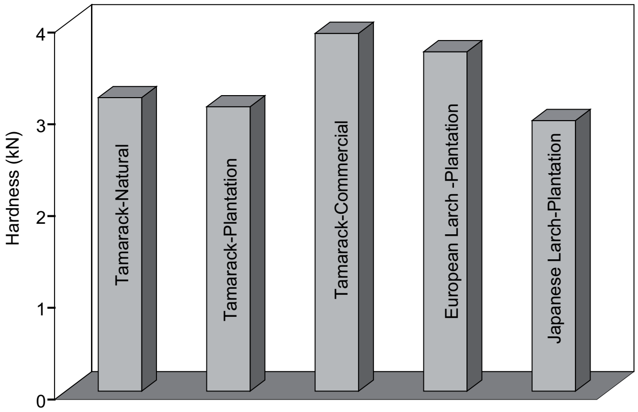

Fig. 6. Hardness of tamarack and exotic larches.

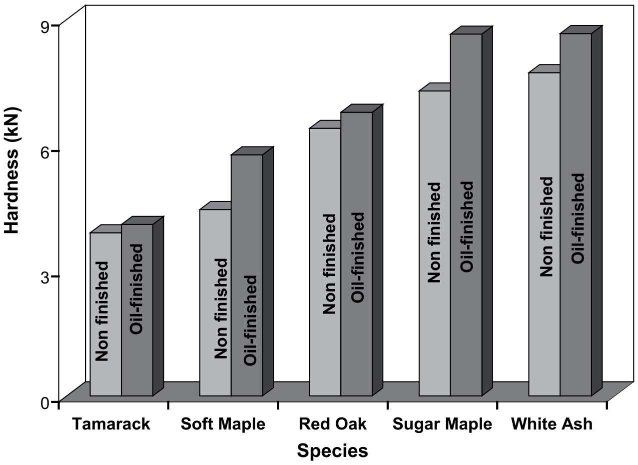

Fig. 7. Comparison of hardness values for different commercial flooring species.

appearance applications, especially flooring. Available information for wood hardness for tamarack and exotic larches is summarized in Figure 6. This illustration indicates that wood from plantation grown larches have comparable hardness values to that from natural stands. Hardness of commercial flooring from tamarack along with data for commercial flooring from different hardwoods are summarized in Figure 7. Surface finishing with oil (Figure 7) or varnish improves the wood hardness.

Abrasion resistance, referred herein as wear index, of tamarack and the main hardwood species used for flooring are shown in Figure 8. The wear index of tamarack is low compared to that of studied hardwoods. Oil finishing improved the wear index. For oil-finished tamarack it decreased to reach a value comparable to that of non-finished soft maple.

Color stability upon exposure to UV light is an important appearance quality attribute for both interior and exterior applications because it may produce significant color variation and thus, aesthetical damages. In Figure 9, we present the color change after 30 days exposure of four species (tamarack, European larch, red oak and white ash) to a weathering cycle which simulates interior long term exposure. The results are presented in terms of ΔE according to the CIELab system. A ΔE value inferior to 2 indicates a color change barely perceptible to the naked eye. Data from Figure 9 indicate that drying operation did not affect the wood color for the 4 studied species. However, after weathering a significant color change (ΔE> 20) is found. The ΔE values indicate that without finishing or protection coating the wood colour is not stable. Finishing wood with UV resistant systems will improve this property.

Conclusions

Because of its particular attributes, special care and special procedures should be applied for proper processing of larch wood. Wood properties of plantation grown larch are inferior to those from natural stand but belong to the same range of variation as plantation grown species from the same group. Surface characteristics of larch wood are inferior to those of high density hardwoods but are similar or to medium density hardwoods such as soft maple and birches. The weathering behaviour of larch wood is typical of that of natural wood and color stability as shown from the color change data. Finishing improves the surface and the appearance properties of larch wood.

Acknowledgements

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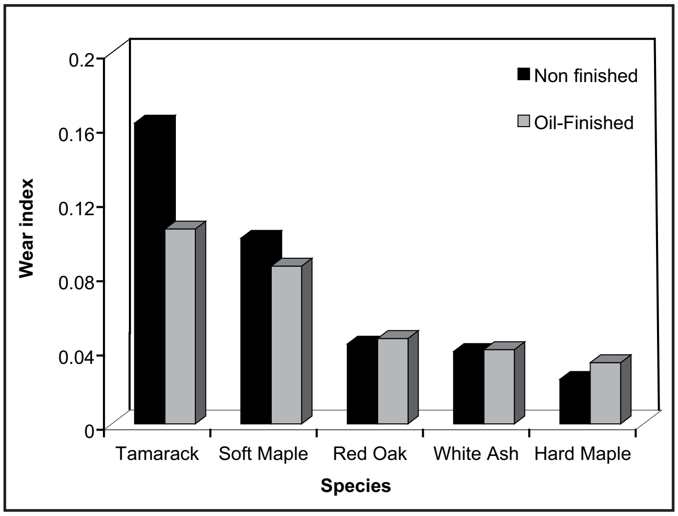

Fig. 8. Wear index for different commercial flooring species.

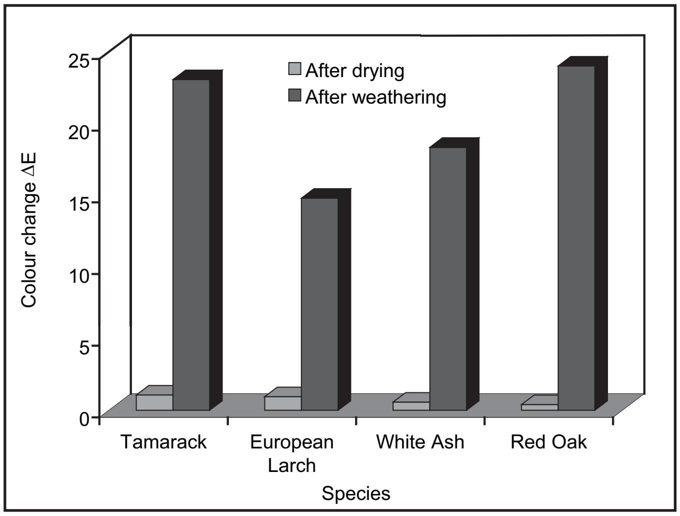

Fig. 9. Color change after dring and weathering of tamarack, European larch, ash and oak.

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Notes

Notes

The International progeny test of Siberian larch

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A major part of the boreal forest on the Eurasian continent is larch. Forty percent of the Russian forest area is dominated by larch (Milyutin and Vishnevetskaya 1995). Larch is also an important part of the forest in China, Korea, Mongolia, Tibet and Japan as well as in the mountain forests of eastern Europe and western North America.

Larch was one of the first natural introductions of forest trees in Scandinavia after the glaciations 9000 years ago but disappeared for unknown reasons already in prehistoric time (Kullman 1998). Larch was re-introduced by man into Scandinavia and other west European countries in the 18th century. The first larch introduced by man in Scandinavia was European larch (*Larix decidua* Mill.). Some 50 years later Siberian larch (*Larix sukaczewii* Dyl.) was introduced in northern Sweden and Finland.

The main reason for the early interest and introduction of larch in western Europe was primarily the wood quality. The timber of larch was early utilised as construction material for buildings, bridges, ships, etc and was appreciated for its mechanical strength and resistance to decay (Chubinski 2003, Jacques *et al.* 2002, Kharuk and Kulikova 1997). Larch has unique timber properties. In Russia larch is called "the oak of the north". Larch develops heartwood at an early age and the proportion of heartwood in the tree is bigger than in Scots pine at similar age and growing conditions.

The international research on genetics and breeding of larch has focused on European larch *(L decidua)*, Japanese larch *(L kaempferii)* and American western larch *(L occidentalis)*. However, the largest distribution and the largest gene pool of larch are to be found in Russia. Within this area there are at least four main species, several sub-species and natural hybrids of larch (Dyllis 1947). The early introduction of Siberian larch into Finland, Scandinavia and Iceland was *Larix sukaczewii*, the most western of the Russian larch species and originated probably from the manmade Raivola forest 50 km west of St Petersburg which was raised from seed sources partly from the Archangelsk area and partly from Ural (Redko 1995, Ilvessalo 1923). This genetic material has been propagated and used in northern Scandinavia for 200 years and is well adapted but has a limited genetic origin. In order to improve stem quality, timber properties and site adaptation to a wide range of habitats, access to the big gene pool in Russia has been discussed since the 1950s. In 1992 this discussion resulted in the beginning of a fruitful co-operation between two Scandinavian organisations: Swedish University of Agricultural Sciences, Umeå and Helgeland Forest Society, Mosjöen, one Japanese organisation Akita Prefectural University, and four Russian research institutes: V N Sukaczew Institute of Forests, Krasnoyarsk, Archangelsk State Technical University, Archangelsk, Bashkirian Botanical Garden Institute, Ufa and Institute of Biology, Magadan.

Collection of larch seed, representing the northern area of larch distribution in Siberia, was organized and accomplished by the Russian research institutes in the period 1996-2001. The project was financed by the Scandinavian and Japanese organisations. Seed was collected from 1005 individual trees origination from 45 stands and 17 regions from Kamchatka in the east to Archangelsk in the west. Figure 1 indicates the 17 regions where the larch seed was collected.

The first seedlings of the collected material were produced in 2002 and the first open pollinated family field experiments were established in Sweden and Norway 2003. Lars Karlman will present the first results of the Swedish test plots at this conference. Later this larch seed material was distributed to 14 different countries or provinces around the northern hemisphere and field experiments have been established in many countries. Table 1 gives the list of participants in this world vide progeny test series.

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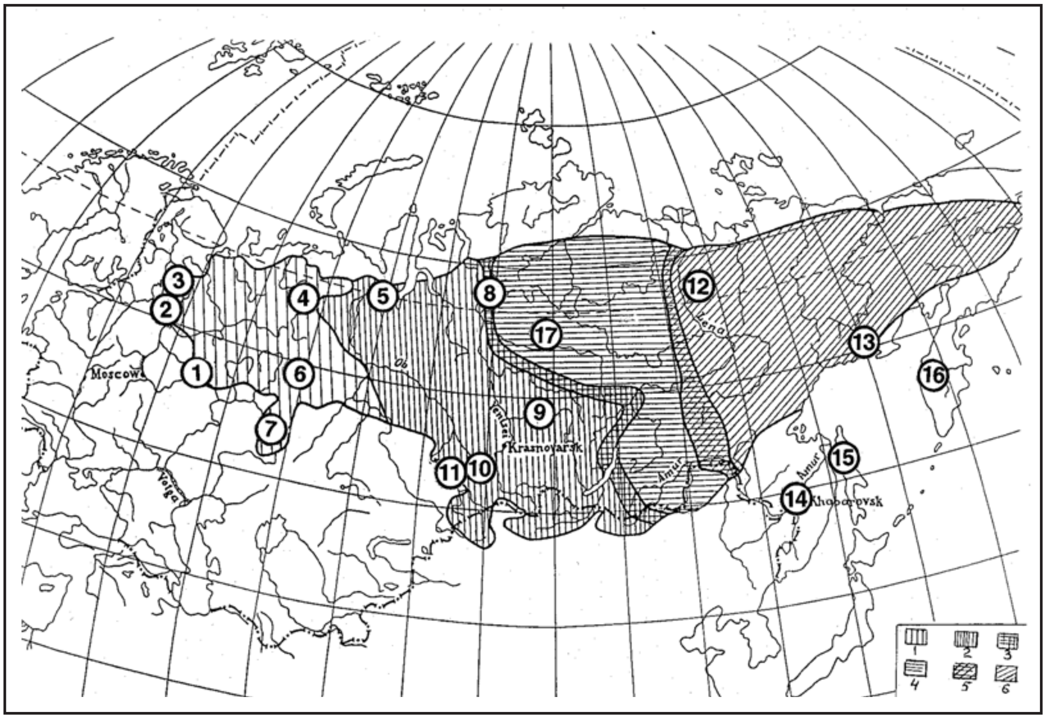

Fig. 1. Regions for collection of larch seed 1996-2001.

Table 1. Participating organisations in the International Progeny test of Siberian larch

Country	Organisation	Responsible scientist	Field tests established
Norway	Helgeland Forest Society, Mosjöen	Jaap Buitink, Gisle Skaaret	2003
Sweden	SLU; Umeå	Owe Martinsson	2003
Finland	METLA	Teijo Nikkanen	2005
Iceland	Iceland Forest Service	Thröstur Eysteinsson	2005
France	INRA	Luc Pâques	
Arkhangelsk	NFRI, Arkhangelsk	Natalia Demidova, Vladimir Barzut	2004
Komi	Komi Science Centre, Syktyvkar	Aleksey Fedorkov	2004
Japan	Kyushu University	Katsuhiko Takata	
China	Beijing University, Beijing	Shen Xi Huan	2004
Alaska	U S Forest Service, Alaska	John Alden	2004
British Columbia	B C Ministry of Forests	Barry Jaquish	
Minnesota	University of Minnesota	Andrew David	2003
Saskachewan	Agriculture and Agri-Food Canada, Indian Head, Saskatchewan	Bill Schroeder	2004
Quebec	Ministry of Forest, Quebec	Mr Lapointe, Martin Perron	

Notes

Notes

Siberian larch provenance trial on three locations in Sweden - Survival and height growth after four growing seasons

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Abstract

A co-operation between three Russian research institutes and two organisations in Scandinavia started in 1996. Seed was collected from 1005 individual trees distributed over 17 regions and 45 larch stands from Kamchatka in the east to Onega in the west. The project was the first international provenance trial of Siberian larch species. Seedlings were produced in 2002 and family field tests of all families including a total of 51 300 seedlings were established on three sites in Sweden in 2003. Test plantations of this material were also established in Norway, Iceland, Finland, France, northwest Russia (Komi and Archangelsk), China, Japan, Alaska, Minnesota, British Columbia, Saskatchewan, and Quebec.

The experimental material included four larch species; *Larix sukaczewii* Dyl., *Larix sibirica* Ledeb., *Larix cajanderi* Mayr. and *Larix gmelinii* Rupr. The average survival rate after four growing seasons on the three locations was between 60 and 75 %. The highest survival was in the northernmost site. The best adaptation in Sweden, so far has been shown by provenances originating from north western Russia (*Larix sukaczewii*). Material of the most northern and continental origin in north central Siberia did not perform well with high mortality and bad height growth. The fast growth of two of the eastern provenances of *Larix gmelinii* (Chabarovsk and Sachalin) is interesting. In the most southern site, these provenances demonstrated a mean height of 235 cm respectively 210 cm, which is 57-77 % above mean height of provenances in that site. The long term vitality and growth of these provenances must be followed up carefully before any recommendations can be done.

Key words

International family field test, Larch (*Larix sp*. Mill,), survival, growth rate, adaptation

Introduction

Larch (*Larix sp.* Mill.) is a major component of the boreal and alpine coniferous forests of Eurasia. The major distribution of larch is to be found in Russia where Siberian larch species covers about 40 % of forested area. The larches of Russia are of specific interest because of their huge area of distribution, great genetic variation and good stem quality (Martinsson 1992, Milyutin and Vishnevetskaya 1995, Putenikhin and Martinsson 1995, Abaimov *et al.* 1998). Larch also had a natural distribution in Scandinavia nine thousand years ago but disappeared for unknown reasons in prehistoric time (Kullman 1998). It was reintroduced by man in the 18th century and has for a long time attracted interest from forestry in Scandinavia (Martinsson 1992). The aim of this study was to find optimal ecologic adaptation and best productivity of Siberian larch for breeding and forestry in Sweden.

Material

In phase 1 of the project seed was collected in 17 regions and 1005 individual trees from Kamchatka in the east to Onega in the west (Figure 1). A detailed report on the seed collection was published in 2002 (Abaimov *et al.* 2002). Material for the Swedish and Norwegian field experiments were brought up in 2002 in Alstahaug nursery in central Norway. In addition to this, seed of the same material was distributed to 11 more participants resulting in a circumpolar participation of this progeny series of Eurasian larch species.

In the spring of 2003 1-year-old container-seedlings were planted on three main sites and ten small sites in Sweden. This report is describing the rate of survival and height growth in the three main sites after four growing seasons, *i.e.* October 2006.

The three main sites for field experiments in Sweden are located in Österbymo, Särna and Järvträsk. The geographic localities and properties of the three sites are explained in Table 1.

Each experimental locality was established as 60 sub-plots of 30x40 m on each site. 300 seedlings, representing one region, were planted in each subplot with spacing 2x2 m. One family (progeny of one selected tree) was represented by 3 or 4 seedlings in three replications on each site. Three commercial Swedish and Finnish seed sources and two Russian collections (No. 18-22) were included as standards in the experiments. For this preliminary report mean values are given for regions of origin, not for each family number. Each region sometimes also contains more than one provenance and covers a wide geographic area (Table 2).

Results

Österbymo

The survival rate between provenances was uneven on this site (table 3). The highest survival was shown by provenances from western Russia – *Larix sukaczewii*. Provenance No. 1 and the control Ivanov both originating from latitude 57 N had a survival rate over 90 %. Other provenances that have shown good adaptation so far were No. 2, 6, and No. 7. Also *Larix gmelinii*, provenance No. 14 and 15 had high rate of survival. The highest mortality was shown by northern and continental provenances; No. 4, 5, 9, 11, 12 and the control Irkutsk. The hybrid larch from Maglehem had a good survival on this site. The most fast growing larch trees are to be found in Österbymo. Mean height for the site after four growing seasons was 133 cm. *L. gmelinii* provenance No. 14 and 15 had the highest mean height of 235 cm respectively 210 cm. Their height development is only matched by the hybrid larch with a mean height of 207cm. The best of *L. sukaczewii* are the southern provenances whereas the northern provenances grow slow. No. 2 from Plesetsk and Lassinmaa had, however, both good survival and a reasonable height growth. *L. sibirica* showed bad adaptation with high mortality and intermediate growth.

Särna

On this experimental site at altitude 540 m, *L. sukaczewii* demonstrated the best survival. Best survival among provenances had Lassinmaa, provenance No. 3, No. 4 and Ivanov, all originating from western Russia. Another provenance that has a reasonable survival is *L. gmelinii* No.16 from Kamchatka. The over all mean height in Särna four growing season after planting is 66 cm. The four provenances with the highest mean height in Särna are No. 15 from Sachalin, No. 18 Lassinmaa, No. 14 Chabarovsk and No.1 Nishnij Novgorod. The very northern provenances No. 12 Yakutia and No. 5 Salechard demonstrated the lowest mean height. In Särna most provenances had high rate of mortality and the bad results might have been influenced by poor scarification.

Järvträsk

On this harsh site with temperature sum of 650 d.d. the survival rate among provenances was the most even. Highest survival was demonstrated by *L. sukaczewii*. Although the provenances of originated from such a wide range as Lat. N 52º- 66º, the survival did not differ so much between them. Best survival of provenances has No. 2, No. 3 and No. 5 and the controls Ivanov and Lassinmaa. *L. cajanderi* perform uneven, No. 12 has the lowest survival of all provenances on this site while No. 13 are among the best. The mean height for the site is 72 cm. Best height development is shown by *L. gmelinii* provenance No. 14 and 15. Among *L. sukaczewii*, provenances from Ivanov and Lassinmaa had the best height growth.

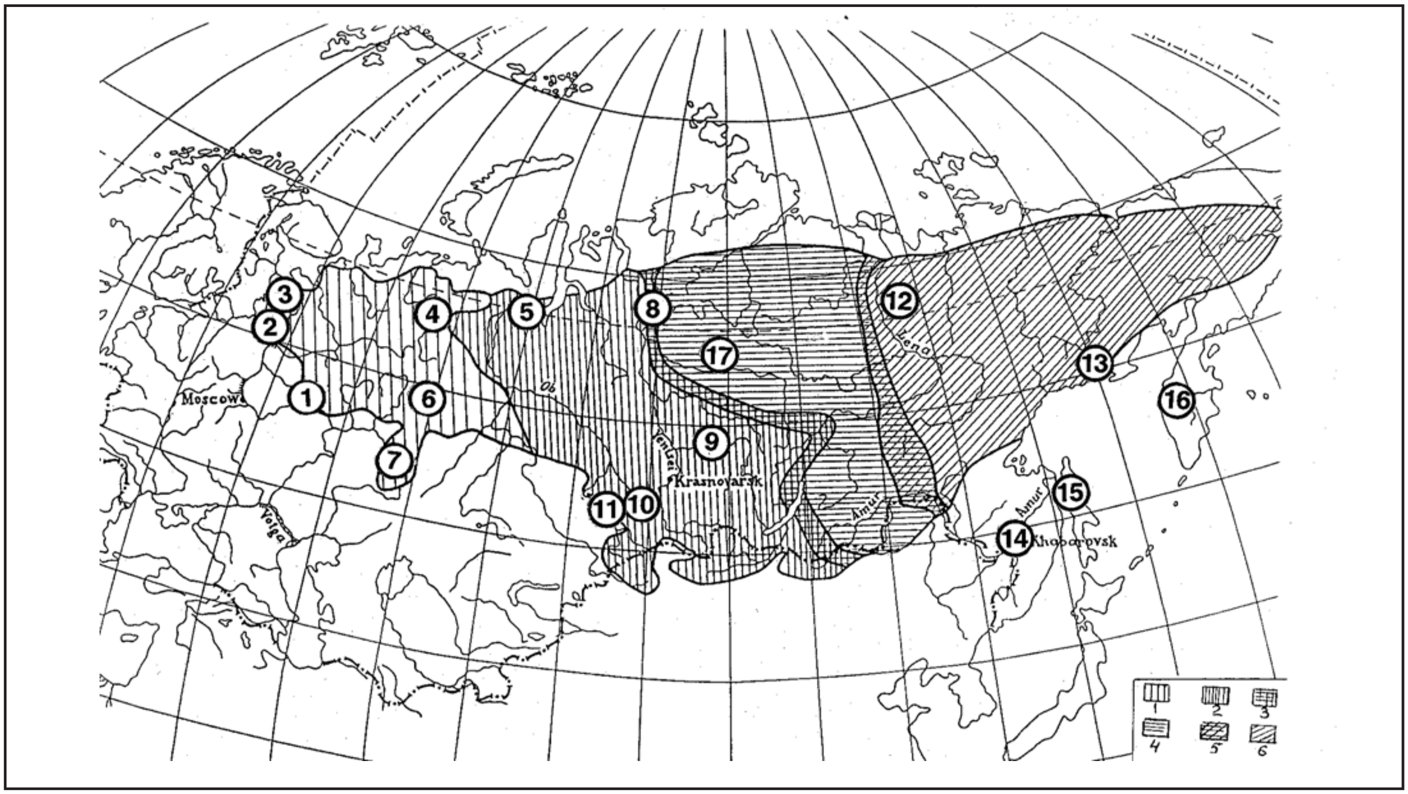

Fig. 1. 17 seed collection regions in Russia. The six different patterns are indicating larch species and their hybridisation zones 1= *Larix sukaczewii* Dyl., 2= *Larix sibirica* Ledeb., 3= *Larix x czekanowskii*, 4= *Larix gmelinii* Rupr., 5= *Larix gmelinii x Larix cajanderii*, 6= *Larix cajanderii* Mayr.

Table 1. Localities and site properties of the three main test sites in Sweden

Locality	Lat. N	Long. E	T-sum d.d.	Altitude, m	Topography	Soil
Österbymo	57° 47’	15° 37’	1160	250	Slight southern slope	Gravelly morain
Särna	61° 31’	13° 00’	725	540	Slight western slope	Stony morain
Järvträsk	65° 11’	19° 31’	650	410	Steep eastern slope	Sandy morain

Table 2. Seed sources and their origin in the three Swedish main test sites. Österbymo, Särna and Järvträsk

Region	Latitude N	Longitude E	Alt. m	No of selected plus trees	Species
1. Nishnij Novgorod	57° 30´	45° 10’	-	66	*Larix sukaczewii*
2. Plesetsk	62° 09´-63° 05´	40° 19´-40° 25´	100-120	63	*Larix sukaczewii*
3. Onega	63° 10´-64° 30´	38° 15´-43° 30´	110	7	*Larix sukaczewii*
4. Petchora	66° 00´	57° 48´	-	64	*Larix sukaczewii*
5. Salechard	63° 41´-66° 56´	65° 45´-66° 44´	40-130	60	*Larix sukaczewii*
6. Perm	55° 43´-57° 30´	55° 27´-60° 27´	160-480	72	*Larix sukaczewii*
7. Ufa	52° 13´-55° 45´	56° 58´-60° 07´	370-600	90	*Larix sukaczewii*
9. Boguchany	58° 39´	97° 30´	96-158	75	*Larix sibirica*
10. Novokuznetsk	52° 48´-54° 12´	87° 24´-88° 42´	Mtn.	60	*Larix sibirica*
11. Altai	50° 12´-50° 16´	87° 3´-87° 54´	1580-1630	78	*Larix sibirica*
12. Yakutia	66° 45´-66° 51´	123° 21´-123° 22´	70-90	60	*Larix cajanderi*
13. Magadan	59° 20´-59° 30´	148° 30´-152° 30´	60-100	75	*Larix cajanderi*
14. Khabarovsk	49° 08´-49° 12´	149° 00´	90-125	60	*L. gmelinii* var. *olgensis*
15. Sachalin	Ca 50°	Ca 142-143°	-	60	*L. gmelinii* var. *japonica*
16. Kamchatka	Ca 57-58°	Ca 160°	-	60	*L. gmelinii* var. *kamchatcka*
17. Evenkiya	64° 17´-64° 19´	100° 13´-100° 16´	270-310	75	*Larix gmelinii*
18. Lassinmaa Seed orchard (Fi)	62° 04´	25° 09´	107		*Larix sukaczewii*
19. Ivanov Seed stand (Ru)	Ca 57°	Ca 41°	-		*Larix sukaczewii*
20. Irkutsk Seed stand (Ru)	Ca 52°	Ca 104°	-		*Larix sibirica*
21. Maglehem Seed orchard (S)	55 °46	14 °10	20		*Larix eurolepis*
22. Östteg Seed orchard (S)	63 °48	20 °16	10		*Larix sukaczewii*

Table 3. Number of initially planted seedlings, survival and mean tree height after 4 growing seasons

Provenance	Österbymo			Järvträsk			Särna		
	N of seedlings	Survival %	Mean height, cm	N of seedlings	Survival %	Mean height, cm	N of seedlings	Survival %	Mean height, cm
1 Nishnij Novgorod	900	90,3	174	900	79,7	69	900	57,6	80
2 Plesetsk	900	89,9	121	900	81,4	70	900	69,8	70
3 Onega	524	83,4	99	524	85,3	76	524	78,6	65
4 Petchora	900	19,3	35	900	78,2	56	900	75,0	43
5 Salechard	900	15,6	60	900	83,6	56	900	65,8	37
6 Perm	900	87,7	139	900	73,6	62	900	62,2	77
7 Ufa	900	88,6	162	900	81,8	64	900	64,3	76
9 Boguchany	900	32,1	118	900	72,2	75	900	63,1	63
10 Novokuznetsk	900	56,1	124	900	75,0	81	900	61,9	68
11 Altai	900	2,6	76	900	61,1	42	900	55,9	44
12 Yakutiya	900	0	0	900	36,3	47	900	20,8	23
13 Magadan	900	77,7	142	900	84,4	89	900	62,8	65
14 Chabarovsk	900	88,3	236	900	77,2	111	900	35,8	84
15 Sachalin	900	86,4	210	900	79,0	104	900	65,1	102
16 Kamchatcka	900	63,4	113	900	79,4	65	900	71,6	66
17 Evenkia	-	-	-	-	-	-	473	30,3	38
18 Lassinmaa	900	86,9	115	885	83,4	79	900	84,7	78
19 Ivanov	823	93,8	167	900	84,9	83	900	71,7	76
20 Irkutsk	900	26,5	112	448	67,2	60	450	44,2	68
21 Maglehem	900	82,3	207	448	60,2	78	450	22,9	88
22 Östteg	900	82,7	109	900	81,3	76	900	59,0	64
Total	17547	62,1	133	16705	75,7	72	17197	59,8	65

Discussion and conclusions

The best adaptation in Sweden, so far has been shown by provenances originating from north western Russia *(Larix sukaczewii)*. Provenances of this species had the highest survival on all three sites. Material of the most northern and continental origin did, however, not perform well with high mortality and poor height growth. This trend is seen also in northern and continental provenances of *L. sibirica* and *L. cajanderi*. The reasons for the bad survival of these provenances could be a too early start of growth in the spring and that the young trees thereby get damaged by spring frost. The bad height growth in northern provenances could be explained by early growth cessation due to changed light conditions in the new environment (Simak 1979). Comparing the nearby related *L. sukaczewii* and *L.sibirica*, the results from this early evaluation are in accordance with previous provenance research done in Finland and Iceland where the former species clearly shows better adaptation than the more continental provenances of *L. sibirica* (Eysteinsson and Skúlason 1995, Hagman 1995).

L. cajanderi was represented by two distinct separated provenances, one from Yakutia with an extreme cold continental climate and one from Magadan with a more maritime climate. Of these two provenances, the one from Magadan showed much better adaptation. Provenance No.12 from Yakutia had high mortality on all sites and was extinct from the most southern site. *L. gmelinii* has demonstrated good adaptation so far in Järvträsk and outstanding growth in Österbymo, but has high mortality in Särna.

It is still however early in the evaluation stage and these trends need to be confirmed during longer periods. The fast growth of two of the eastern provenances of *Larix gmelinii* (Chabarovsk and Sachalin) is interesting. In the most southern site, these provenances demonstrated a mean height of 235 cm respectively 210 cm. The long term vitality and growth of these provenances must be followed up carefully before any recommendations can be done.

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Notes

Western Larch Tree Improvement in British Columbia

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Western larch (*Larix occidentalis* Nutt.) forms a small, but locally important component of British Columbia's forest resource. The B.C. western larch tree improvement program began in 1987 with the goals of (1) developing a constant supply of seed that has been bred to produce trees with improved volume and wood quality, while maintaining acceptable levels of adaptation and genetic diversity; and (2) conducting research to guide the breeding and seed production program. The program is based on a system of recurrent selection for general combining ability. Program components in the first cycle of breeding include: 1) phenotypic selection in natural stands in two seed planning zones: East Kootenay (EK) and Nelson (NE); 2) wind-pollinated progeny testing; 3) soil-based seed orchards; and 4) controlled crossing to produce full-sib families for second-generation selection. Since 1987, a total of 609 parent trees have been selected in wild stands in the two seed planning zones. Grafts of all parent trees have been established in breeding orchards and clone banks near Vernon. Wind-pollinated families of 607 of these selected parents are included in four series of progeny tests on 17 sites, the oldest of which is 16-years-old. In spring 1990, two clonal first-generation seed orchards were established in Vernon. Both orchards began producing collectable seed crops in 1998 and since then have produced large crops annually. The largest seed crop (181.6 kg/ 400 lbs) was collected in 2002 and produced enough seed to grow 40.9 million seedlings. This represented about a six year supply of improved seed. In 2007, nearly 70 percent of BC's western larch planting program originated from improved seed orchards with Genetic Worths (average parental breeding values) for tree volume ranging from 15 to 30 percent. Second-generation crossing for each zone uses a combination of small (4 x 4 tree) disconnected factorials for high breeding value (BV) parents and single-pair mating for lower BV parents. This crossing will result in 243 full-sib families for testing and reselection in each zone. To date, about 65% of the crossing is complete. We expect to begin establishing full-sib second-generation progeny tests in 2009. On-going western larch research projects focus on seed orchard flowering, crown management, seed orchard mating systems, range-wide provenance testing, realized genetic gain testing, and studies of molecular genetic variation.

Notes

Peculiarities of sex and somatic embryogenesis in vitro of Siberian larch

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Abstract

The great perspectives are opened by using methods of culture in vitro for studying regularities of embryological processes and especially embryogenesis. The cultivation of megagamethophytes, immature embryos and annual vegetative shoots of Siberian larch on MS and *SG medium added with 2.4-D and 6-BAP in different concentration allowed to management embryo morphogenesis and embryo dormancy, to cause cell proliferation and to get somatic embryogenesis. Inoculation of explants in stages of proembryo and early embryo promoted the development of nonembryogenic callus in micropilar part of megagamethophytes. Somatic embryogenesis in vitro begins from development of embryonal-syspensor mass (ESM) at cotyledons stage of zygotic embryo. ESM consisted from long embryonal tubes and small embryonal cells. Somatic embryos developed as a result of transfer on MS medium with reduced hormone concentration. Morphogenesis of somatic embryos has the properties of zygotic embryos. Zygotic embryogenesis begins from one initial cell – zygote and somatic embryo arises from own initial (or initial group cells) from pericolumn. Zygotic and somatic embryos pass the same stages of development: proembryogeny, early embryogeny and late embryogeny. Zygotic and somatic embryos consist of three cell groups: embryonal mass, embryonal tubes and suspensor cells. Cultivation of annual vegetative shoots allowed to get ESM and embryo-like structures. Structure of embtyoids differed from zygotic and somatic embryos. Success of somatic embryogenesis is due to genotypes of donor trees. Explants of larch trees with high reproductive potential produce ESM and somatic embryos more actively.

Introduction

The great perspectives are opened by using methods of culture *in vitro* for studying regularities of embryological processes and especially embryogenesis. Somatic embryogenesis in conifer species is a potential tool for the mass propagation of improvement forest trees. It also provides an ideal experimental process for investigating the mechanisms of expression of totipotency in plant cell. Embryogenic cell lines maintain their competence for a long period of time and give rise to genetically uniform and normal plant populations. Somatic embryogenesis has been reported for a few larch, spruce and pine species (Hakman, Fowke, 1985; Durzan, Gupta, 1987; Klimaszewska, 1989; von Aderkas *et al.*, 1990; Letu *et al.*, 1994; Klimaszewska, Cyr, 2002; etc).

The Larix genera species characterized by fast growth and good wood quality and wide ecological plasticity. Larch is the main forest forming species in Russia. Larch species occupies 263 mln/ha (38,5 percent of Russian forests). But Siberian larch (*Larix sibirica* Ledeb.) characterized by irregular crop on the many-year cycles and low seed quality (Iroshnikov, 2004; Koropachinski, Milutin, 2006). Besides, brachiblasts of Siberian larch trees damaging by larch bud midge. As a result crop and seed yield is reduced (or absolute absent). Somatic embryogenesis of Siberian larch has still not been reported.

The aim of work was to initiate the somatic embryogenesis from Siberian larch embryos and annual vegetative shoots and to make a cytological analysis of this process.

Materials and Methods

The 30-40-years old trees of Siberian larch, growing at the Arbooretum of the Institute of Forest (Krasnoyarsk) as well in natural conditions in Khakasiya and Baikal Lake coast were as the objects of the study. Material of study was megagamethophytes, immature embryos and annual vegetative shoots of resistant to damage by larch bud midges of Siberian larch.

Megagamethophytes dissected out from the seed, annual vegetative shoots clean off needles and surface sterilized with 0,1% hypocloride Na for 20 min, followed by 10 % hydrogen peroxide for 10 min and then rinsed three times with sterile distilled water.

Induction of embryonal mass (EM)

Megagamethophytes and immature embryos were cultured on basal MSG medium (Becwar *et al.*, 1990), containing 1.45 gl^{-1} glutamine and 0.1 gl^{-1} inositol. Medium were supplemented with 30 gl^{-1} sucrose, 2 mgl^{-1} 2,4-D, 1 mgl^{-1} BA and solidified with 7gl^{-1} Difco Bacto-agar.

Annual vegetative shoots of Siberian larch subject to cold treatment on ½ MS medium without hormones. After 3 days they were subcultured on ½ MS medium added with 2.4-D and 6-BAP in different concentration.

Five - ten megagamethophytes, embryos, segments of vegetative shoots were cultured in each flask on 20 ml of induction medium and incubated in darkness at 25° C. The presence of EM was determined by morphological observations and cytological test.

Proliferation of EM

The EM obtained were transferred to proliferation medium that consisted of MSG supplemented with 2 mgl^{-1} 2,4-D, 0,5 mg^{l-1} BA , 20 mgl^{-1} sucrose and 4 gl^{-1} Gelrite. The cultures were incubated in darkness at 25° C and subcultured every 2 weeks.

Maturation of somatic embryos

After subcultivation on proliferation medium, pieces of EM were transferred into MSG medium without growth regulators but containing 1% activated charcoal, 15 gl^{-1}

sucrose and solidified with 4gl^{-1} Gelrite. Flasks with EM pieces were placed under cool-white fluorescent light (16 / h / photoperiod). After 7 days the EM were transferred onto MSG medium with the following combinations of growth regulators and 30 gl^{-1} sucrose:

0 mgl^{-1} IBA + 0 mgl^{-1} ABA

0,2 mgl^{-1} IBA + 5,3 mgl^{-1} ABA

0,2 mgl^{-1} IBA + 15 mgl^{-1} ABA

The growth regulators (IBA and ABA) and L- glutamine were filter sterilized and added to cooled media after autoclaving.

Statistical analysis

Six pieces of EM were used in each experiment and each experiment was repeated three times. Reliability of data obtained was tested by ANOVA.

Cytological analysis

For cytological analysis explants were fixed by Karnua solution (96% alcohol and acetic acid). Preparations were stained with hematoxylin using Heidenhein procedures and safranin solution.

The preparations were examined by KS300 Imaging system microscope (Germany). Cytological changes were registered by Nicon camera (Japan).

Results and Discussions

Larch embryogenesis in vivo. There are 3 stages of embryo development in Larix as another species of *Pinaceae* family. Proembryogeny - all the stages before the elongation of suspensor; early embryogeny – all the stages after the elongation of suspensor and before the establishment of root generative meristem and late embryogeny - establishment of the polar meristems (root and shoot) and the development of embryo following this events (Singh, 1978; Tretyakova, 1990; von Aderkas *et al.*, 1991).

Proembryogenesis begins from division of zygote nucleus. The four free nuclei descend to the base of archegonium, and become arranged in layer. The nuclei divide with vertical spindles to give rise to eight nuclei arranged in two layers of four each: pU and pE.The two tiers go through the internal division as usual, to form four tiers. The lowest two comprise the E group (E and E1), followed by S and U tier. The cells of the S tier do not elongate but go through an aborrtive meristematic activity. This tier is designated as dS (disfunctional suspensor). The upper four cells of the E group elongate to function as a suspensor (embryonal suspensor) and the lower four cells form the embryonal mass.

Early embryogenesis begins with elongation of suspensor cells and pushing out of embryonal cells in corrosion cavity of megagametophyte. The E layer continues to divide and ptoduces more cells of the same types. Several distinct phenomena like elongation of the suspensor, cleavage of the embryonal mass, initiation of polyembryony, formation of the young embryonal mass, proliferation of the suspensor cells and formation of “rosette embryos” occur during early embryogeny stage. Elongation and development of suspensor cause cleavage of embryonal units. The secondary suspensor is of three types: embryonal suspensor, suspensor tubes and embryonal tubes. Embryonal tubes are group of unequally elongating cells derived from proximal end of embryonal mass. As the embryonal mass enlarges the secondary suspensor becomes very massive, due to the elongation of the proximal cell of the mass. In Larix one of embryonal cells outgrows the others and gives rise to embryonal cells (embryonal mass). The secondary suspensor is composed of embryonal tube cells produced by the embryonal mass. So, larch zygotic embryogenesis shows a phenomenon called delayed cleavage polyembryony, in which one part of embryo tier in the proembryo outgrows the rest.

Late embryogeny is characterized by the development of distinct meristems. The first to appear is the root meristem. The proximal portion of the embryo matures before the shoot meristem. The cells of the rounded portion of the embryonal mass divide in all directions. The surface cells contribute to the suspensor at the edges, and later to the root cap, but the cells in the middle eventually become the focal zone, which will give rice to the embryonic shoot apex. Cotyledons develop from epidermal layer and the shoot apical meristem develops between the cotyledons.

Somatic embryogenesis from immature Siberian larch embryos. Somatic embryogenesis *in vitro* begins from development of embryonal-syspensor mass (ESM). The heist response was obtained for immature Siberian larch zygotic embryos from the stage where no cotyledonary primordia were visible to the stage where embryos had cotyledonary primordia. 70 % of such embryos formed embryonal mass in 5-10 days (Fig.1).

In these period somatic cells of Siberian larch immature zygotic embryos in the area of pericolumn began to elongate and turn into mononuclear and binuclear

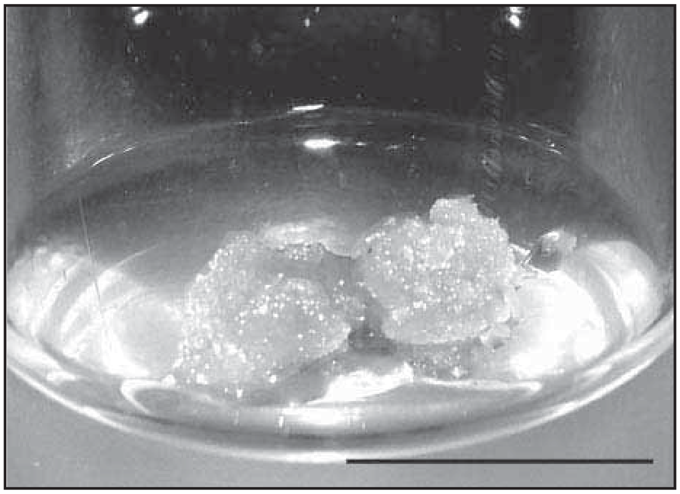

Fig. 1. Formation of embryonal mass from immature zygotic embryos of Siberian larch (20 days of culturing). Scale is 1 cm.

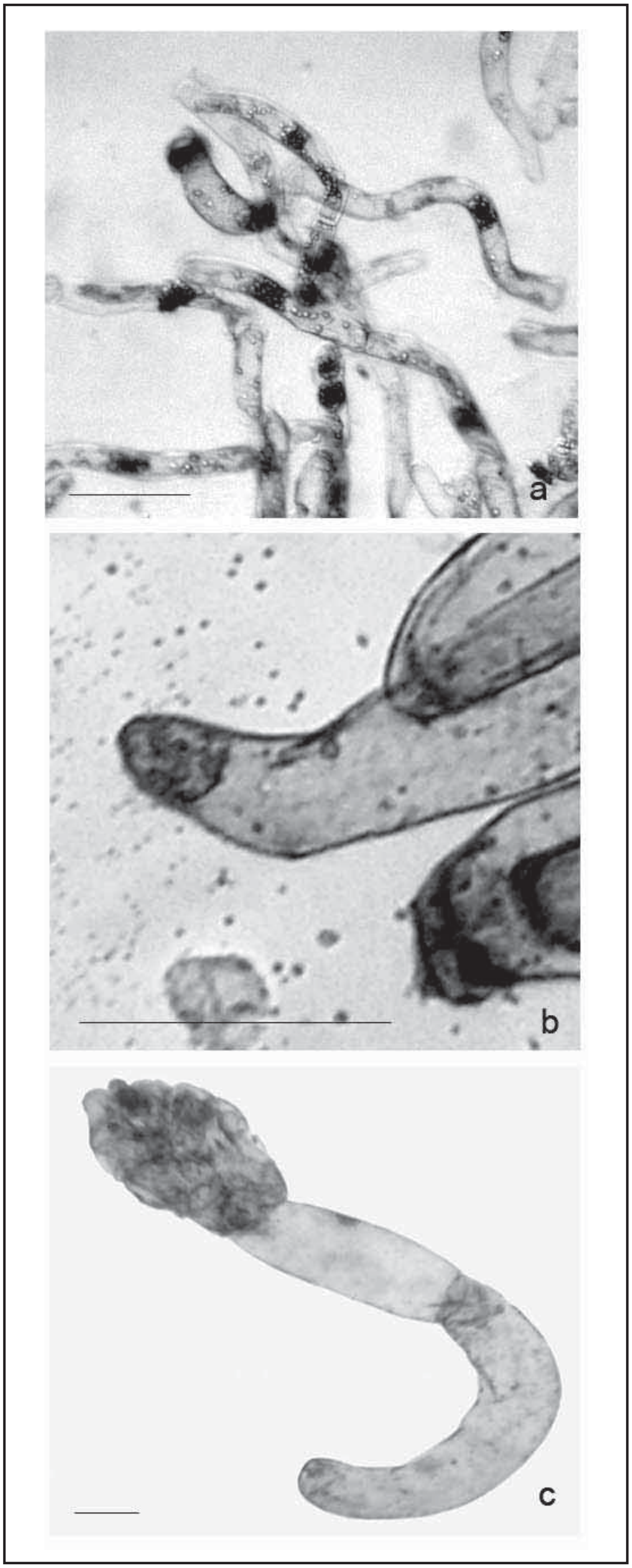

Fig. 2. Development stages of Siberian larch embryonal mass: formation of embryonal tubes (a), asymmetric division of embryonal tubes at formation of embryonal cell (b), clusters of embryonal cells and embryonal tubes (c). Scale is 50 mkm.

“embryonal tubes” (Fig.2a). Asymmetric division of embryonal tubes leads to the formation of a small cell at one of the poles (Fig.2b). Few-celled aggregates have formed in 15 days and were composed of elongated cells and adjacent to them group of small meristematic cells (Fig.2c).

Somatic embryos developed as a result of transfer on the MS medium with reduced hormone concentration. The somatic embryos at globular stage with well-formed suspensor were obtained from immature zygotic embryos in 20 days. They became visible at the surface of embryonal mass in 2 months of culturing. Torpedo-like Siberian larch somatic embryos with suspensor and beginning of differentiation were obtained in 2 months (Fig. 3).

Maturation of somatic embryos was more successful on MSG medium with 15 mgl^{-1} ABA (Fig. 4).

Somatic embryogenesis from mature Siberian larch embryos. It was found two types of explant development at mature embryos culturing. At first type – the development of cotyledons and nonembryogenic callus was observed (30%). At this type no any somatic embryos were found.

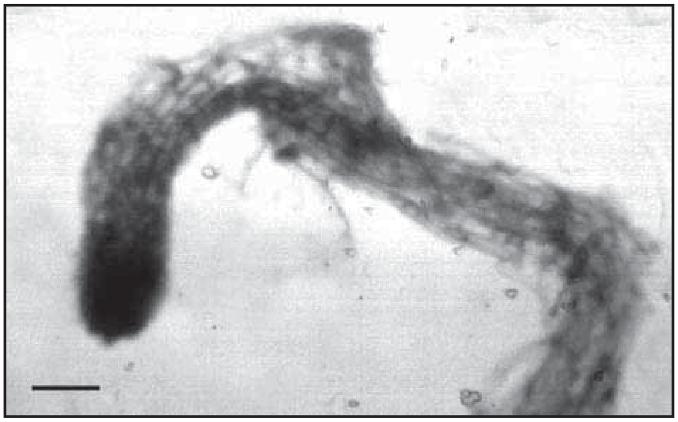

Fig. 3. Siberian larch somatic embryo. Scale is 100 mkm.

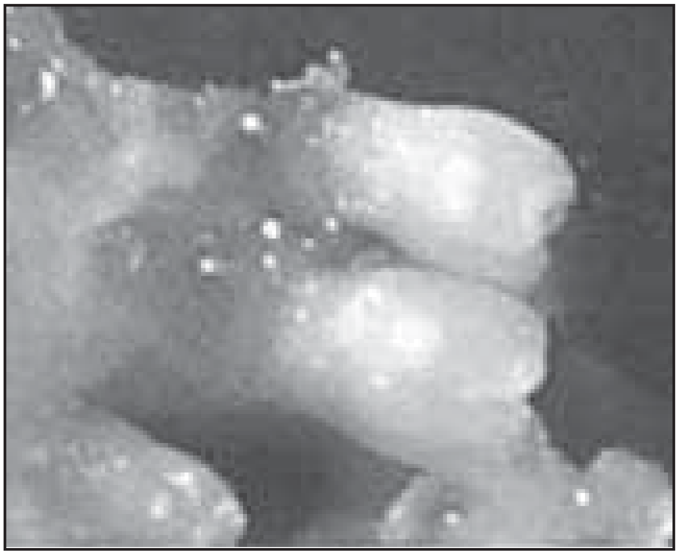

Fig. 4. Maturation of Siberian larch somatic embryos.

At second type the cotyledons were not develop and embryonal mass formation was occurred (46%). Torpedo-like somatic embryos were obtained from mature zygotic embryos in 4 months of culturing.

It was observed that genotypes of Siberian larch have different capacity to somatic embryogenesis (Fig.5).

Somatic embryogenesis from annual vegetative shoots. Embryogenic callus was obtained from segments of vegetative shoots of Siberian larch in 15-20 days of culturing. Cytological analysis show cell conglomerates consisted of two types of cells – elongated and isometric. In the 30 days embryo-like structures appeared. They include embryonal mass cells, embryonal tubes cells and suspensor-like cells (Fig.6).

Thus, morphogenesis of somatic embryos has the properties of zygotic embryos. Zygotic embryogenesis begins from one initial cell – zygote and somatic embryo arises from own initial (or initial group cells) from pericolumn

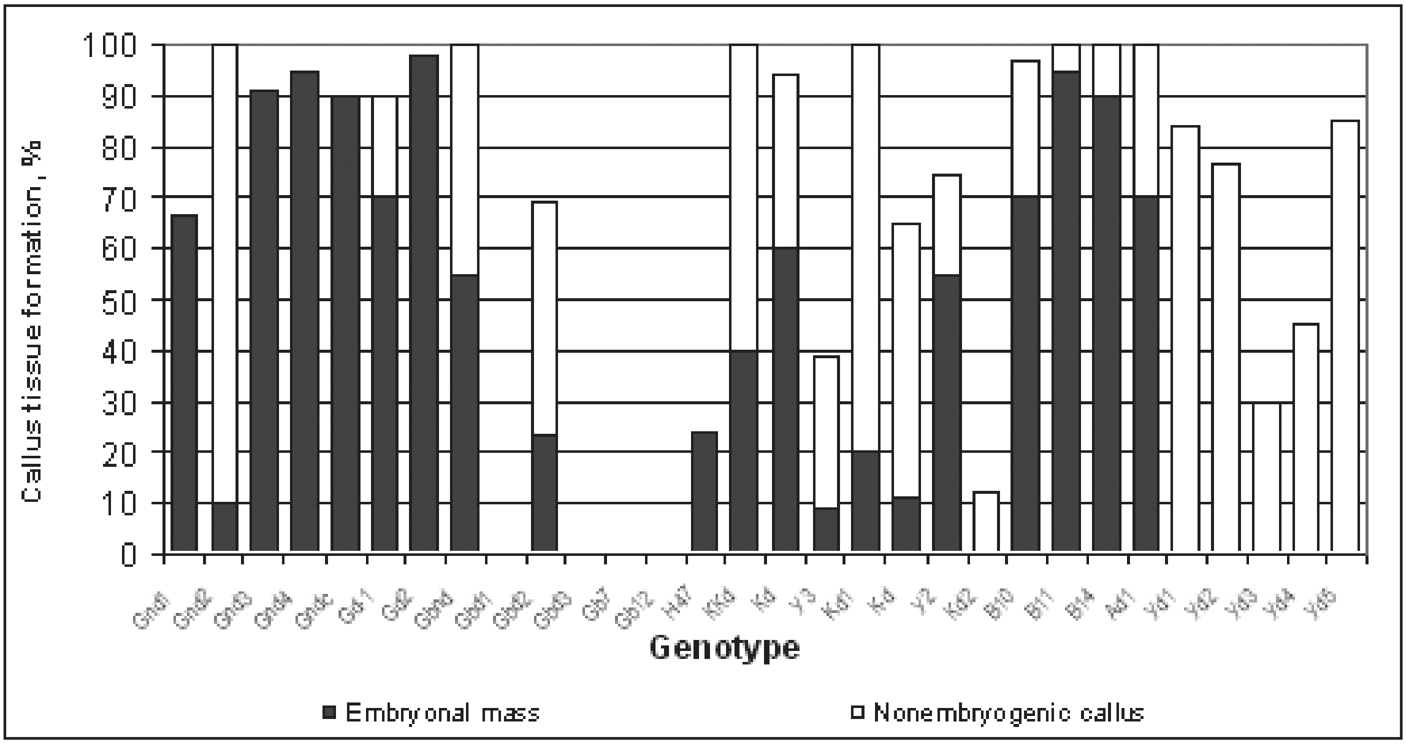

Fig. 5. Formation of embrygenic and nonombryogenic callus from immature zygotic embryos of different Siberian larch genotypes.

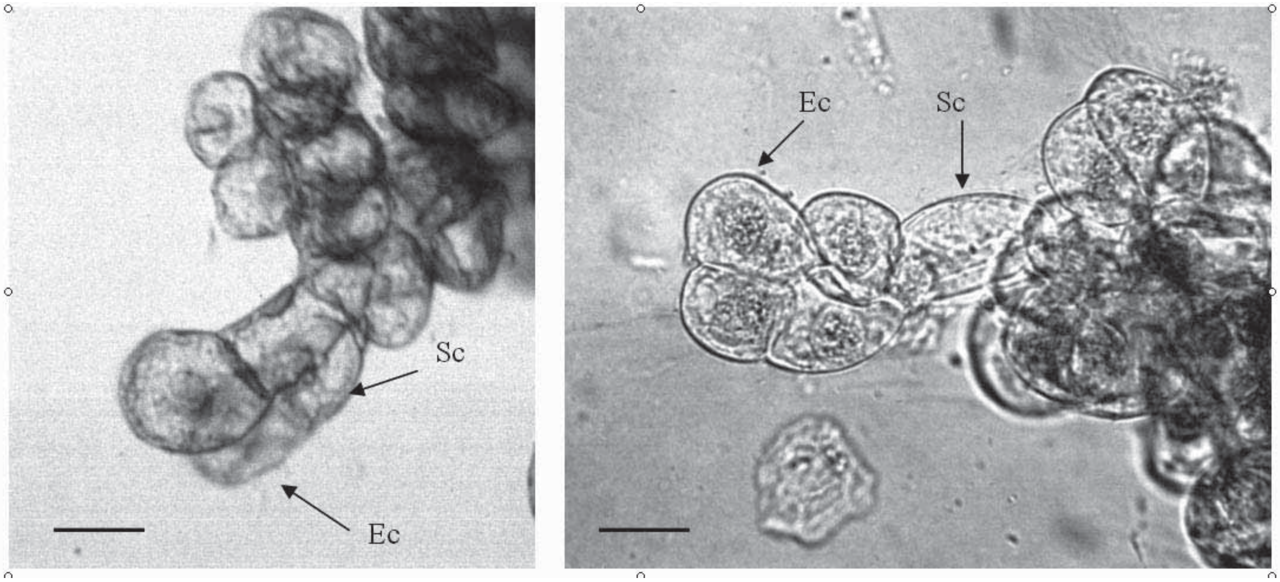

Fig. 6. Embryo-like structures obtained from annual vegetative shoot segments of Siberian larch (Ec - embryonal cell, Sc - suspensor cell). Scale is 60 mkm.

too. Zygotic and somatic embryos pass the same stages of development: proembryogeny, early embryogeny and late embryogeny. Zygotic and somatic embryos consist of three cell groups: embryonal mass, embryonal tubes and suspensor cells. Cultivation of annual vegetative shoots allowed to get EM and embryo-like structures. Structure of embtyoids was differented from zygotic and somatic embryos. Success of somatic embryogenesis is due to genotypes of donor trees. Explants of trees with high reproductive potential produce EM and somatic embryos more actively.

In general cells of generative and vegetative organs of Siberian larch can proliferate in culture in vitro and form ESM, somatic embryos and embryoids.

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Notes

Nursery Propagation and Establishment Best Practice for Larch in Britain

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We summarise the results of a series of experiments, including a synthesis of data from previous Forest Research publications, to provide information regarding recent advances in propagation of hybrid larch using cutting production systems. Furthermore, we provide an update on best practice for the successful establishment of hybrid, European and Japanese larch. Recent research has shown that cutting stock plant management, cutting rooting environment and the correct manipulation of subsequent nursery practices can increase the yield of marketable nursery stock. These improvements in vegetative propagation are described. A series of experiments studied physiological attributes (using plant quality tests) and early forest performance of cuttings and transplants of Japanese, European and hybrid larch. Physiological attributes included root growth potential (RGP), root membrane function (REL), shoot and root frost hardiness, and resistance to cold-storage. The optimum time for handling, cold storage and planting is described. In the nursery, undercutting and wrenching improved subsequent survival. In the field, planting position and weed control influenced growth and survival. The use of cuttings did not lead to changes in height or diameter growth when compared to transplants of the same genetic origin. Rooting pattern showed some dependence upon propagation technique and the implications for future stability are discussed.

Key words: Larch, propagation, physiology, out-planting, rooting

Notes

Verification of the morphological distinction method of hybrid larch seedlings by DNA markers

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Abstract

The hybrid larch between Kuril larch (*Larix gmelinii* var. *japonica*) × Japanese larch (*L. kaempferi*) is an important afforestation species in Hokkaido, Japan, because of rapid juvenile growth, straight stems, and resistance to bark gnawing by mice. In order to produce desirable hybrid seedlings, the precise distinction of these seedlings is important. However, continuous variations in morphological and phenological traits occur across *L. gmelinii* var. *japonica* × *L. kaempferi* (GL) and *L. gmelinii* var. *japonica* × *L. gmelinii* var. *japonica* (GG) seedlings. A clear distinction of hybrid seedlings obtained by open pollination is very difficult. Therefore, we used DNA markers to verify and improve the morphological and phenological distinction method. We collected seeds from a common hybrid seed orchard and a single maternal clone seed orchard (SMC). The DNA analysis revealed that the hybridization rates of individual ramets in the 1-year-old seedlings in the common hybrid seed orchard were 5.8–39.4% (mean, 23.2%). On the other hand, in the SMC orchard, the hybridization rates of individual ramets were 71.9–89.1% (mean, 83.5%). The reasons for the high hybridization rates in the SMC orchard might be relative abundance of Japanese larch pollen and self-incompatibility of the Kuril larch. The seedling height, number of sylleptic branches, and the day of terminal bud set in both orchards differed significantly between the GG and GL seedlings. However, continuous variations in all traits were observed across the GG and GL seedlings. A clear distinction of hybrid seedlings based on only one phenotypic trait was impossible. The contamination rates of the GG seedlings in the hybrid seedlings discriminated by the phenotypic methods considerably differed among the families and the seed orchard types, and the contamination and hybridization rates before discrimination were negatively correlated. These results suggest that enhancing pollen production of the Japanese larch and eliminating low hybridization clones of the Kuril larch are important to improve the quality of hybrid seedlings.

Introduction

The Japanese larch *(Larix kaempferi)* is one of the most important afforestation species in the boreal temperate zone of Japan because of its ability to grow rapidly. However, the Japanese larch has some disadvantages for afforestation in northern Japan, including its sensitivity to bark gnawing by mice and the fact that its stems are not straight. Therefore, various interspecific crosses of this genus, especially those distributed in East Asia, have been tested in Hokkaido, an island in the north of Japan. This study showed that the F1 hybrid of Kuril larch *(Larix gmelinii* var. *japonica)* × Japanese larch exhibited the most desirable traits, i.e., rapid juvenile growth, straight stems, and resistance to mice. In order to produce desirable hybrid seedlings, the precise distinction of hybrid seedlings is important because the stand volume of *Larix gmelinii* var. *japonica* is half that of the hybrid at 30 years of age. However, variations in morphological and phenological traits are continuous across *L. gmelinii* var. *japonica* × *L. kaempferi* (GL) and *L. gmelinii* var. *japonica* × *L gmelinii* var. *japonica* (GG) seedlings. Clear distinction of hybrid seedlings generated from open pollination is very difficult. Recently, reliable diagnostic molecular markers have been developed (Acheré *et al.* 2004, Zhang *et al.* 2004), but their usage in the production of hybrid seedlings is impractical. Therefore, we applied DNA markers to verify and improve the morphological and phenological distinction method.

A new type of clonal hybrid seed orchard was established in Hokkaido after alternately cutting a Japanese larch seed orchard (Kuromaru *et al.* 2003) that consists of a single Kuril larch clone and multiple Japanese larch clones. In this new type of hybrid seed orchard with a single maternal clone (SMC), the hybridization rate was expected to be higher than that in the common hybrid seed orchard because the Kuril larch is self-incompatible, and the pollen of the Japanese larch is relatively more abundant than that of the Kuril larch.

The objectives of this study were to verify and improve distinction methods based on the morphological and phenological traits of hybrid larch seedlings by using DNA markers in the 2 types of hybrid seed orchards.

Materials and Methods

1) Study seed orchards

This study was carried out in 2 types of hybrid seed orchards in Hokkaido Prefecture (43°N, 143°45′E). The first type was a common hybrid seed orchard that was established in 1974. Currently, the tree heights of *L. gmelinii* var. *japonica* and *L. kaempferi* are approximately 18 and 16 m respectively. The second type was the SMC hybrid seed orchard that was established as *L. kaempferi* seed orchards in 1961, and in 1994 and 1996, the *L. kaempferi* trees were alternately removed and a single clone of *L. gmelinii* var. *japonica* was replanted. The Nakashibetsu-3 clone was planted in the 1st, 4th, and 5th blocks and the Nakashibetsu-5 clone, in the 2nd and 3rd blocks. Among hybrid larches, the growth and wood quality of the progenies derived from these 2 clones are superior. Currently, the tree heights of *L. gmelinii* var. *japonica* and *L. kaempferi* in the SMC hybrid seed orchard are approximately 6 and 22 m respectively.

2) Plant materials and determination of seedling phenotype

In 2004, seeds were collected from 5 *L. gmelinii* var. *japonica* clones in the common seed orchard and from 2 *L gmelinii* var. *japonica* clones in the SMC orchard (Table 1). The Nakasizuka-5 clone was included in both orchards. In May 2005, we sowed the seeds in the nursery at the Hokkaido Forestry Research Institute (43°28′N, 141°85′E). In August, we marked the seedlings with small plastic tags for individual identification. In May 2006, we replanted the seedlings at 36 trees/m^2.

The seedling height and number of sylleptic branches were measured in October 2005 and 2006. The day on which the bud set developed on the terminal shoot was also investigated every week in September 2005 and 2006.

3) DNA analysis

Needle tissues were sampled for DNA analysis in early October 2005. To discriminate the hybrid seedlings, we used 2 chloroplast DNA markers that showed interspecific polymorphism in *L. gmelinii* var. *japonica* and *L. kaempferi*. One DNA marker was cpDNA *Il-TaqI* that amplifies the rbcL region with primer *Il* and is digested by the restriction enzyme *TaqI* (Acheré *et al.* 2004). The other was a paternally inherited microsatelite marker (Zhang *et al.* 2004).

Results and Discussion

1) Hybridization rate

Based on previous studies on phenotypic data, the average hybridization rate for a particular year was considered to be approximately 60%. However, the hybridization rate in 2004 was expected to be lower than that in an average year because the pollen fecundity of the Japanese larch was poor. According to the DNA analysis, the hybridization rates of individual ramets in the 1-year-old seedlings in the common orchard were 5.8–39.4% (mean, 23.2%), which was low, as expected. On the other hand, in the SMC orchard, the hybridization rates of individual ramets were 71.9–89.1% with a mean of 83.5%.

The variation in the hybridization rates was larger between the seed orchards than within them. Ennos and Qian (1994) and Acheré *et al.* (2004) suggested that the factors influencing hybridization rate were pollen fecundity and the differences in flowering times between the 2 species. In the SMC orchard, the tree heights of *L. gmelinii* var. *japonica* and *L. kaempferi* differed greatly, and they were 6 and 22 m respectively. Therefore, the pollen fecundity of *L. kaempferi* might be much higher than that of *L. gmelinii* var. *japonica*. This might be the main reason for the high hybridization rates observed in the SMC seed orchard. In addition, self-incompatibility is also an important factor influencing the hybridization rate because the *Larix* sp. is a self-incompatible species, GG cross did not produce any seeds in the SMC orchard.

Our results showed that for hybridization, the pollen fecundity was more important than the differences in the flowering times between the 2 species. However, the hybridization rates varied between the clones in the common orchard. Moriguchi *et al.* (in preparation) also revealed that the hybridization rates of seeds in the common orchard differed significantly from those of the clones. In order to increase the hybridization rate, it is necessary to remove clones exhibiting low hybridization rates from the common orchard.

Table 1. Plant materials and hybridization rates of 1-year seedlings

Hybrid seed-orchard type	Name of clone	Ramet no.	No. of progeny seedlings analyzed	Hybridizaion rates of 1-year seedlings
Common	Kabaoka-484	2	118	0.127
	Nakashibetu-5	5	109	0.394
	Nakashibetu-660	3	118	0.305
	Rubeshibe-28	4	104	0.058
	Rumoi-5	1	116	0.267
			Average	0.232
Single maternal clone	Nakashibetu-3	2	120	0.800
		4	118	0.805
		29	117	0.863
		33	120	0.858
		35	96	0.854
		69	120	0.800
	Nakashibetu-5	44	119	0.891
		61	120	0.717
		64	110	0.891
			Average	0.830

2) Phenotypic differences between GL and GG seedlings

Compared to GG seedlings, GL seedlings are generally larger, have more branches, and develop terminal buds later. The mean heights (±standard deviation) of 1-year-old GG and GL seedlings from the common orchard were 8.3 ± 2.9 and 9.6 ± 3.4 cm respectively. Number of branches in the 1-year-old seedlings from the common orchard showed an L-shaped distribution with the mode at the 0th branch class in both the GG and GL seedlings. By September 9, bud sets were observed in 85% 1-year-old GG seedlings (n = 434) and 25% GL seedlings (n = 131). The mean heights (±standard deviation) of 2-year-old GG and GL seedlings from the common orchard were 38.5 ± 12.3 and 48.5 ± 14.1 cm respectively. The mode of the sylleptic branches in the 2-year-old seedlings in the common orchard was at the 0th branch class in GG and at the 4th–7th branch classes in GL. By September 19, bud sets were observed in 83% 2-year-old GG seedlings (n = 390) and 20% GL seedlings (n = 119). All traits investigated in the 1- and 2-year-old seedlings from the common orchard differed significantly between the GG and GL seedlings. However, the variations in all traits were continuous across the GG and GL seedlings. Clear distinction of hybrid seedlings based on only 1 phenotypic trait was impossible.

GG and GL seedlings from the SMC orchard showed similar differences as those from the common orchard, except with regard to the number of sylleptic branches in the 2-year-old seedlings. The mode of the sylleptic branches in the 2-year-old GL seedlings from the SMC orchard was at the 0th branch class but at the 4th–7th branch class in the 2-year-old GL seedlings from the common orchard.

3) Verification of the distinction method

In commercial nurseries, hybrid seedlings are discriminated via 2 steps based on morphological and phenological traits. The first step is to measure the height 1–year–old seedlings, and the second step is to measure the height and branches of 2–year-old seedlings. In our study, according to the DNA analysis of 509 seedlings from the common orchard, 119 were hybrid seedlings. When the hybrid seedlings were defined as those with a height ≥ 6 cm at 1 year and ≥ 30 cm at 2 years, and ≥ 1 sylleptic branches at 2 years (criterion 1), 90 of the 119 hybrid seedlings determined by the DNA analysis were correctly discriminated as hybrid, and 127 of 390 GG seedlings in the common orchard that were determined by DNA analysis were incorrectly discriminated as hybrid (Fig. 1). When we changed the criterion of the 2-year-old seedling height to 40 cm and 50 cm (criterion 2 and 3, respectively), the number of GG seedlings incorrectly discriminated as hybrid decreased to 85 and 40, respectively. However, the number of seedlings correctly discriminated as hybrid also decreased to 83 and 58, respectively. The contamination rates of GG seedlings in the seedlings discriminated as hybrids based on morphological and phenological traits were 58.5% for criterion 1, 50.6% for criterion 2, and 40.8% for criterion 3. In practice, these contamination rates were very high. The rate of incorrect discrimination for both GL and GG were the lowest with criterion 4 (height of a 1-year-old seedling ≥ 6 cm, that of a 2-year-old seedling ≥ 30 cm, and the bud set in a 2-year-old seedling bud after September 19) However, adaptation of the day of bud set is difficult in commercial seedling production because the duration of observation is limited. The contamination rates of GG seedlings in the hybrid seedlings were considerably different among families (e.g., 23–83% for criterion 2), and the contamination and hybridization rates before discrimination were negatively correlated. Therefore, selection of the maternal clone effectively decreases the contamination rate.

For the SMC orchard, the contamination rate of GG seedlings after discrimination based on the phenotypic criterion was remarkably low (4%) because the hybridization rate was high even before discrimination (83.5%). Seedlings in this orchard had fewer sylleptic branches than those in the common orchard. The mode of sylleptic branches in the hybrid seedlings of the SMC orchard was 0. This caused incorrect discrimination when the number of sylleptic branches was included as a discrimination criterion. In criterion 3, of a total of 764 hybrid seedlings determined using DNA analysis, 474 were correctly discriminated as hybrid. On other hand, when the number of sylleptic branches was not used as criterion 3, 573 hybrid seedlings were correctly discriminated as hybrid, and the incorrectly discriminated GG seedlings increased from 14 to only 26. This result showed that the criteria for hybrid seedling discrimination should be modified when seedlings are derived from a few specific clones.

Pâques *et al.* (2006) reported that incorrect discrimination of European and Japanese larch hybrid seedlings changed from 2% or less to 27% depending on the orchard seedling lot. They suggested that the reasons for incorrect discrimination might be errors or biases in trait evaluation and the insufficient discriminating power of some of the selected traits or molecular markers. All traits investigated in this study differed significantly between the GG and GL seedlings. However, variations in these traits were continuous across the 2 species. The main reason for the incorrect discrimination in this study was probably the insufficient discriminating power of the selected traits. There are mainly 2 types of incorrect discriminations: (1) GL seedlings discriminated as GG seedlings due to bad growth and (2) GG seedlings discriminated as GL seedlings due to good growth. The former is not a problem because seedlings exhibiting bad growth should be removed regardless of whether they are GG or GL. In the latter case, however, we have to be cautious because the future wood production of GG trees is generally worse than that of GL trees, despite the rapid seedling growth of GG. The contamination rates of GG seedlings remarkably decreased as the hybridization rates increased. Therefore, seed orchard improvements that enhance hybridization, such as establishing SMC seed orchards or removing low hybridization clones in common hybrid orchards, are important to improve the quality of hybrid seedlings.

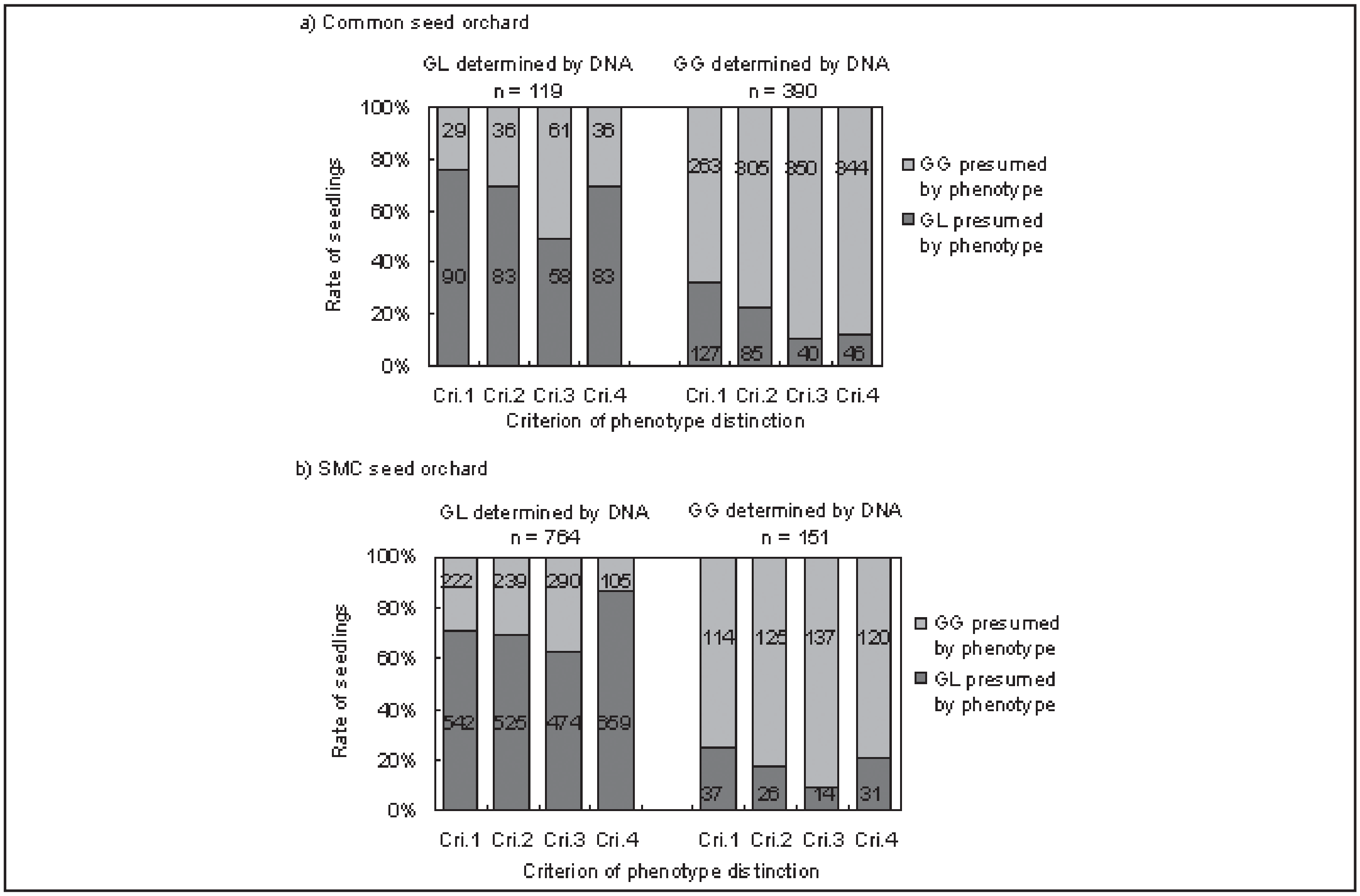

Fig. 1. Rate of seedlings discriminated by their phenotype in (a) the common seed orchard and (b) SMC seed orchard. The 4 left bars indicate hybrid crosses (GL: L. gmelinii var. japonica × L. kaempferi), and the 4 right bars indicate within-species crosses of L. gmelinii var. japonica as determined using DNA analysis. The light and dark gray areas indicate hybrid and within-species crosses discriminated based on the phenotype. The numbers in the bars indicate the number of seedlings of each category.

Criterion of phenotype distinction:

Cri.1: 1-year seedling height ≥ 6 cm, 2-year seedling height ≥ 30 cm, and 2-year seedling sylleptic branch number ≥ 1.

Cri.2: 1-year seedling height ≥ 6 cm, 2-year seedling height ≥ 40 cm, and 2-year seedling sylleptic branch number ≥ 1.

Cri.3: 1-year seedling height ≥ 6 cm, 2-year seedling height ≥ 50 cm, and 2-year seedling sylleptic branch number ≥ 1.

Cri.4: 1-year seedling height ≥ 6 cm, 2-year seedling height ≥ 30 cm, and 2-year seedling bud set after September 19.

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Notes

Notes

Annex/Annexe

Notes

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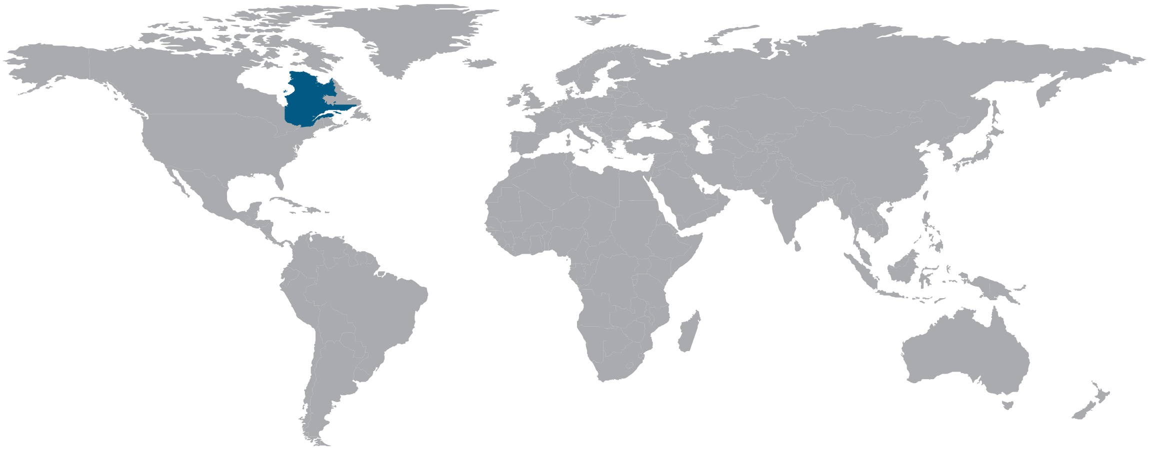


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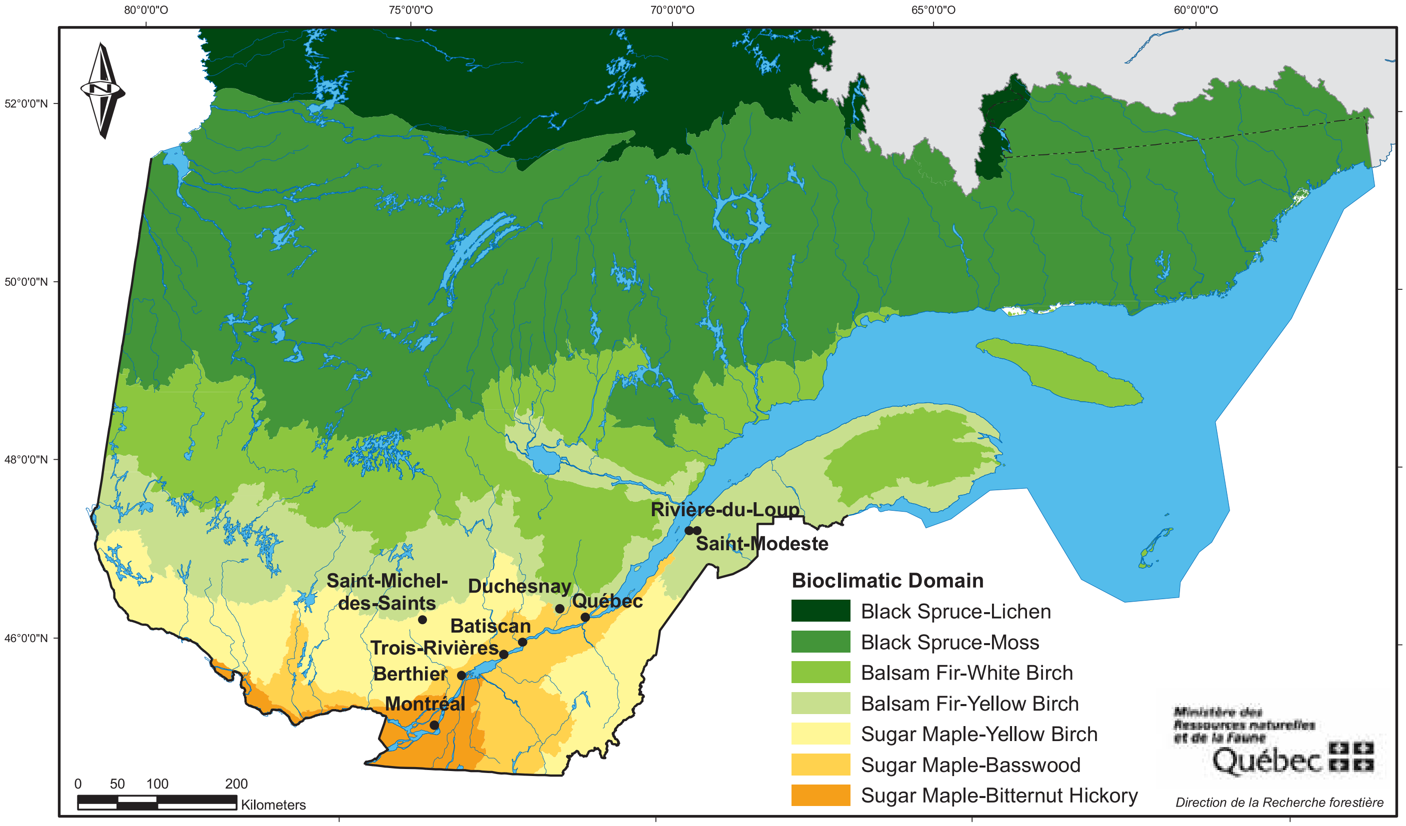
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Duchesnay
Québec
Batiscan
Trois-Rivières
Berthier
Montréal
Bioclimatic Domain
Black Spruce-Lichen
Black Spruce-Moss
Balsam Fir-White Birch
Balsam Fir-Yellow Birch
Sugar Maple-Yellow Birch
Sugar Maple-Basswood
Sugar Maple-Bitternut Hickory
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