

LATVIJAS VALSTS MEŽZINĀTNES INSTITŪTS "SILAVA"  
LATVIAN STATE FOREST RESEARCH INSTITUTE "SILAVA"

# MEŽZINĀTNE

## **Speciālais izdevums**

LVMI Silava sadarbībā ar SNS un IUFRO  
organizēto starptautisko konferenču  
**KOPSAVILKUMU KRĀJUMS**

## ***Special issue***

## **ABSTRACTS**

*for international conferences  
organised by LSFRI Silava  
in cooperation with SNS and IUFRO*

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## KOPSAVILKUMU KRĀJUMS

*Special issue of scientific proceedings "Mežzinātne" No 25(58)'2012*

### ABSTRACTS

*of international conferences organised by LSFRI Silava  
in cooperation with Nordic Forest Research Co-operation Committee (SNS) and  
International Union of Forest Research Organisations (IUFRO)*



norden

Nordic Forest Research  
Co-operation Committee (SNS)



### OSCAR un IUFRO (WP 3.05.00)

Ziemeļvalstu un Baltijas valstu mežsaimniecības tehnoloģiju zinātniskā  
konference OSCAR 2012, 2012. gada 24.–26. oktobris, Rīga, Latvija

*The Nordic Baltic conference on forest operations – OSCAR 2012,  
October 24–26, 2012, Riga, Latvia*

### AdapCar un IUFRO (WP 2.02.00)

konference "Adaptācijas un pielāgošanās ģenētiskie aspekti meža veselības, koksnes  
kvalitātes un biomasas ražošanas kontekstā", 2012. gada 3.–5. oktobris, Rīga, Latvija  
conference "Genetic aspects of adaptation and mitigation: forest health, wood quality and  
biomass production", October 3–5, 2012, Riga, Latvia

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conference “*Genetic aspects of adaptation and mitigation: forest health,  
wood quality and biomass production*”,  
October 3–5, 2012, Riga, Latvia

Jansons Ā.	Priekšvārds	139
	Preface	140
	Agenda	142
Stenlid J.	Mechanisms of resistance to pests and diseases	145
Hamberger B., Ohnishi T., Hamberger B., Seguin A., Bohlmann J.	Development of a genomic toolbox to dissect localisation, regulation and biosynthesis of conifer diterpene resin defences	146
McKinney L.V., Nielsen L.R., Hansen J.K., Kjær E.D.	Presence of natural genetic resistance in common ash ( <i>Fraxinus excelsior</i> ) to <i>Hymenoscyphus</i> <i>pseudoalbidus</i>	147
Stener L.-G.	What is going on regarding genetic studies of pest resistance on broad-leaved species in Sweden?	148
Karlsson B.	Breeding for resistance against root rot in Norway spruce	149
Persson T.	Genotype by environment interaction for survival, growth and <i>Cronartium</i> resistance in northern Scots pine	150
Bräuner-Nielsen U.	Genetic variation in resistance to <i>Dreyfusia</i> <i>nordmanniana</i> in Nordmann Fir	151
Swedjemark G., Almquist C.	Development of screening methods for resistance to <i>G. abietina</i> in Scots pine clones to be applied in existing breeding programmes	152
Jansons Ā., Baliuckas V.	<i>Pinus sylvestris</i> populations and families resistance to <i>Heterobasidion annosum</i> and possibilities for breeding in Latvia	154
Hansen J.K., Harding S., Ravn H.P.	Role of resistance to green spruce aphid in Sitka spruce for growth and survival	155
Krutovsky K.V.	Forest Genomics for Mitigating Climate Change and Breeding Resilient Trees	156
Danusevicius D., Baliuckas V., Kavaliauskas D., Stulginskis A.	Norway spruce provenance variation in precocious flowering and tolerance to needle pest <i>Nematodus abietinus</i>	157

Steffenrem A.	Epigenetics in the forest – practical observations and considerations	159
Kjær E., Hansen L.N., Hansen J.K.	Adaptive potential of nine native woody species in relation to climate change	160
Zeps M., Jansons Ā.	Selection of hybrid aspen for future climatic conditions	161
Jansons Ā., Baumanis I.	Gain from use of Scots pine seed orchard material	162
Siliņš I., Šmits A., Jansons Ā.	Use of weather change indicators in pest outbreak prediction	163
Pulkkinen P., Varis S.	Between and within species differences of adaptive traits – how to select the best trees for future?	165
Jansons Ā., Voronova-Petrova A., Zeps M., Kānberga-Siliņa K., Ruņģis D.	Genetic diversity of bread planting material in comparison to natural regeneration: case studies from Latvia	167
Puriņa L., Sisenis L., Krišāns O., Jansons Ā.	Improved genetic material for a future climate – new species – new genotypes (case study of <i>Pinus contorta</i> in Latvia)	168
Dutkowski G.W., Kerr R.J., Tier B., Li L., Costa e Silva J., Ivković M., Potts B.M., McRae T.A.	Next generation breeding values	169
Ruņģis D., Veinberga I.	Molecular genetic tools for research and breeding of forest tree species	170
Wu H.X.	How should we weight wood properties and quantity in forest tree breeding programs?	171
Harju A., Venäläinen M., Haapanen M.	Associations between growth and resistance-related heartwood traits in Scots pine	172

## STENDA REFERĀTI

### POSTERS

Zhao T., Krokene P., Hu J., Christiansen E., Björklund N., Långström B., Solheim H., Borg-Karlson A.-K.	Fungal inoculation and methyl jasmonate application induced highly variable terpene accumulation in Norway spruce	174
Voronova-Petrova A., Jansons Ā., Ruņģis D.	Retrotransposon-like sequences expressed under various stressors in Pine genome shows different similarity level to known mobile genetic elements	175
Gaile I., Ruņģis D.	<i>In vitro</i> expression of the recombinant TLP protein from <i>Pinus sylvestris</i> and study of its antimicrobial activity	176

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Šķipars V., Beļeviča V., Kānberga-Siliņa K., Ruņģis D.	Use of resistance-linked gene copy number variation analysis in selection of <i>Heterobasidion annosum</i> resistant Scots pine	178
Kānberga-Siliņa K., Jansons Ā., Ruņģis D.	Expression of phenylpropanoid pathway genes in Scots pine ( <i>Pinus sylvestris</i> L.) in open-pollinated families with differing wood densities during early and late wood formation	179
Bitenieks K., Korica A., Bacāns Ž., Nikolajeva V., Baumanis I., Veinberga I., Ruņģis D.	Identification of microorganisms found in discoloured birch ( <i>Betula pendula</i> L.) wood	180
Šķipars V., Kjos M., Nagy N.E., Krivmane B., Ruņģis D., Fossdal C.G.	Gene expression of defence related genes in parenchyma and resin duct samples of Scots pine obtained by laser capture microdissection	181
Šmits A., Siliņš I., Jansons Ā.	Most significant dendrophagous pests for Latvian forestry, review of invasion and outbreak risks in relation with climate changes in near future – next 50 years	182
Gailis A., Jansons Ā.	Silver birch ( <i>Betula pendula</i> Roth.) provenance differences in trial in Latvia	183
Gailis A., Augustovs J., Purviņš A., Jansons Ā.	Differences of Latvia's silver birch ( <i>Betula pendula</i> Roth.) provenances	185
Lazdiņa D., Bārdule A., Zeps M., Bārdulis A.	Effect of wood ash and waste water sludge fertilization on ingrowth and annual increment of poplar cuttings	187

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## AdapCar un IUFRO (WP 2.02.00)

konference

**Adaptācijas un pielāgošanās ģenētiskie aspekti meža veselības,  
koksnes kvalitātes un biomasas ražošanas kontekstā  
2012. gada 3.–5. oktobris, Rīga, Latvija**

*conference*

***Genetic aspects of adaptation and mitigation: forest health,  
wood quality and biomass production  
October 3–5, 2012, Riga, Latvia***

### Priekšvārds

Meža ģenētikas, selekcijas un atjaunošanas perspektīvo pētījumu centrs AdapCAR ir viens no Ziemeļvalstu meža pētījumu sadarbības komitejas (*Nordic Forest Research Co-operation Committee – SNS*) tikla projektiem, tas ir izveidots laika periodam no 2011. līdz 2015. gadam, un to vada profesors *Erik Dahl Kjaer* no Kopenhāģenas universitātes Dānijas meža un ainavu institūta. AdapCAR mērķis ir uzlabot zināšanas par koku pielāgošanos globālajai sasilšanai. Klimatiskajiem apstākļiem ģenētiski nepiemēroti koki ir vairāk pakļauti slimību riskam, un ģenētiskajai variācijai kopā ar adaptīvām pazīmēm ir būtiska loma koku veselības saglabāšanā mainīga klimata apstākļos. Vispārējais AdapCAR mērķis ir atbalstīt ģenētisko resursu apgūšanu un praktisku pielietojšanu.

Šī projekta specifiskais mērķis ir uzlabot sadarbību (tajā skaitā apmaiņu ar idejām) un veicināt jaunu meža ģenētiskās izpētes un selekcijas projektu attīstību Baltijas jūras reģionā, lai:

- iegūtu labāku izpratni par to, kā koki pielāgojas klimata izmaiņām;
- veicinātu uzkrātā CO<sub>2</sub> apjoma un biomasas pieaugumu;
- sekmētu meža reproduktīvā materiāla uzlabošanu un pielietojšanu, kas nodrošinātu augstvērtīgu produkciju, labu veselību, kā arī minimālus klimata izraisītus postījumus un stabilitāti ilgtermiņā;
- uzlabotu Baltijas jūras reģiona pētniecisko vidi, veicinot savstarpēju saikni starp molekulāro bioloģiju un genomu izpēti apstākļos, kad ģenētisko resursu uzlabošana apsaimniekošana, aizsardzība un izmantošana ir vispārārtzīta

AdapCAR organizē ikgadēju tikšanos Baltijas jūras reģiona partnerorganizācijām un uzaicina citu valstu ekspertus, kuri katrs pēta kādu specifisku adaptācijas aspektu.

Otrā AdapCAR tikšanās tika organizēta kā IUFRO starptautiska zinātniska konference *“Genetic aspects of adaptation and mitigation: forest health, wood quality and biomass production”* („Adaptācijas un pielāgošanās ģenētiskie aspekti meža veselības, koksnes kvalitātes un biomasas ražošanas kontekstā”) pielietojot starpdisciplināru pieeju, iesaistot zinātniekus ne tikai no meža selekcijas virziena, bet arī no molekulārās ģenētikas un meža patoloģijas jomām.

Konferences dalībnieki no 10 valstīm uzstājās ar divdesmit sešām mutiskām prezentācijām un vienpadsmit posteru prezentācijām. Galvenās prezentāciju tēmas:

- kaitēkļu un slimību rezistences mehānismi;
- kaitēkļu un slimību rezistences ģenētiskā variācija;
- meža genomu izmantošana klimata izmaiņu ietekmes mazināšanai un pielāgoties spējīgu (elastīgu) koku selekcionēšanai;
- nākotnes klimatam piemērots ģenētiskais materiāls;
- selekcija ar mērķi uzlabot koksnes kvalitāti un biomasas apjomu.

Mēs vēlamies pateikties SNS par finansiālu atbalstu AdapCAR tīklam un šai konferencei, kā arī IUFRO Virzienam 2.00.00 „Fizioloģija un ģenētika” par atbalstu. Pasākums un daļa no prezentētajiem pētījumiem finansēti no Eiropas Sociālā fonda projekta „Ģenētisko faktoru nozīme adaptēties spējīgu un pēc koksnes īpašībām kvalitatīvu mežaudžu izveidē” (Nr. 2009/0200/1DP/1.1.1.2.0/09/APIA/VIAA/146).

Salaspils, 01.10.2012

Ā. Jansons

Latvijas Valsts mežzinātnes institūts “Silava”

...

## ***Preface***

*Centre for Advanced Research in forest genetics, breeding and regeneration for adapting and mitigating climate change AdapCAR is one of the SNS (Nordic Forest Research Cooperation Committee) networks, established for a period from year 2011 to year 2015, lead by professor Erik Dahl Kjaer, Forest & Landscape Denmark, University of Copenhagen. Scientific motivation behind AdapCAR is the need to increase knowledge about the adaptation of forest trees to global warming. Maladapted trees are prone to diseases, and the genetic variability associated with adaptable traits is essential for the health of trees in a changing climate. The overall aim of AdapCAR is to support development and implementation of good practices for use and management of forest genetic resources.*

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*The specific objective of the project is to enhance collaboration including exchange of ideas, and development of new projects in forest genetic research and breeding in the Nordic region in order to:*

- obtain better understanding of how trees adapt to climate change;*
- contribute to increased CO<sub>2</sub> sequestration and biomass production;*
- promote development and deployment of forest reproductive material that will combine high value production with and superior health, minimum climatic injuries and long term stability;*
- contribute to a Nordic research environment, where the mutual interdependency between genetic and genomic research, wise genetic management, effective improvement, and corresponding and timely deployment are recognized.*

*AdapCAR organizes a yearly meeting of the partner organizations from Baltic Sea region and invited experts from other countries, that each focuses on specific aspects of adaptation.*

*Second AdapCAR meeting is organized in a format of IUFRO international scientific conference “Genetic aspects of adaptation and mitigation: forest health, wood quality and biomass production” and will focus on interdisciplinary approach, involving scientists not only from forest tree breeding (quantitative genetics), but also molecular genetics and forest pathology.*

*Twenty six oral presentations and eleven poster presentations were made by participants from 10 countries. The following topics are covered:*

- Mechanisms of resistance to pests and diseases*
- Genetic variation in resistance against pests and diseases*
- Forest genomics for mitigating climate change and breeding resilient trees*
- Genetic material for a future climate*
- Breeding for quality and biomass production*

*We would like to thank the SNS for the financial support of the AdapCAR network and this conference and IUFRO Division 2.00.00 Physiology and Genetics for the contribution. Funding from European Social Fund's Project “Importance of Genetic Factors in Formation of Forest Stands with High Adaptability and Qualitative Wood Properties” (No 2009/0200/1DP/1.1.1.2.0/09/APIA/VIAA/146) to the event and part of the studies presented is also acknowledged.*

*Salaspils, 01.10.2012*

*Ā. Jansons*

*Latvian State Forest Research Institute “Silava”*



## Agenda

October 3, 2012	8:30	8:45	<b>Opening of the conference</b> Representatives of AdapCAR network and LSFRI Silava
	8:45	9:30	<b>Mechanisms of resistance to pests and diseases</b> Jan Stenlid. Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences (Keynote speaker)
	9:30	10:15	<b>The genetic basis for diterpene defence in Sitka spruce</b> Björn Robert Hamberger, Department of Plant and Environmental Sciences, Faculty of Life Sciences, University of Copenhagen (Keynote speaker)
	10:15	10:30	<b>Break</b>
	10:30	11:00	<b>Genetic variation in resistance against pests and diseases</b> <b>Utilization of DNA assay as a tool for phytopathological monitoring in nurseries and field trials</b> Oleg Baranov, Forest Institute of National Academy of Science of Belarus, Laboratory of Forest Genetics and biotechnology, Laboratory of Forest Genetics and biotechnology, Belarus
	11:00	11:30	<b>Adaptive potential of ash (<i>Fraxinus excelsior</i>) populations against the novel emerging pathogen <i>Hymenoscyphus pseudoalbidus</i></b> Erik D. Kjær and Lea Vig McKinney, Forest & Landscape Denmark, University of Copenhagen
	11:30	12:00	<b>What is going on regarding genetic studies of pest resistance on broad-leaved species in Sweden?</b> Lars Göran Stener, SKOGFORSK
	12:00	12:55	<b>Lunch break</b>
	12:55	13:00	<b>Introduction to Trees4Future</b> Egbert Beuker, METLA, Finland
	13:00	13:30	<b>Breeding for resistance against root rot in Norway spruce</b> Bo Karlsson, SKOGFORSK
	13:30	14:00	<b>Genotype by environment interaction for survival, growth and <i>Cronartium</i> resistance in northern Scots pine</b> Torgny Persson, Skogforsk
	14:00	14:30	<b>Aphid resistance in Nordmann fir</b> Ulrik Bräuner-Nielsen, Forest & Landscape Denmark, University of Copenhagen
	14:30	14:45	<b>Break</b>
	14:45	15:15	<b>Development of early genetic testing methods for resistance to <i>Gremmeniella abietina</i> in Scots pine</b> Gunilla Swedjemark, SKOGFORSK

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	15:15	15:45	<b>Genetic difference in Scots pine resistance against root rot</b> Aris Jansons LSFRI Silava, Virgilijus Baliuckas, Forest Institute, Lithuanian Centre for Agriculture and Forestry
	15:45	16:15	<b>Role of resistance to green spruce aphid in Sitka spruce for growth and survival</b> Jon Kehlet Hansen, Forest & Landscape Denmark, University of Copenhagen, Susanne Harding, The Royal Danish Garden Society, and Hans Peter Ravn, Forest & Landscape Denmark, University of Copenhagen
	16:15	16:45	<b>Plenum discussion: Strategies to prepare for new pests and diseases</b>
	16:45		<b>Cooperation in Norway spruce breeding</b>
	October 4, 2012		<b>Genetic material for a future climate</b>
	8:45	9:30	<b>Forest Genomics for Mitigating Climate Change and Breeding Resilient Trees</b> Kostya V. Krutovsky, Department of Ecosystem Science and Management, Texas A&M University (Keynote speaker)
	9:30	10:00	<b>Norway spruce provenance variation in precautious flowering and tolerance to needle pest <i>Nematodus abietinus</i></b> Darius Danusevicius, Faculty of Forest and Ecology, Aleksandras Stulginskis University and Virgilijus Baliuckas, Forest Institute, Lithuanian Centre for Agriculture and Forestry
	10:00	10:30	<b>Epigenetics in Norway spruce</b> Arne Steffenrem and Harald Kvaalen, Norwegian Forest and Landscape Institute
	10:30	10:45	<b>Break</b>
	10:45	11:15	<b>Adaptive potential of nine native woody species in relation to climate change</b> Erik D. Kjær, Forest & Landscape Denmark, University of Copenhagen
	11:15	11:45	<b>Selection of hybrid aspen for future climatic conditions</b> Mārtiņš Zeps and Āris Jansons, LSFRI Silava
	11:45	12:00	<b>Discussion</b>
	12:00	13:00	<b>Lunch break</b>
	13:00	18:00	<b>Excursion</b> <b>Gain from use of Scots pine seed orchard material</b> Āris Jansons and Imants Baumanis, LSFRI Silava <b>Use of weather change indicators in pest outbreak prediction</b> Ingars Siliņš, Agnis Šmits and Āris Jansons, LSFRI Silava

October 5, 2012	9:00	9:45	<b>Genetic material for a future climate</b> (continued) <b>Genetic difference in adaptive traits of forest trees</b> Pertti Pulkkinen, METLA (Keynote speaker)
	9:45	10:15	<b>Genetic diversity of bread planting material in comparison to natural regeneration: case studies from Latvia</b> Āris Jansons, Angelika Voronova-Petrova, Mārtiņš Zeps, Krista Kānberga-Siliņa, LSFRI Silava
	10:15	10:45	<b>Improved genetic material for a future climate – new species – new genotypes (case study of <i>P. contorta</i> in Latvia)</b> Linards Sisenis, LUA, Līga Puriņa, Oskars Krišāns and Āris Jansons, LSFRI Silava
	10:45	11:00	<b>Break</b>
	11:00	11:45	<b>Breeding for quality and biomass production</b> <b>Next generation breeding values</b> Greg W. Dutkowski and RJ Kerr, PlantPlan Genetics, c/- School of Plant Science, University of Tasmania, B Tier and L Li, Animal Genetics and Breeding Unit, University of New England, J Costa e Silva, Instituto Superior de Agronomia, Lisbon, M Ivković, CSIRO, BM Potts, School of Plant Science and CRC for Forestry, University of Tasmania, and TA McRae, PlantPlan Genetics, c/- School of Plant Science, University of Tasmania (Key note speaker)
	11:45	12:45	<b>Lunch break</b>
	12:45	13:15	<b>Molecular genetic tools for research and breeding of forest tree species: Latvian perspective</b> Dainis Rungis, LSFRI Silava
	13:15	14:00	<b>How should we weight quality and quantity in forest tree breeding programs?</b> Harry Wu, Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences (Key note speaker)
	14:00	14:30	<b>Associations between growth and resistance related heartwood traits in Scots pine</b> Anni Harju, Martti Venäläinen and Matti Haapanen
	14:30	14:45	<b>Break</b>
	14:45	15:15	<b>Discussion on strategies for development of future trees in the Nordic/Baltic countries</b>
	15:15		<b>Closing of the conference</b>

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## MECHANISMS OF RESISTANCE TO PESTS AND DISEASES

*Jan Stenlid<sup>1</sup>*

Trees as well as other plants have a multitude of defence mechanisms at different organisational levels towards pests and diseases. These typically consist of preformed and induced structural and chemical characters but can also include nonsynchronous phenology between host and pest or pathogen. Constitutive resistance is all those factors that are present in the host prior to challenge. They can be structural, including mechanical barriers such as bark, cuticle or wax layers on leaves. Preformed chemical resistance components can be macromolecules, e.g. lignin, or extractable low molecular weight metabolites, e.g. the stilbene pinosylvins in pines. When a tree is exposed to a pathogen or a herbivore, several responses are triggered. The intruder can be recognised through innate immunity and signals are transmitted via signalling pathways including salicylic acid, jasmonate or ethylene. The downstream responses include activation of hypersensitive cell death and formation of reactive oxygen species, phenolic substances and resin production. Subsequently, new polyphenol rich cells, traumatic resin ducts and later on periderm are formed. Previous exposure to a pathogen can also affect the level of resistance for future attacks. Such acquired resistance can either be expressed locally or systemically.

The resistance can be specific for a given pest or pathogen (race-specific or vertical resistance) or general (basal or horizontal resistance). Trees are also able to tolerate disease, meaning that they are infected but the disease is not causing a major phenotypic disadvantage in terms of vitality or growth capacity. Resistance can be costly and tolerance can provide an evolutionarily stable strategy to handle a moderate pathogen pressure.

Breeding for resistance is an attractive approach for sustainable management of pests and diseases. Recent advances in genetics and genomics can help to provide markers for resistance breeding and at the same time maintaining genetic diversity for other traits in the population.

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<sup>1</sup> Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences, Box 7026 S-75007 Uppsala Sweden, E-mail: Jan.Stenlid@slu.se

## DEVELOPMENT OF A GENOMIC TOOLBOX TO DISSECT LOCALISATION, REGULATION AND BIOSYNTHESIS OF CONIFER DITERPENE RESIN DEFENCES

*Björn Hamberger<sup>1\*</sup>, Toshiyuki Ohnishi<sup>2</sup>, Britta Hamberger<sup>1</sup>,  
Armand Seguin<sup>3</sup> and Jörg Bohlmann<sup>4</sup>*

Diterpene resin acids are major defence chemicals in conifers. The structural diversity and chemical functionalisation of resin diterpenoids is generated through sequential activity of diterpene synthases (diTPS) and cytochrome P450 monooxygenases (CYP). We have developed a genomic toolbox to gain insights into the localisation, regulation and evolution of the biosynthetic route to diterpenoids. We identified a conifer specific class of P450s, CYP720B, which is highly diverse and consists of 38 known genes clustering in four distinct clades. We have isolated ten full-length cDNAs for CYP720B genes from Sitka spruce, and started their functional characterisation. For example, the promiscuous and multifunctional Sitka spruce CYP720B4 catalyses three consecutive oxidations of a range of diterpenoids en route to resin acids. Distinct expression of CYP720B family members in trees in response to treatment with methyl jasmonate, across different organs, tissues and in single cell types isolated by laser microdissection indicates both neo- and subfunctionalisation. Both a diTPS and seven members of the CYP720B family are highly expressed in cells lining cortical resin ducts, the major location of constitutively formed diterpene resin acids. We have used this knowledge in an attempt to modulate the biochemical capacity of terpenoid production *in vivo*. For this, we have targeted a panel of genes involved in terpenoid biosynthesis through stable RNA interference, including diTPS and CYP720B4. A significant reduction in transcript abundance of the target genes was accompanied by specifically altered accumulation of diterpenoids. To improve our still limited understanding of the evolution, structure and gene space of conifer genomes, we built a BAC library and sequenced the genomic regions carrying diterpene biosynthetic genes. The results of our work provide insights into the genetic repertoire and evolutionary origin of diterpenoid chemical diversity in conifer defence.

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## **PRESENCE OF NATURAL GENETIC RESISTANCE IN COMMON ASH (*FRAXINUS EXCELSIOR*) TO *HYMENOSCYPHUS PSEUDOALBIDUS***

*Lea Vig McKinney<sup>1</sup>, Lene Rostgaard Nielsen<sup>1</sup>,  
Jon Kehlet Hansen<sup>1</sup> and Erik Dahl Kjær<sup>1\*</sup>*

The devastating disease caused by the pathogenic fungus *Hymenoscyphus pseudoalbidus* (the sexual form of *Chalara fraxinea*) is currently threatening stands of *Fraxinus excelsior* in most of Europe. In Denmark the disease has been present since 2003 and severe dieback symptoms have been observed since 2005. We have assessed the impact of the disease on native Danish trees by estimating the presence of resistance in natural populations using clonal and progeny trials. Strong genetic variation in susceptibility to natural infections was observed among the clones and progenies with narrow sense heritability  $h^2 = 0.37-0.52$  and additive genetic coefficient of variation  $CVA = 0.37-0.61\%$ . The data suggests that 2-5% of trees in Danish ash population have substantial resistance to the new disease. This fraction is probably too low to avoid population collapse in natural stands, but the result provides prospects for future maintenance of the species through artificial selection of healthy individuals in the landscape.

A breeding programme is presently being initiated in Denmark based on phenotypic screening and selection with high selection intensity of healthy trees. Pilot tests have revealed that low-susceptibility clones develop substantially smaller necroses compared to susceptible clones when subjected to controlled inoculations, and we will therefore utilise controlled inoculations for early testing. In the presentation we also discuss the role of international cooperation in the breeding of ash.

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## WHAT IS GOING ON REGARDING GENETIC STUDIES OF PEST RESISTANCE ON BROAD-LEAVED SPECIES IN SWEDEN?

*Lars-Göran Stener*<sup>1</sup>

There are two major studies involving genetic resistance to pests on broad-leaved species in Sweden:

- 1) clonal differences in susceptibility to the dieback of *Fraxinus excelsior* in southern Sweden and
- 2) testing of resistance towards the most serious pathogens on hybrid aspen and poplars.

Ash dieback damage was assessed and analysed on 16-22 year-old grafts in two ash seed orchards. The grafts originated from 106 plus-tree clones selected from 27 stands in southern Sweden based on their phenotypes. The results obtained indicate that ash dieback disease is strongly genotypically controlled. There was considerable genotypic variation among individuals. None of the clones seemed to be totally resistant, but some exhibited reduced susceptibility and retained this resistance after six years under heavy infection pressure. Together with the high heritability of resistance, strong age x age correlations and weak genotype x environment interactions, this suggests there is good scope for breeding less susceptible trees for the future.

Hybrid aspen and poplars are fast growing species with large potential to increase the amount of renewable resources for energy purposes. However, there are only few tested clones available in southern Sweden, and further north such material is missing. In addition our knowledge about resistance to serious pathogens such as *Xanthomonas populi* (bacterial canker) and *Melampsora larici-populina* (leaf rust) on poplars and *Entoleuca mammatum* (stem canker) on hybrid aspen is very poor. Thus new field tests of new candidates will be established as well as resistance testing of the commercial material and of new candidates. The project is financed by the Swedish Energy Agency and is carried out by Skogforsk in cooperation with SLU and JILU in Sweden and INBO in Belgium.

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## BREEDING FOR RESISTANCE AGAINST ROOT ROT IN NORWAY SPRUCE

*Bo Karlsson*<sup>1</sup>

Genetic response to artificial and natural infections by *Heterobasidion* sp has shown sufficient variation to consider the pathogen in breeding activities. Broad sense heritability estimates ( $H^2$ ) were found to be of the same magnitude as for growth traits (0.15-0.30).

Clones from clonal forestry programmes were tested through inoculations in young plantlets in the nursery as well as in young trees in clone trials and the most resistant clones were selected for breeding and further testing.

Clones showing low infection frequencies after natural infections in older clone trials were also selected.

Healthy trees from heavily infected stands were selected at the time for final cut and grafted and included in the breeding population.

A large screening project on plus-tree grafts using branch inoculations was carried out and the results will be used in a large association mapping project.

An important remaining issue is the correlation between early inoculation results and the degree of natural infections in mature trees.

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## **GENOTYPE BY ENVIRONMENT INTERACTION FOR SURVIVAL, GROWTH AND CRONARTIUM RESISTANCE IN NORTHERN SCOTS PINE**

*Torgny Persson*<sup>1</sup>

Data from 71 field trials (7- to 31-year-old trees) of full-sib or open-pollinated offspring from Scots pine (*Pinus sylvestris* L.) plus-trees were used to estimate the amount of genotype by environment interaction for survival and tree height in northern Sweden. In addition, inventories of pine blister rust (*Cronartium flaccidum* and *Peridermium pini*) were made in two of the progeny trials, motivated by the widespread infection by these fungi during the last decade in young commercial pine stands in northern Sweden. The field trials were located at sites spanning a wide range of environmental conditions. The genotype by environment interactions were derived using the type B genetic correlation between the same traits measured in different trials, estimated by multivariate REML analyses. In total, 59 and 117 type B correlations were calculated for survival and tree height, respectively.

The type B genetic correlations for survival and tree height averaged 0.62 and 0.66, respectively, indicating a moderate genotype by environment interaction for both traits, although there was substantial variation among pairs of sites. The single type B correlation between pine blister rust resistance measured in the two field trials was 0.85 ( $\pm 0.14$ ), indicating a strong genetic association in rust resistance between these sites. The genetic correlation between pine blister rust resistance and survival/tree height (measured on relatives in non-infected environments) was found to be weak and non-significant.

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## GENETIC VARIATION IN RESISTANCE TO *DREYFUSIA* *NORDMANNIANA* IN NORDMANN FIR

*Ulrik Bräuner-Nielsen*<sup>1</sup>

Nordmann fir (*Abies nordmanniana* (Stev.) Spach.) is the main Christmas tree species in Denmark and early 10 mio. trees are produced mainly for export. Attacks from the silver fir woolly adelgid (*Dreyfusia nordmanniana* Eckst.) are a major problem for the production and pesticides are applied regularly in a majority of the production stands. The adelgid causes a severe needle curling and yellowing of the needles emerging on current year needles and severely reduces the value of the crop.

Genetic differences between Nordmann fir provenances, families within provenances and their suitability as host plants for the silver fir woolly adelgid (*Dreyfusia nordmanniana* Eckst.) were investigated based on artificial infestations of 63 families originating from three provenances and four provenance samples a total of 2100 seedlings planted in a single field trial. Results after two years were compared to an assessment 10 years later. Using early artificial infestation seems to be a promising tool for evaluating genetic differences in infestation levels and the damage at the end of a Christmas tree rotation after 10 years. Results of correlation and heritability's will be presented. Furthermore recent observations in a clonal seed orchard based on natural infestation confirms a rather strong genetic component in proportion of trees showing damage due to needle curling.

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## DEVELOPMENT OF SCREENING METHODS FOR RESISTANCE TO *G. ABIETINA* IN SCOTS PINE CLONES TO BE APPLIED IN EXISTING BREEDING PROGRAMMES

*Gunilla Swedjemark<sup>1\*</sup> and Curt Almquist<sup>1</sup>*

The fungus *Gremminiella abietina* is widespread in pine forests in Europe, North America and East Asia and its economic importance is significant. The pathogen is common on lower shaded branches but may, when circumstances are favourable, attack branches higher up in the crown and eventually kill the tree. Weather conditions during the summer and suitable temperatures during the winter are essential for the development of the disease; cool and rainy seasons favour epidemic outbreaks of *G. abietina*.

Scots pine (*Pinus sylvestris*) stands in large areas of Sweden were attacked by *G. abietina* in 2001 following weather conditions favourable to the pathogen. A total of 330 000 ha was damaged according to the Swedish National Forest Inventory. In an ongoing study at Skogforsk, *G. abietina* damages were assessed in five genetic field trials and one clonal seed orchard. The results show large genetic variation in susceptibility and significant genetic parameters in clones and families of Scots pine. There were no correlations between attack frequency and growth characters.

Previous studies have shown significant differences between clones when inoculating twigs with mycelium of *G. abietina*. The length of the necrosis in the shoot has been the indicator of resistance/susceptibility in the host to the pathogen. Breeding for resistance to *G. abietina*, is not practiced today in *P. sylvestris* but may be considered in the future. One reason is that climate scenarios for Scandinavia show warmer and more humid winters. This will create favorable conditions for *G. abietina* which could respond with more frequent large scale outbreaks in the future.

In the present study, we wanted to correlate the results after artificial inoculation of *G. abietina* to the damage frequency after natural outbreaks of the pathogen. We used the clones in a seed orchard in Northern Sweden consisting of 34 clones randomly distributed in the orchard. In the outbreak in 2001, significant differences between the clones had been noted. The trees were ca 40 years old and ca 10 m high. Twigs were inoculated with mycelia of *G. abietina* and incubation time was 90 days. The twigs were then moved to the laboratory, length of the necrosis were noted, isolation of the fungus was achieved and Relative and Quantitative PCR on the tissue was performed to find the presence of *G. abietina* DNA.

About 75% of the inoculated twigs contained *G. abietina* DNA and none of the

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uninoculated controls. We found no correlation between the length of necrosis, presence of fungi and previous results from the natural outbreak 2001. There was no correlation between the presence of fungal DNA and the length of the necrosis.

The conclusion from this study is that artificial inoculation with mycelia is not a useful method to screen for resistance to *G. abietina* in Scots pine trees. Previously, the main character to measure resistance in inoculated Scots pine twigs has been the length of the necrosis. This study shows that necrosis is not a suitable parameter for measuring resistance in such experiments.

## **PINUS SYLVESTRIS POPULATIONS AND FAMILIES RESISTANCE TO HETEROBASIDION ANNOSUM AND POSSIBILITIES FOR BREEDING IN LATVIA**

*Āris Jansons<sup>1\*</sup> and Virgilijus Baliuckas<sup>2</sup>*

The productivity, branch diameter, branchiness, stem straightness, spike knots and damage by root rot of 154 Scots pine (*Pinus sylvestris* L.) half-sib families from 13 Latvian populations were evaluated in the field trial at age 38.

28 different *Heterobasidion annosum* genotypes were registered on an area of 1.55 ha. But only 12 genotypes were more abundant. Root rot kriging estimates were used to select damage plots in the field trial. An average radius of the damage plot was 11 m and the area – 108 m<sup>2</sup>. 28% of total observations were included to the damage plots. Genetic parameters of root rot were calculated using only records from damage plots. Individual heritability for resistance to root rot was as high as for tree height (0.24). Correlations of family breeding estimates between resistance to root rot and the other traits analysed were very weak and not significant. Negative association of resistance to root rot with tree height and positive with branchiness or branch diameter might be noted.

Matrices of similarity of resistance index of family as a proportion of alive to dead trees and distance to damage plot centre were formed and Mantel test was performed. The test resulted in significant correlation (0.19) indicating that probability to select families with higher resistance to *H. annosum* is not low.

We applied typical Scots pine breeding methodology in Latvia for selection of best families and trees. Tree height, volume and some restrictions to quality traits are being used. The results showed that such selection would not change average damage by root rot estimates, but would drastically decrease resistance index. If the average of family damage by root rot or tree resistance index were separately included to the selection index formula by weighing them equally as productivity traits then increase in resistance to root rot comprised 18% or in case of resistance index – 52%. Genetic gain estimates in the other traits were quite robust to inclusion of resistance trait to selection index. Tandem selection seems to be very effective only if resistance estimates are included to family selection index in second stage after the first with 30% of top resistant family selection. The possibilities to use different approaches are discussed.

**Keywords:** *Scots pine, population, half-sib family, root rot, growth, resistance*

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## ROLE OF RESISTANCE TO GREEN SPRUCE APHID IN SITKA SPRUCE FOR GROWTH AND SURVIVAL

*Jon Kehlet Hansen<sup>1\*</sup>, Susanne Harding<sup>2</sup> and Hans Peter Ravn<sup>1</sup>*

Sitka spruce (*Picea sitchensis* Bong Carr.) is in Denmark mainly planted at poor sites and sites near the Sea due to its tolerance to salt spray and better growth compared to Norway spruce. A main problem for the species is recurrent defoliations of the green spruce aphid after mild winters that reduce growth, but seldom leads to death. The frequency and severity of green spruce defoliations may increase in the future due to predicted higher spring temperatures that allows the aphid population number to increase rapidly, while predators are still low in number.

This study aimed at investigating the importance of resistance to green spruce aphid for the growth and survival of Sitka spruce in the light of the foreseen climate change.

Wood cores were sampled from 81 trees from families of five open pollinated trees, which showed different degrees of defoliation after aphid feeding in 1990 in a field trial located at sandy poor soil. The breeding value for defoliation was estimated for the 81 trees and related to the annual growth of the trees from 1990 to 2009. Furthermore, the defoliation was investigated in four field trials established in 1984 with 151 Sitka spruce clones, which showed significant defoliation in two of the field trials after aphid feeding in 1990.

In the progeny test, a significant regression was found between the breeding values for defoliation of the 81 trees and annual growth from 1990 to 2009 and with the 10% most resistant trees having about 17% higher annual growth. The importance of the defoliation breeding value was less pronounced in years with poor precipitation in the growing season, which perhaps reflects that older needles contribute little to growth in years with drought.

In the field trial series with 151 clones, a selection of the 20% most resistant clones would possibly improve growth about 13% in conditions similar to the field trial most severely attacked by aphids. However, the genetic correlation of defoliation at the most severely attacked field trial with growth in the other field trials where site conditions were better was lacking and indicates that the aphid resistance at least in mid-aged populations at better sites is of minor importance.

The breeding program of Sitka spruce is divided in two breeding populations. One is aiming at poor sites with high risk of frost and wind and one at more fertile sites with less risk of frost and the results of this study stresses the importance of including aphid resistance in breeding population aiming at poor sites.

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## FOREST GENOMICS FOR MITIGATING CLIMATE CHANGE AND BREEDING RESILIENT TREES

*Kostya V. Krutovsky*<sup>1</sup>

Forest population and landscape genomics, molecular ecology and ecogenomics, and other genomics approaches, tools and resources provide researches now with unprecedentedly new opportunities to study plant genetic adaptation to environmental stresses at the genome-wide level and to do genomic selection for frost tolerance, drought, disease and pest resistance, and other important adaptive traits using simultaneously thousands of genes and millions of genetic markers that represent the entire genome variation. Global climate change, accelerating human population growth rate and, respectively, the growing demand for construction tree products and energy wood feedstock require a more efficient tree breeding. Conservation of forest tree resources has also become even a more serious problem. The growing demand for an alternative, available, renewable, and ecologically friendly source of energy significantly increases the role of forests and population and ecological genomic studies of their adaptive, ecological, and economic potential. The accelerating global climate change can exceed the adaptive potential of pine forests, promote wildfires and pest invasions, and lead to forest contraction or even disappearance on large areas. However, some forest tree populations growing now in the regions that have ecological conditions similar to the future changes are likely to possess the adaptive potential necessary for spreading adaptation into other regions with similar ecological conditions expected in the future. Unfortunately, phenotypic plasticity and the historically established gene flow between remote populations can be insufficient for fast natural propagation of genetic adaptations. In this connection, the role of conservation management and especially that of assisted migration (also called assisted colonization, managed relocation or translocation done by physically moving the plant material (pollen, seeds and seedlings) to other regions where this material is better adapted to the future environments) increases. Modern methods and tools of population, landscape, and ecological genomics allow to study the genetic basis of adaptation in forest tree species and also detect the main genes responsible for important adaptive and economic traits that can be used in assisted gene migration and tree breeding for biomass growth, pest and disease resistance, water use efficiency, cellulose content, and other traits important for adaptation and for bioenergy and biofuel production. The recent data on Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) and loblolly pine (*Pinus taeda* L.) populations studied for thousands different genes are presented to illustrate population and ecological genomic studies in conifers.

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## NORWAY SPRUCE PROVENANCE VARIATION IN PRECOCIOUS FLOWERING AND TOLERANCE TO NEEDLE PEST *NEMATODUS ABIETINUS*

*Darius Danusevicius*<sup>1\*</sup>, *Virgilijus Baliuckas*<sup>1</sup>,  
*Darius Kavaliauskas*<sup>1</sup> and *Aleksandras Stulginskis*<sup>2</sup>

The information on the genetic variation of precocious flowering is scarce in Norway spruce and is mainly based of clonal variation in seed orchards. We have observed early flowering in a series of provenance tests in Lithuania and report the results of this assessment. Objective of our study was to assess the provenance variation in early flowering of Norway spruce in a provenance field test in central Lithuania. The provenance field test was established in 2001 at central Lithuania. 120 provenances were tested in spring 2007 being at the age of 11, the trees produced female flowers and cones for the first time in the field tests in Kazlu Ruda and Telsiai. In 2008 to 2010, no flowering was observed in this field test in spite of abundant flowering of spruce in 2008 in Lithuania. Given this was the first flowering at an early age of 11 years and no flowering three years in a row, we assume that this was a precocious flowering event. In total, approximately 5% (ca 200 trees) of the trees were flowering in the field test. Trees were healthy, no external damage was observed on the trees. This may indicate that the stress causing the precocious flowering may have occurred during the bud differentiation period in July of the previous year. The supposed climatic induction of precocious flowering is also supported by the occurrence of flowering of the same set of provenances in the other test of the same series in Telsiai (ca 250 km apart in north western Lithuania in 2007).

Flowering among trees was variable with the number of cones per tree varying between 1 and 58 cones. The ANOVA on number of cones per tree revealed a significant effect of provenance, whereas the block effect was not significant. The plot of regional means for the proportion of flowering trees together with the correlation analysis showed that the southern provenances possessed relatively more flowering trees and more cones per tree than the northern provenances. We attribute this provenance variation in early flowering to their difference in phenology caused by the transfer effect. Among other traits phenology includes timing of bud differentiation into vegetative, flower and latent buds in July of the proceeding year. In Norway spruce, the phenology traits are all strongly correlated with each other and due to the transfer effect, the timing of physiological processes of southern origins of Norway spruce occurs earlier than that of local and northern origins. If climatic conditions in July of the proceeding year are favorable for differentiation into flower buds of a population with

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certain phenology rhythm, then contribution of this population to the genetic composition of the future generations may be greater. These considerations require a further analysis to connect the flowering variation with variation in phenology and climatic variation during the July of the preceding year (2006).

In spring 2011, this same test was severally damaged by the needle eating sawfly *Nematodus abiensis*. Almost all trees were affected. In summer 2011, Tree height was measured and the damage was scored in classes. The results revealed that northern provenances were less damaged than southern provenances. This also means that on the provenance mean level the taller, late flushing trees were damaged more than the shorter in early flushing trees.

In conclusion, our study indicates that the first flowering event in the ontogeny of a set of 120 Norway spruce provenances in a field test showed that early flowering is provenance depended and southern provenances possessed more flowering trees and more cones per tree than northern provenances. We attribute this finding to the phenology differences of the provenances during the time of bud differentiation the year before. This variation in timing of bud differentiation was caused by the transfer effect. Presumably, a flower-inducing stress manifested at the bud differentiation stage of southern provenances stronger than of the northern provenances.

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## EPIGENETICS IN THE FOREST – PRACTICAL OBSERVATIONS AND CONSIDERATIONS

*Arne Steffenrem<sup>1\*</sup> and Harald Kvaalen<sup>1</sup>*

Temperatures and photoperiod during reproductive stages and seed maturation affects phenotypic expression of phenology traits of Norway spruce (*Picea abies*). This long lasting change in traits important for climatic adaptation is assumed to be epigenetic effects possible due to regulation of gene expression. But the underlying mechanisms causing the ‘memory’ are yet to be fully understood.

This phenomenon has consequences for climatic adaptation both in ‘natural’ and ‘domesticated’ populations. Of practical significance is the effect of temperatures during seed maturation in forests, seed orchards and clonal archives. We have several observations of seed-year dependent variation in phenology and general performance of spruce both in nurseries and in forests. We will look at data from an experiment designed to study the phenology and growth of seedlings from seed harvested the very warm seed year of 2006 compared to previous seed years that were cooler. In short term nursery trials we found that “2006 year seedlings” had delayed bud set the first growth season, delayed bud flush the following spring and delayed bud set the second season. In a long term progeny field trial, comprising control seed lots from provenances harvested in 1970, 1989 and 2006, we found that bud flush were delayed on 2006 year seedlings when scored after three seasons in field (five years from seed). The temperature dependent variation has several implications for seed production and the geographic localization of seed orchards. However, variation in phenology traits also significantly affects growth and performance of the progenies in field. This has major implications for a breeding program since it adds restrictions to mating and testing procedures in the field.

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## ADAPTIVE POTENTIAL OF NINE NATIVE WOODY SPECIES IN RELATION TO CLIMATE CHANGE

*Erik Kjær<sup>1</sup>\*, Lars Nørgård Hansen<sup>1</sup> and Jon Kehlet Hansen<sup>1</sup>*

Bud burst and leaf senescence was studied among families from open pollinated trees originating from natural populations of *Fraxinus excelsior*, *Betula pubescence*, *Malus sylvestris*, *Cornus sanguinea*, *Rosa dumalis*, *Acer campestre*, *Prunus avium* and *Quercus petraea*. Additionally, bud burst and leaf senescence was studied among *Tillia cordata* clones from natural populations.

The objective of the study was to investigate the degree of local adaption of bud burst and leaf senescence of populations and the potential of the natural populations through natural selection to adapt bud burst and leaf senescence to changed climate conditions.

For all species, populations were significant different as regards bud burst and leaf senescence. Bud burst development of populations was for three of the species significantly related to the estimated minimum temperatures at the population sites in April, May or June. Leaf senescence development of populations was only poorly explained by estimated autumn temperatures at population sites. The genetic variation within populations in bud burst was generally so large that a selection in one generation could possibly change the timing of bud burst as much as observed between the most extreme populations as regards bud burst, while the genetic variation of leaf senescence within populations was more moderate. The difference in estimated minimum temperature in May between population sites ranged from 0.5-1.3 °C, i.e. the populations would possibly over one generation be able to adapt to a change in temperature of this range. Regional climate models points towards a possible increase of more than 2 °C in 2071-2100 depending on development in greenhouse gasses. For species like *Quercus petraea* and *Fagus sylvatica* with a long generation time an adaptation to this change would be difficult and for these species it could likely be of advantage to import more southern populations that could take advantage of a longer growing season.

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## SELECTION OF HYBRID ASPEN FOR FUTURE CLIMATIC CONDITIONS

*Mārtiņš Zeps<sup>1</sup> and Āris Jansons<sup>1</sup>*

Climate change models suggest that temperatures rise of temperature that by the end of the century can reach 4°C in winters and 3°C in spring and autumn. Increase of frequency of very hot seasons is predicted. Projections indicate that rainfall will increase in the winter, early spring and autumn, but during vegetation period will remain practically unchanged.

Hybrid aspen (*Populus tremuloides* x *Populus tremula*) has very high productivity and therefore is one of the most promising alternatives for establishment of plantations on abandoned agricultural lands in Baltic Sea region countries. In order to ensure selection of appropriate clones for predicted changes in climatic conditions as well as to forecast possible changes in productivity and risks for hybrid aspen clones used currently it is essential to obtain detailed knowledge on formation of its increment.

Therefore aim of the study is to assess length of annual height increment, factors affecting it and pattern of its development. Results from hybrid aspen plantation consisting of 15 clones, measured during 4<sup>th</sup> and 5<sup>th</sup> growing season.

Tree height at the beginning of vegetation period was significant factor influencing length of height increment ( $R^2=0.25$ ) and height growth intensity ( $R^2=0.22$ ). Height increment was slightly longer for early flushing clones ( $111\pm5.4$ cm) than for late-flushing ( $101\pm7.1$  cm), but was not related to length of used vegetation period. Intensity of height growth was strongly related to daily mean temperature ( $R^2=0.87$ ;  $R^2=0.76$ ) and in 4<sup>th</sup> growing season had two peaks– at the beginning of growth season (end of May) and from July till end of August. Height growing intensity peak of 5<sup>th</sup> growing season has similar timing than second peak in 4<sup>th</sup> growing season.

Precipitations influence to height grows varied notably between seasons.

Results suggest, that hybrid aspen will be capable to efficiently utilize increase in length of vegetation period and that there is notable variation among clones in response to meteorological conditions and room for selection of most suitable clones.

**Keywords:** *Productivity, Clones, Adaptation, Populus*

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## GAIN FROM USE OF SCOTS PINE SEED ORCHARD MATERIAL

*Āris Jansons<sup>1</sup>\* and Imants Baumanis<sup>1</sup>*

Seed orchards are established to ensure steady seed supply and deliver the genetic gain (improvement of values of economically important traits) from seed orchard to forests. Currently all Scots pine seed material for plant production is collected from seed orchards, most of them first round (phenotypically selected), but some also second round (progeny tested). Due to long breeding cycle and relative high costs to carry it out as well as establishment and maintenance costs of seed orchards it is important to estimate the genetic gain while using seed orchard seeds.

Realized genetic gain values have been estimated from 2 sets of experiments:

- a) trial of average seed orchard and stand samples, established in year 1983 with one year old seedlings, using seeds from years 1979–1981. Material was collected in altogether 24 first round seed orchards and 8 stands, distributed in different regions of Latvia. Initial spacing  $2 \times 1$  m, 6 replications, 20–40 tree block plots, no thinning carried out prior to measurement;
- b) 12 progeny trials, 25–35 years old (planted with distance  $2 \times 1$  m, mostly un-thinned) that together contains more than 400 plus-tree and clone open pollinated families and stand seed lots as a controls.

Due to strong competition effect only results from dominant trees (highest tress representing optimal density at certain age in Latvia, ranging from 800 per ha to 1000 per ha) were used for analysis. In this way also influence of survival differences on the yield estimates are minimized.

Results reveal that progenies from seed orchard in western part of Latvia at the age of 26 years exceeds the yield of the forest stand progenies from the same region by 22% on average. It is more than was found in previous measurement at the age of 21 year – 18.8% (Baumanis *et al.*, 2003). Gain in stem straightness was negligible (below 5%) due to very limited problems with and variation in this trait for Latvian Scots pines. Similar differences for the traits were found also for eastern provenance region.

Further improvement of the yield can be reached with progeny testing – families whose mother trees will be (or are) recommended for second round seed orchards exceeds the growth of the stand progenies (control lots) on average by 27% in western provenance region and by 24% in eastern (median form data from all experiments; median age 32 years). Differences in branch thickness and stem straightness remains relative unchanged, except for branch thickness for western provenance region, where further improvement (reduction of average value by 5%) was achieved.

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## USE OF WEATHER CHANGE INDICATORS IN PEST OUTBREAK PREDICTION

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Weather changes are important for mortality, fecundity, food consumption, health, reproduction and mating success of insects and therefore can be used to predict insect pest outbreak probabilities in next year.

We collected large amount of information from available literature about different, complex meteorological parameters which often affect survival success of common forest pest species. Such changes as air, soil temperatures; air, soil humidity; wind speed and precipitation, also level of snow have been taken in consideration, analysing survival of different pest species and their parasitoids to predict next year outbreak possibilities.

According to available information, major factors which affect pest mortality in winter, are sudden and in periodicity long (longer than 2 weeks) black frost with extremely low temperatures (-20 – -36 °C) in combination with low humidity (under 60%) and these conditions effectively limits approximately 15–30% of all overwintering insects in bark (xyphidrids, scolytids, cerambicids), in forest soil till 15 cm deep – mostly in preimago stages. Those particular conditions affect leaf beetle mortality significantly – usually dies 18–25% of overwintering imago. Survival of pests depends on such beneficial weather conditions as long persistence (during 3 or more months) of relatively high air humidity (50% or more) with high winter temperature during 3 or more months – close above 5 °C. If snow level is close to 40cm persist with these conditions – there is much greater chance of outbreak absence in next year. But those conditions are inappropriate for ground – overwintering insect preimagos as moth pupae, sawfly larvae – because of high activity of cold resistant entomophagous fungi and nematodes (mostly *Steinernema* spp.). We concluded that relatively late frost always serves as indicator for increased outbreak possibility in the next year.

We found that conditions affecting pests in spring and autumn are quite the same, but pest development cycles are differently affected. Noticeable delay of vegetation period (for 2 or more weeks) in spring with low air temperature (under 5 °C) and drastic precipitation (more than 500 mm) promote mortality of freshly hatched moth (for example *Lymantriidae*) larvae. Like rain, increased wind speed at the beginning of vegetation period also serves as disturbing factor for pest larvae feeding. High precipitation in valley-like regions and in low altitudes (even under sea level) is critical for survival of overwintering pest preimago. Reasonably

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approximately 10–13% of insects in soil deeper than 15 cm are drained and suffocates. It happens, because dryness of these regions always is low – because of lower wind speed and therefore lower evaporation. But on the contrary invasive alien species survives in those regions which are explained by higher temperatures during the summer. Those conditions adversely affect overwintering preimagos in ground – as usually those became invaded by nematodes (which are mostly active at 3–5 °C). Number of moths and sawflies can be greater in early spring when rise of air humidity is more than 80%, because it disturbs flight of egg parasites from *Teleonidae* and *Aulacidae*. But early and relatively dry spring negatively affects overwintering success of pest eggs. Early frost in autumn usually cuts hibernation of sawflies which also results in higher mortality.

As summer is important reproductive period for lot of species and is the most important key season due the weather conditions which can be used for outbreak predictions.

High air humidity (more than 80%) during June and July can significantly limit moth larvae by increased infection with nucleopolyhedroviruses. Precipitation periods longer than 2 weeks disturbs feeding and by that can kill up to 20% of pest larvae. Survival of bark beetles and weevils also are reduced because of better tree resistance. We found that persistence of these conditions for 3 or more days can limit approximately 10% of all forest pests. Dryness for periods longer than 2 weeks significantly delays development of parasitoid fly's preimagos in forest soil (for example, larvae of *Parasetigena silvestris*) and therefore is beneficial for most of *Lymantriidae* species. After several relatively dry summers (usually 2 or 3) with average precipitation higher than 550 mm possibility of r – strategist species outbreaks in next summer reaches approximately 80–100%. In those outbreaks defoliation of hosts can reach 100% according to suppressed feeding and higher foraging response. Temperatures over 25 °C induce pest larvae feeding more than 5 times as food source also becomes a water source. Furthermore, monthly periods with temperature more than 28 °C induce outbreaks of leaf mining wasps as *Fenusa* sp. and also are beneficial to bark beetles. Outbreaks of scolytids in current and next year can be promoted with high dryness and temperature rise between 35 °C and 40 °C. Therefore, soil temperature over 25 °C reduces activity of entomophagous nematodes close to zero. We found that rise of all-weather indicators over 10% can positively promote survival of *Lymantriidae* larvae and increase outbreak possibility in next year up to 20%.

By available information, we found that outbreaks definitely are affected by seasonal climatic deviations. Still exists dilemma in all climate pest-related models – as species always adapts to new conditions making previous weather indicators unusable for next predictions.

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## BETWEEN AND WITHIN SPECIES DIFFERENCES OF ADAPTIVE TRAITS – HOW TO SELECT THE BEST TREES FOR FUTURE?

*Pertti Pulkkinen<sup>1</sup> and Salla Varis<sup>2</sup>*

During the last five years we have analysed how young seedlings of different Scots pine, Norway spruce, Silver birch, European aspen, English oak and Small-leaved lime origins grow in different climatic conditions established in greenhouses. We have followed growth and hardening development, break up speed of deep-dormancy stage and moreover the dehardening (vernalization). The main conditions used was temperatures and carbon dioxide levels assumed in scenario A1B around the years 2030 and 2100, thus the temperature was about 1.5°C or 4.5°C higher than local present. Respective carbon dioxide levels were 290 ppm and 700 ppm. We also use local average soil moistures and dryer soil moisture levels. Our main aim was to produce basic knowledge about provenance level adaptation of the species and origins used in commercial forestry or in the city parks.

In general results showed that growth conditions during the previous summer has a major effect especially on hardening but also on the possibilities of dormancy-break down and dehardening development of all the studied tree species. On average high temperatures and dry soils tends to slow down the hardening development and increase the risks of dormancy-break-downs with most of the studied material, but high carbon dioxide levels seems to act opposite. High temperatures during the previous growth season seem to delay the dehardening development.

However, there were major differences between species. Species like Norway spruce and Scots pine had f.ex. delayed hardening development in A1B 2100 conditions, but the time difference compared with the same material grown in local, present, conditions was only one to three weeks. The opposite was European aspen. In some experiments aspens start their hardening development in the A1B year 2100 conditions several weeks later compared those grown in outside and actually they were not hardy even on late November. This was also partly due to secondary growth which seems to be more common with higher temperature and more common with species like aspen and spruce than species like pine.

In general it seems to be unwise to import more southern provenances even from areas where the temperatures are about similar that has been predicted to exits in future in our (more northern) conditions. In our experiments origins like North-Poland, North-Germany,

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Southernmost Sweden and Denmark was even worse in future conditions than they are in the present conditions compared with local origins and the result was quite similar in all the species studies.

What should we do? First, our studies cover only the one or two first years of the trees lifespan and even though trees must first survive to be able to grow both the growth and quality is important characteristics not only the survival. This kind of testing techniques can cover some traits connected with the growth rhythm and perhaps we can use the timing of onset of efficient photosynthesis to describe some growth potential of our material, but definitely we need also field-tests with these preselected material based on the results obtained from controlled growth experiments.

If we can't use directly more southern provenances what then? Of course we can use natural selection, thus let the nature to take care of our reforestation, but then we definitely will have material not so usable in forestry than the present one. One possible solution is hybrids. We have seen gain of the hybrids in several times; some of them are better than neither of the parents and we can utilize this also when considering trees adapted to further, not yet existing, conditions. This is possible especially with species we clone, but hybrids can be utilized also with pine by establishing seed orchards in environment where most of the pollen came from surrounding trees producing hybrid crops. We should also focus more attention, like plant breeders has already done, to the plasticity. The new information from genome structures and especially strong role of regulatory genes has favored the idea that plasticity is one of the features we need to study with and select with.

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## GENETIC DIVERSITY OF BREAD PLANTING MATERIAL IN COMPARISON TO NATURAL REGENERATION: CASE STUDIES FROM LATVIA

Āris Jansons<sup>1\*</sup>, Angelika Voronova-Petrova<sup>2</sup>, Mārtiņš Zeps<sup>1</sup>,  
Krista Kānberga-Siliņa<sup>2</sup> and Dainis Ruņģis<sup>2</sup>

Genetic diversity is an important factor to ensure resilience of the stands or plantations towards changes in meteorological factors or pest outbreaks. However, increase of diversity in production population inevitably leads to loss of gain in the traits of interest. Aim of our study was to approach the trade-off between gain and diversity while analyzing the levels of the diversity in natural populations of common aspen and Norway spruce.

Study of aspen was based on assessment of 18 pure stands of common aspen, located in 6 geographically distant regions with highest concentration of aspen stands in order to cover the possible diversity. Stand age varied from 5 to 10 years, within stand sample trees were systematically chosen to cover all the area. Number of clones were delineated by phenotype (bud burst and leaf coloring phenology) and using 6 SSR markers (developed for *Populus nigra* and *Populus tremuloides*).

Study of Norway spruce was based on assessment of average (industrial) sample of progenies of two first round (phenotypically selected) seed orchards (with 50 and 20 clones) using 6 SSR markers. Altogether 541 seedling was genotyped.

Results reveal that number of clones in hybrid aspen stand is ranging from 6.2 to 12.5 per ha and relative high proportion of them (70–90%) are represented with single ramet. Average distance occupied by one clone was 39 m, but ramets of the same clone were found also 170 m apart. Results indicate, that careful assessment of different resilience against different factors needs to be carried for the clones to be used in plantations, since diversity in them will be smaller than in natural stands.

Norway spruce seed orchard progenies in comparison to average samples from forest stands (data from Ruņģis *et al.*, 2006) demonstrated the same number of different alleles with a frequency larger than or equal to 5%: from 4.8 to 5.0 on average. Also Shannon's Information Index, used as measure of diversity, did not differ significantly between the seed orchards and forest stand (ranging from 1.91 to 2.07), neither did the expected heterozygosity. Results reveal, that in open-pollinated seed orchard also 20 clones can ensure similar levels of genetic diversity as found in natural stands and can be recommended for use in praxis.

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## IMPROVED GENETIC MATERIAL FOR A FUTURE CLIMATE – NEW SPECIES – NEW GENOTYPES (CASE STUDY OF *PINUS CONTORTA* IN LATVIA)

Līga Puriņa<sup>1</sup>, Linards Sisenis<sup>2</sup>, Oskars Krišāns<sup>1</sup> and Āris Jansons<sup>1\*</sup>

Predictions of future climatic conditions are uncertain and might be influenced by number of different factors. One of the forest management strategies to increase adaptation in such conditions is to diversify – increase number of genetic entries within species or species used. In Latvia relative large areas are occupied by poor, sandy soil, suitable only for Scots pine. Therefore aim of the study was to evaluate potential to use lodgepole pine in those soil conditions.

Study was based on 18 experiments, including 40 lodgepole pine provenances, established from year 1982 to year 1995 using initial spacing 1 × 2 m and block plots with 4–6 replications. Measurements carried out at different age of the trees: 12–29 years.

Results demonstrate considerably and significantly higher average survival and both radial and height increment of *Pinus contorta* in comparison to *Pinus sylvestris*. Therefore also yield and biomass of lodgepole pines were higher. Significant provenance effect was found to all of the traits determining productivity.

There were no differences in time of formation of height increment among pine species, but *Pinus contorta* formed two branch whorls per season in contrast to just one for *Pinus sylvestris*. Therefore number of branches per meter of stem was significantly higher for lodgepole pine as well as proportion of trees with spike knots and double leaders higher and natural pruning rate lower. These traits would limit the use of *Pinus contorta* timber.

Serious deficiency in resistance against local pests or pathogens were not found in any of the trials.

Results indicate possibilities to use suitable provenances of *Pinus contorta* to diversify options of regeneration of areas on poor, sandy soils.

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## NEXT GENERATION BREEDING VALUES

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The current benchmark for genetic value prediction is program-wide multivariate Best Linear Unbiased Prediction of measured traits, from which harvest age traits are predicted and then combined into economic indices. This is a very flexible framework, but is demanding to implement and not many programs have done so. Models for hybrid populations, calculation of standard errors for harvest traits and indices for genotypes, families and larger deployment units, economic models for risk traits, and integration of synthetic variables derived from markers information into evaluation are all in the process of operational adoption. There are other advances, ranging from accounting for indirect genetic effects to integration of genome-wide molecular information for which there is still much work needed to allow them to be used on a program-wide scale. For operational implementation, both the computational systems and their supporting database systems need to be developed to store and process the increasing amounts of information used.

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## MOLECULAR GENETIC TOOLS FOR RESEARCH AND BREEDING OF FOREST TREE SPECIES

*Dainis Ruņģis<sup>1\*</sup> and Ilze Veinberga<sup>1</sup>*

The amount of genetic diversity within forest tree breeding programs is much higher than in crop species. While the development of molecular tools for forest tree species has been lagging behind crop species, there are currently a large amount of resources developed for major species. The large amount of genetic diversity and other population characteristics has been cited as an advantage for the application of recently developed techniques such as association mapping. The application of molecular tools to forest tree breeding is complicated by the long generation times and the implications of clonal forestry. The use of neutral DNA markers can assist tree breeding programs in identification and characterisation of breeding material, but cannot be used to replace phenotypic selection. Gene expression studies have long been used to elucidate the molecular genetic response of organisms to biotic and abiotic stresses. However, while the genetic variation at the DNA level has been widely studied in forest tree species, the variation in gene expression levels has not.

Results from our laboratory indicate that large variations exist within *Pinus sylvestris* breeding material for gene expression, gene copy numbers as well as for other genetic elements such as retrotransposons, all of which may contribute to the adaptive variation being selected for within these breeding programs. Our results have indicated a correlation between induced expression levels and copy number variation of the TLP gene in Scots pine. We have also examined more indirect indicators of genetic diversity and adaptation such as retrotransposon fragment number variation within Scots pine populations. Given the large amount of genetic variation, the heterogeneous genetic background, and the large number of individuals in forest tree breeding programs, the use of DNA assays is preferable to gene expression analyses. Investigation of the variation in gene expression as well as other genetic factors will allow for a better assessment of the amount of adaptive variation found within forest tree breeding programs as well as developing tools for the more efficient utilisation of this variation.

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## HOW SHOULD WE WEIGHT WOOD PROPERTIES AND QUANTITY IN FOREST TREE BREEDING PROGRAMS?

*Harry X. Wu<sup>1</sup>*

Many conifer breeding programs have entered into advanced generation and breeding against the negative genetic correlation (unfavorable relationship between wood quantity and quality) has become one of the biggest challenges in conifer breeding. Both selection strategy and population mating strategy were proposed for balancing wood properties and quantity in forest tree breeding program. For selection strategy, index selection and estimates of appropriate economic weights for breeding objective traits is the most efficient way to weight wood properties and quantity. To develop economic breeding objective, we first need to identify sources of income and expenditure for wood production system, and then to determine tree biological traits influencing costs and revenues for wood production system. Economic weights could be estimated through profit function or by construction of bio-economic model to link wood production system with biological traits (breeding objective traits).

Sawing studies for wood production objective traits are usually required to estimate the economic weights. Estimates of genetic variances and covariances between selection criterion traits at early age and breeding objective traits at rotation age are also required for the construction of selection index. Examples of adverse genetic correlation in conifers are illustrated and case studies using profit function and bio-economic model for estimating economic weights are presented.

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## ASSOCIATIONS BETWEEN GROWTH AND RESISTANCE-RELATED HEARTWOOD TRAITS IN SCOTS PINE

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Until the era of impregnated timber the heartwood of Scots pine (*Pinus sylvestris* L.) was traditional material in constructions prone to decay. Along with increasing concern about the ecological impacts of wood preservation chemicals, the natural durability of wooden structures has become important again.

In Scots pine heartwood timber, the concentration of stilbenes and total phenolics has proven to be a good predictor of resistance against brown rot fungus *Coniophora puteana* (Schum. Ex Fr.) Karst., a cellar fungus. Thus, the concentration of these extractives could be used to indirectly measure durability of wood.

The goal of our studies has been to describe the phenotypic and genetic variation in the traits associated to natural resistance to decay and to estimate their relation to growth traits. To study preconditions of improving the quality and quantity of Scots pine heartwood timber by means of selective breeding, we estimated phenotypic and genetic parameters for stem diameter, wood density, the amount of heartwood and the concentration of stilbenes and total phenolics in the heartwood.

Phenotypic variation in all the traits was large. For stilbenes and total phenolics, as well as wood density heritability was higher than 0.5, and thus a large part of the variation was attributed to genetic differences among the families. As for stem diameter and the amount of heartwood the heritabilities ranged from 0.1 to 0.3, and the environmental effect was more pronounced.

The phenotypic and genetic correlations between stem diameter, wood density, and the heartwood traits were generally small, which indicates them to be mostly genetically independent on each other.

Based on this preliminary study, we conclude that it would be possible to select for trees with a larger proportion of heartwood and better decay resistance without causing a significant loss in volume growth. Possibilities of vegetative propagation will be discussed.

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## **POSTERS**



## **FUNGAL INOCULATION AND METHYL JASMONATE APPLICATION INDUCED HIGHLY VARIABLE TERPENE ACCUMULATION IN NORWAY SPRUCE**

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Spruce bark beetle *Ips typographus* (L.), the most serious killer of mature Norway spruce *Picea abies* (L.) Karsten, is often associated with several phytopathogenic fungi including *Ceratocystis polonica* (Siem.) C. Moreau to overcome tree resistance. Conifers have constitutive and inducible resistance against invader. Induction of terpenoid-based oleoresin has long been considered important in conifer defense against bark beetles, but it has been difficult to demonstrate a direct correlation between terpene levels and resistance to bark beetle colonization. To gain a deeper understanding of conifer defence mechanisms against bark beetle, 60 mature Norway spruce trees in a pure stand were randomly assigned to *C. polonica* inoculation (Cp), methyl jasmonate (MeJA) or control treatment in 2008. Bark sample for chemical analysis was taken from each tree and analyzed by a gas chromatography-mass spectrometry (GC-MS) and a two-dimensional GC. The attacks of bark beetles in all the trees were checked in the end of the season.

Cp inoculation and MeJA application induced very strong terpene accumulation. The terpene levels in the phloem of Cp- and MeJA- treated trees were significantly higher than that of control trees 14 days and 35 days after treatment. Correspondingly, significantly less treated trees died from bark beetle damage compared to control trees. Interestingly, the terpene accumulations and beetle attacks were highly variable among the trees subjected to Cp inoculation. Trees with low induced terpene levels had 20 fold more beetle attacks and 38 fold more beetle tunneling than trees with high terpene levels. Cp inoculation induced terpene accumulation in Norway spruce inhibits bark beetle colonization in a dose-dependent manner, evidencing that terpene induction may be instrumental in tree resistance. In addition, the observed highly variable terpene induction suggests trees differ extensively on their resistance. Further works should be done to select trees with resistance to bark beetle for future forestry.

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## RETROTRANSPOSON-LIKE SEQUENCES EXPRESSED UNDER VARIOUS STRESSORS IN PINE GENOME SHOWS DIFFERENT SIMILARITY LEVEL TO KNOWN MOBILE GENETIC ELEMENTS

*Angelika Voronova-Petrova<sup>1\*</sup>, Āris Jansons<sup>2</sup> and Dainis Ruņģis<sup>1</sup>*

Retrotransposon activity could be a major factor in genome instability, rearrangements and therefore also plasticity of the plant genome and adaptation to changing environmental conditions. This study examines retrotransposon-like sequences in the expression profile of stressed Scots pine (*Pinus sylvestris* L.) trees. Our initial results suggest the existence of several groups of active retrotransposons in the Scots pine genome, which share differing levels of similarity with known elements from other plant species (Voronova *et al.*, 2011). Naturally occurring stresses such as heat shock and insect infection as well as treatment with specific chemicals like abscisic acid and salicylic acid were tested. Sequence analyses and mobile element identification was performed by searching within several nucleotide and protein databases. Retrotransposon-like sequences are widely transcribed as many are also present in EST (Expressed Sequence Tag) databases of various plant species. Searches of nucleotide sequence databases reveal several elements that have characteristic structural features of full-length LTR (Long Terminal Repeat) retrotransposons. The expressed retrotransposon-like sequences isolated after exposure to different stresses were often found to belong to different families of mobile elements. Specific primers were used to evaluate expression of retrotransposon-like sequences between samples subjected to various stresses. The amplification with specific primers shows cross amplification of some fragments. It has not been clarified, how many copies from the identified retrotransposon-like sequences are activated in the complex pine genome, as translation of even a single element can cause transcription of many more non-autonomous retrotransposons with truncated sequences. Further studies of retrotransposon activation in pine could increase understanding of genomic rearrangements in response to stress conditions.

Voronova A., Jansons A., Ruņģis D. 2011. Expression of retrotransposon-like sequences in Scots pine (*Pinus sylvestris*) in response to heat stress. *Environmental and Experimental Biology*, 9: 121–127.

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## **IN VITRO EXPRESSION OF THE RECOMBINANT TLP PROTEIN FROM *PINUS SYLVESTRIS* AND STUDY OF ITS ANTIMICROBIAL ACTIVITY**

*Ilze Gaile<sup>1</sup> and Dainis Ruņģis<sup>1\*</sup>*

One of the most important inducible defense mechanisms is the biosynthesis of pathogenesis – related (PR) proteins. Members of the PR – 5 groups are called thaumatin – like proteins (TLPs) because their amino acid sequences are homologous to thaumatin, a sweet – tasting protein from the West African shrub *Taumatococcus danielli*. Many PR – 5 proteins are induced in plants in response to infection by pathogens, osmotic stress, treatment with abscisic acid, ethylene, salicylic acid and wounding. Members of this group have been shown to have antifungal activity against a broad spectrum of fungal pathogens. Previous results from our laboratory have shown that TLP gene expression increases in *P. sylvestris* after inoculation with *Heterobasidion annosum*; therefore the effect of TLP protein on growth of *H. annosum* was studied.

The *Pinus taeda* TLP gene sequence was used to design primers for amplification of the full-length TLP gene from *P. sylvestris* (PsTLP). The full-length PsTLP has an open reading frame of 606 bp, encoding a protein of 202 amino acids, molecular weight approximately 22.5 kDa, including 16 cysteine residues which are characteristic of L-type TLPs. The disulfide bridges formed by these cysteine residues have important role in maintaining the protein stability and correct folding and preserving high activity under extreme temperatures and pH conditions. In addition, the deduced protein has a thaumatin family signature G-x-[GF]-x-C-x-T-[GA]-D-C-x(1,2)-[GQ]-x(2,3)C. Also sequence has 5 conserved amino acids residues responsible for the acidic cleft with known antifungal activity. This conserved acidic cleft is comprised of five amino acids (R, E, D, D, D) and is believed to be involved in binding to  $\beta$ -1,3-glucan on the fungal cell membrane, resulting in a targeted interaction between host TLP and the fungal cell, which leads to permeabilization of the fungal cell membrane and disruption of the osmotic balance inside hyphal cells, resulting in cell rupture.

To study the effect of *Pinus sylvestris* TLP protein on fungal growth, the coding sequence was expressed *in vitro* in a cell – free expression system. The advantages of *in vitro* translation system include time saving, the possibility to produce proteins that are toxic and the possibility of using PCR products as templates for protein synthesis. This greatly accelerates the protein production process, because no cloning steps are required. In order to examine the effect of the *in vitro* expressed TLP, an agar plug containing mycelia of *H. annosum* was placed in the

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center of agar plates and incubated for 1 week. The PsTLP at various concentrations were applied to sterile filter paper discs surrounding the plugs 1 cm away from the rim of the mycelia colony. After 2 weeks incubation at 25°C, no clear inhibition zones were seen but in the plate with 100 µl protein, *H. annosum* mycelia were lighter and thinner than in the plate with 15 µl of protein. Work is continuing to obtain sufficient amounts of protein for more effective antifungal activity assay and to determine if the expressed His tag may have a negative effect on protein activity. Also, the protein may have incorrect folding or be without necessary post-translational modifications which the *in vitro* expression system cannot introduce.

## **USE OF RESISTANCE-LINKED GENE COPY NUMBER VARIATION ANALYSIS IN SELECTION OF *HETEROBASIDION ANNOSUM* RESISTANT SCOTS PINE**

*Vilnis Šķipars<sup>1</sup>, Viktorija Beļeviča<sup>1</sup>,  
Krista Kānberga-Siliņa<sup>1</sup> and Dainis Ruņģis<sup>1\*</sup>*

Gene copy number variation (CNV) has been found to be common in the human genome, however, in plants, it has not been as widely studied. CNV analysis in *Pinus sylvestris* has been undertaken using real-time PCR in our laboratory. Genes analysed include the thaumatin-like protein (TLP) gene, defensin 2, and *Pinus sylvestris* pinosylvin synthase gene, as well as some genes involved in the lignin biosynthesis pathway. Elevated copy number of the TLP gene was demonstrated to coincide with higher increase of TLP gene expression in inoculated samples compared to samples with lower TLP gene copy number. CNV analysis of material in the pine breeding program will reveal the extent of this variation and enable determination of correlation with resistance. One of the main problems for real-time PCR CNV analysis in Scots pine is the selection of reference individuals with defined gene copy numbers. However, further investigations will allow for the identification of individual trees with pre-determined gene copy numbers, and the use of multiple control genes will increase the confidence of detected CNV polymorphism. In this poster we compare two strategies of molecular genetics assisted selection towards *H. annosum* resistant Scots pines – use of gene copy number variation compared to use of gene expression analyses.

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## EXPRESSION OF PHENYLPROPANOID PATHWAY GENES IN SCOTS PINE (*PINUS SYLVESTRIS* L.) IN OPEN-POLLINATED FAMILIES WITH DIFFERING WOOD DENSITIES DURING EARLY AND LATE WOOD FORMATION

*Krista Kānberga-Siliņa<sup>1</sup>, Āris Jansons<sup>2</sup> and Dainis Ruņģis<sup>1\*</sup>*

Scots pine (*Pinus sylvestris* L.) is a species of great economic importance in the Northern hemisphere. Wood volume and quality are the most important aspects of commercial forestry production, and studies of wood formation are becoming more important in order to increase the value and efficiency of forestry production. The phenylpropanoid pathway produces various compounds with diverse functions both for plant defence against biotic and abiotic stress as well as structural development. One of main roles is monolignol production for lignin biosynthesis, which is a crucial aspect of wood formation. For this study three candidate genes involved in lignin biosynthesis were selected: phenylalanine ammonia-lyase (*PAL1*), cinnamyl alcohol dehydrogenase (*CAD*) and cinnamoyl-CoA reductase (*CCR*). Wood density was measured because it is one of main wood quality parameters. Candidate gene expression was studied in selected open-pollinated Scots pine families in which wood density was measured to investigate the expression of these genes during early wood (EW) and late wood (LW) formation and whether correlation between these genes and wood density can be established. Wood density values for selected trees were similar within tree families but differed significantly between families with high and low wood density ( $p=1,06E-20$ ). Significant gene expression differences between early and late wood formation was detected for the *CAD* gene in trees with low wood density ( $p=0,00179$ ). It was three times lower during late wood formation compared to early wood formation. Expression of the *PAL1* gene was five times higher during EW formation in trees with higher wood density. A positive correlation was detected between *PAL1* and *CCR* gene expression during EW formation ( $r=0,804$ ) and during LW formation ( $r=0,466$ ).

**Keywords:** *CAD, CCR, Scots pine, PAL1, Pinus sylvestris, wood density*

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## IDENTIFICATION OF MICROORGANISMS FOUND IN DISCOLOURED BIRCH (*BETULA PENDULA* L.) WOOD

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'Latvijas Finieris' manufactures plywood exclusively from birch wood. Discolouration of birch wood does not affect the structural properties of the plywood; however, it decreases the value of the product as lighter coloured plywood is more desirable. Wood discoloration has been studied in birch and other tree species, however, the causal microorganism and mechanism of discolouration has not been fully elucidated. Discolouration of wood can be enhanced by artificial or natural branch thinning, which presumably allows microorganisms to colonise the stem. In this study, we utilised two approaches to study the microorganisms found in discoloured birch wood.

The first was PCR amplification of total DNA extracted from birch wood using universal ribosomal fungal primers, followed by sequencing of cloned PCR products and comparison to sequence databases. The second approach utilised denaturing gradient gel electrophoresis (DGGE) to compare fungal and bacterial profiles between discoloured and normal wood. In addition, we utilised traditional microorganism culturing techniques to isolate fungi and bacteria from discoloured birch wood. These isolated microorganisms, as well as further fungi ordered from the CBS Fungal biodiversity centre (Utrecht, Netherlands) were used for inoculation experiments on birch wood blocks as well as living trees. The interaction of birch wood extracts with microorganisms was also examined. During thinning in experimental birch plantations, discoloration of wood was identified already in 10 year-old birch trees. In addition, variation in discoloration was found between families, indicating a possible genetic component, and allowing for the possibility of breeding for reduced susceptibility to discolouration.

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## GENE EXPRESSION OF DEFENCE RELATED GENES IN PARENCHYMA AND RESIN DUCT SAMPLES OF SCOTS PINE OBTAINED BY LASER CAPTURE MICRODISSECTION

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Laser Capture Microdissection (LCM) in combination with gene expression analyses can give insights in expression of specific genes in different tissue types, even small groups of cells like resin ducts. Still this approach is not widely used in research of conifers. Use of LCM in combination with RNA amplification and gene expression analysis for characterising Scots pine thaumatin-like protein gene (*TLP*), defensin 2 (*Def2*), pinosylvin synthase (*PsBBs*), and *Pinus sylvestris* resistance gene (*PsACRE*) expression in parenchyma and resin ducts is described. Results were compared to results obtained from whole cross-section cut samples processed without RNA amplification. We find that trends of gene expression changes in response of wounding and inoculation with *Heterobasidion annosum* are similar for LCM and whole cut samples. Results presented in this work indicate increase of expression of *TLP* and *PsBBs* genes after wounding and inoculation and no change or decrease of expression of the *Def2* and *PsACRE* genes.

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## **MOST SIGNIFICANT DENDROPHAGOUS PESTS FOR LATVIAN FORESTRY, REVIEW OF INVASION AND OUTBREAK RISKS IN RELATION WITH CLIMATE CHANGES IN NEAR FUTURE – NEXT 50 YEARS**

*Agnis Šmits<sup>1</sup>, Ingars Siliņš<sup>1</sup> and Āris Jansons<sup>2</sup>*

There are almost countless forest pest species known to science. We did literature survey that contains information about 36 most significant dendrophagous pest species which are abundant or potential to Latvian region. Those species are selected by studying their potential of harm to forestry by managing a lot of information (2 543 internet sources and 136 scientific publications).

In this literature survey we concentrated information on 14 most aggressive forest species: *Altica ambiens*, *Agelastica alni*, *Fenusa dohrnii*, *Xiphydria camelus*, *Lymantria dispar*, *Lymantria monacha*, *Ips typographus*, *Hylobius abietis*, *Bupalus piniarius*, *Neodiprion sertifer*, *Euproctis chrysorrhoea*, *Diprion pini*, *Panolis flammea*, *Dendrolimus pini*. We included information about biology, mass outbreaks and natural enemies of each species. Also information about pest and therefore natural enemy affecting climatic conditions were summarized and taken in consideration.

We found that coleopterids such as bark, leaf beetles and weevils are mostly affected by parasitoid wasps and cold resistant nematodes, but moths usually are affected by NPV (nucleopolyedroviruses) and also by parasitoid wasps. Relatively high air humidity (more than 70%) and precipitation with combination of cold weather significantly affects a pressure on parasitoid wasps, but enhance invasion by NPV. Therefore, long, dry periods negatively affects pest mortality by nematodes. Also we found that most of reviewed pests can tolerate heat near up to +40 °C and cold near under -20 °C. Interesting that only 5 species of the list was potentially invasive – meanwhile the biggest outbreak risk is expected from native species.

By looking at available information about pest outbreak cycles and known trends in climate change, – in this information research we concluded that coniferous trees will be affected firstly by increased pest outbreaks and invasive alien species will be more abundant in Latvian region in next 50 years. Based on our study, we believe that obtained information are usable for modelling dispersal of reviewed species.

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## SILVER BIRCH (*BETULA PENDULA* ROTH.) PROVENANCE DIFFERENCES IN TRIAL IN LATVIA

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Birch dominates significant part of Latvia's forest stands and has a high economic importance due to processing capacity (mainly plywood industry) developed in our country. Most of the birch stands are regenerated naturally, without much attention paid to the traits of seed source (stands or solitary trees). During the last decade demand for birch seed material for plant production has been increasing, especially for regeneration of former agricultural lands. It has driven interest to use also foreign seed material. Results from provenance trials could address both the importance of selection of seed sources for natural regeneration and possibilities for seed material transfer. However, only few provenances tests are established in Latvia, since until the end of 1980<sup>th</sup> the potential of this species was neglected and no active breeding work had been carried out. Aim of the study is to analyze the existing material in order to characterize differences between provenances in productivity and quality.

Study is based on results from trial, established in central part of Latvia (56°N, 24°E) that consists of 16 geographically distant provenances from Poland, Latvia and Finland, covering the latitude range from 52°N to 61°N. Provenance is represented by a single stand where seeds from randomly selected 20 trees are collected.

Trees are located in *Hylocomiosa* forest type in 6 replications, using 24 tree block plots. Initial spacing 2×1.2 m, one year old seedlings used, no thinning carried out prior to measurements at the age of 37 years.

To calculate significance ANOVA and Chi-Square tests have been used.

Analysis reveals that provenance is a statistically significant factor affecting survival of the trees, ranging from 11.1% (provenance Sääksmäki, Finland) to 62.5% (Saldus, Latvia). Average survival was the highest for Latvian provenances (50%) followed by Polish (41%) and Finnish (16%).

Provenance was a statistically significant factor influencing also tree height, diameter and stem volume. Average tree height at the age of 37 years ranged from 12.1 m to 20.3 m, birches from Latvia being significantly higher ( $18.2 \pm 0.5$  m) than those from Poland ( $15.8 \pm 0.7$  m) or Finland ( $14.7 \pm 1.4$  m). Height growth in the trial on average was slower than that observed in birch stands on fertile soils in Latvia (based on data from 258 NFI sample plots) indicating the relative poor soil conditions for the species in the trial). Set of dominant trees (tallest trees within each block-plot with density corresponding to 520 per ha) reveals even larger

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difference between provenances but the trend remains unchanged. Provenance ranking by tree height corresponds well with that observed already at the age of 5 years ( $r=0.87$ ) indicating possibilities for rather early elimination of worst-performing material. Similar differences as for height are observed also in analysis of tree diameters: Latvian birches are thicker ( $13.0\pm0.5$  cm) than Polish ( $10.7\pm0.7$  cm) or Finnish ( $9.9\pm1.2$  cm).

Observed variances in these parameters are a cause of notable differences in yield ranging from  $49\text{ m}^3\text{ ha}^{-1}$  to  $298\text{ m}^3\text{ ha}^{-1}$ . Taking into consideration that the data about survival in the first years after trials establishment were not available and that there could have been random factors affecting the result, the yield was recalculated, assuming a 30% survival rate (corresponding to  $1200\text{ trees ha}^{-1}$ ) – the density close to that recommended by thinning models. The differences between groups of provenances from particular countries decrease in this case but the tendency remains the same. In comparison with results in ordinary birch stands on fertile soils, the yield is higher in trial ( $298\text{ m}^3\text{ ha}^{-1}$  vs  $228\text{ m}^3\text{ ha}^{-1}$ ), however, if the survival is reduced to 30% assuming the same average tree diameter – lower. Significant differences between Latvian birch provenances were detected in traits determining productivity.

Proportion of trees with spike knots ranges from 11% (Finnish) to 20% (Latvian) and 23% (Polish). For particular Latvian provenances it is from 12% to 30% and does not have a significant correlation with tree height.

Results indicate the best performance of local birch provenances and the need for selection of appropriate seed sources (at provenance level) also if natural regeneration is considered to ensure high quality and productivity of the next generation stand.

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## DIFFERENCES OF LATVIA'S SILVER BIRCH (*BETULA PENDULA* ROTH.) PROVENANCES

*Arnis Gailis<sup>1</sup>\*, Juris Augustovs<sup>1</sup>, Andis Purviņš<sup>1</sup> and Āris Jansons<sup>1</sup>*

Birch is one of the most widespread tree species in Latvia (30% from the area). During the past century it was not viewed as valuable tree species in most of the countries around the Baltic Sea, therefore limited number of experiments were carried out. In Latvia, intensive breeding process of the species was started from the year 1995 with selection of candidate-stands, their inventories and selection of plus trees for establishment of seed orchards and progeny trials. Currently these two seed orchards cover almost all the demand for birch seeds that has been constantly growing mainly due to afforestation of former arable lands and, in recent years, also planting in forest. Established progeny trials can provide information not only for selection of new set of tested seed orchard clones, but also general trends in comparison of provenances across Latvia. Aim of the study is to analyze the differences and geographical patterns of provenances.

Material was collected from plus trees and good quality trees from 37 stands representing all regions of Latvia. In 3 trials altogether 921 open-pollinated family was used, majority of them were represented in two (331) or all three (316) trials, but portion (274) only in one trial. Therefore number of families per site ranges from 613 to 653. Design was either large block plots (32 trees) in 4 replications or single tree plots. All trials were established on abandoned agricultural land with initial spacing 2×2 m or 2×2.5 m using one-year-old container plants.

For the analysis only 6 or 10 highest trees per block-plot or family were used.

Results reveal that average tree height in different test sites reaches 6.3 m to 7.6 m, diameter of the thickest branch up to two meters height is on average 10mm and branch angle in the middle part of the green crown 45°. Frequency of trees with spike knots varies greatly among trials due to both genetic and environmental reasons: from 14% up to 38%.

The differences in mean height of each population, representing the productivity, were plotted against the distance among populations independently from the direction. Results reveal no specific trend, indicating, that specific characteristics of particular populations (or trees representing them) are more important than the geographical distance in such a relatively small area as Latvia.

Statistically significant height differences were found between populations from western and eastern provenance regions in all test sites ranging from 2.6% to 5.5%. Larger differences were found among populations in particular locations, reaching up to 11-25%.

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Population effect was significant to all analyzed productivity and quality traits. However, also very large within-population variation was detected, indicating a notable potential in further selection, that is in line with relatively high heritability's coefficients for the traits of interest: for height  $h^2=0.40$ , for branch angle  $h^2=0.32$ , for branch diameter  $h^2=0.18$ .

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## EFFECT OF WOOD ASH AND WASTE WATER SLUDGE FERTILIZATION ON INGROWTH AND ANNUAL INCREMENT OF POPLAR CUTTINGS

*Dagnija Lazdiņa<sup>1\*</sup>, Arta Bārdule<sup>1</sup>, Mārtiņš Zeps<sup>1</sup> and Andis Bārdulis<sup>1</sup>*

Aim of study is to estimate suitability of poplar coppice's plantations for energy wood production and reaction of cuttings ingrowth and growing to fertilization under first growing season.

Four Italian poplar clones (AF2, AF6, AF7, AF8) and local introduced at previous century was planted in May 2011. Field was fertilized with 10 t<sub>DM</sub> ha<sup>-1</sup> waste water sludges from local municipality and stabilized mixed soft and hard woods ash 6 t<sub>DM</sub> ha<sup>-1</sup> from city boiler house. Experiment was carried in three replicates. The fertilizers were milled at soil layer depth of 0–20 cm. Management of weed (mowing of weeds) was done twice. Cutting dieback during vegetation season 2011 and annual increments was analyzed in spring 2012.

Wood ash fertilization improved growth and surviving of poplars clones AF 7 and AF 8 at first season. Fertilization of waste water sludge had effect of minor growth increase to clone AF2, but significantly decrease growth of clone AF 8 and survivability of all clones. Waste water contains a lot of N that caused more vigorous shoots which were later on affected by *Melampsora medusae*.

Clone AF6 had better results at control plots. Wood ash increase survival rate of clones AF2, AF7, AF8, but differences were not significant.

Frost damages was not detected, just some minor damages caused by artiodactyla, mainly in parts more closely to forest and here, no significant different effect between fertilizers.

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