Towards a European Douglas-fir breeding population for long term breeding

Daniel Michaud, Jean-Charles Bastien, Leopoldo Sanchez Rodriguez

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CONTENT

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ORAL PRESENTATIONS
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Keynote Lecture
Climate change and agile tree breeding

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The apparent misalignment between forest tree populations' migration rates and environmental changes, mediated by climate change, necessitated human intervention through the physical movement of genotypes to new environments (assisted migration), thus newly established populations would develop in favourable conditions. This process is critical and perfect matching between the moving genotypes and the constantly changing environments is required for maladaptation avoidance. To reduce any detrimental effect caused by: 1) movement of trees to a contrasting thermal environment, 2) drastic photoperiod shift, and 3) epigenetic aftereffects associated with reproductive material transfer; here I propose an *in situ* selection approach at the species' leading latitudinal front for minimizing poleward movement. Under this scheme, existing natural northern peripheral populations will play a dual role in providing already tested and adapted genotypes to fluctuating environment and selection will be exclusively based on adaptive attributes such as cold and drought tolerance and timing of growth initiation and cessation, attributes known for their high genetic control, thus spatial and age effects are minimal. Genetic evaluation of the selected individuals will be based on affordable large batteries of anonymous DNA markers obtained through NGS methods and innovative genomic-based quantitative genetics analytical approaches such as genomic best linear unbiased prediction and genome-based prediction methods (genomic selection) that simultaneously utilize large genomic information to explain the observed phenotypic variability of complex polygenic traits. The proposed genetic analyses are perfectly suited to unstructured populations where prior knowledge on genealogy is often lacking. The selected individuals, in turn, would form the raw material for the production of adapted stock for planting at the new favourable environmental conditions often located at the species' latitudinal front edge. A brief review of the classical recurrent selection scheme and examples of the genomic-based evaluation methods using structured and unstructured forest tree populations will be presented.

Keynote Lecture
Seed orchards and supporting breeding

Dag Lindgren
Swedish University of Agricultural Sciences, UPSC, Sweden

Seed orchards are the main outlet and work horse for forest tree breeding. Seed orchard programs should be supported by long term breeding, this lecture assumes this has been going on for some decades.

Seed orchards often use grafted genotypes with high estimated breeding values. With Norway spruce, where vegetative propagation of juvenile material is easy, current seed orchard deployment is based on clonal tests. For Scots pine progeny tested genotypes have been common, but progeny testing is a painfully slow for advanced generation breeding. Recent orchards are sometimes a mixture of tested clones and untested offspring from good parents. It seems inefficient to avoid a low percentage of related clones in advanced generation seed orchards.

Very small sublines for complete elimination of relatedness does not seem optimal, but options to select unrelated should decline rather slow over generations and to some degree remain forever.

The "optimal" number of unrelated clones is often in magnitude twenty. Higher number of clones may be more common when clones are related.

It is efficient to deploy clones in optimal proportions, it is not recommended to strive for exactly equal proportions.

Long term tree breeding benefits from "breeding populations" large enough to allow among parent (among family) selection. Synchronisation of breeding stock management and seed orchard establishment is desirable.

There are often advantages in deploying new cohorts of recently selected clones. Seed orchards become often too old considering the advancement in breeding stock.

Genetic thinning is seldom worth the trouble, but selective harvest seems an attractive option.

Pollen contamination reduces gain, a general remedy has not been found. Crops from young "contaminated" seed orchards may be competitive to mature genetically outdated seed sources.

Seed harvest cost is a major management concern.

Keynote Lecture

Opportunities for plantation deployment and genetic improvement of hybrid forest trees

Gary R. Hodge
Department of Forestry and Environmental Resources, North Carolina State University, USA

Hybrid trees from a variety of genera (e.g., *Pinus, Eucalyptus, Populus,* and *Larix*) have been used for many years, but only occupy a small fraction of the total plantation area in the world. With climate change, increasing disease threats, and economi c forces pushing commercial forestry to more marginal sites, hybrid varieties offer some advantages in adaptation, growth, disease resistance, and wood properties. In the past 15 years, Camcore (N.C. State University) has produced a total of 23 pine hybrid combinations and a total of 66 field trials have been established in South America and Africa. Several of these hybrids show commercial potential, including *P. caribaea x P. tecunumanii, P. greggii x P. tecunumanii*, and *P. patula x P. tecunumanii.* Forestry companies in South Africa are beginning to plant the *P. patula x P. tecunumanii* hybrid operationally. The hybrid has an advantage over the pure species *P. patula* in terms of resistance to *Fusarium circinatum*, but it also offers advantages in growth and wood quality. A cooperative breeding and testing effort for this hybrid, involving eight organizations in southern Africa, has been organized by Camcore. Eucalypt hybrids also have a long history of commercial use in forestry, with perhaps the best known being *E. grandis x E. urophylla.* There is an overwhelming number of potential hybrid combinations that might be of interest, so collaborative efforts to make eucalypt hybrids of a number of interesting combinations are being initiated. There are substantial differences between eucalypts and pines in terms of reproductive biology, amenability to vegetative propagation, and genetic architecture which impact hybrid breeding, testing and deployment strategies. Despite long experience with hybrids, many important questions remain unanswered, about such things as reproductive barriers to hybridization, the quantitative genetics of hybrids, and optimal breeding strategies that should be used.

Keynote Lecture

Back to the forest: Predicting evolutionary change under environmental change

Antoine Kremer
Functional ecology and genomics, INRA, France

Along the current hot debate on adaptation of trees under climate change, the need for monitoring or predicting evolutionary change *in natura* has become of utmost importance. There is indeed experimental evidence that trees may undergo significant genetic change at contemporary time scales for various traits, based on the large body of experimental data demonstrating the wide genetic variation residing in natural tree populations. The main questions however remain as to which trait will actually be affected by climatic change, and how large the genetic change will be. I will extend traditional quantitative genetic approaches implemented under controlled experimental designs (progeny tests) and elaborate on the estimation of genetic parameters that would allow to make predictions of genetic changes *in situ* under so called natural conditions. Under directional selection driven by climate change, theoretical predictions of genetic changes will depend on the heritability of the trait and the selection gradient, but will be buffered by the amount of gene flow with neighbouring populations. I will further examine how heritability can be estimated by using recent approaches based on genomic predictions of (i) genetic relatedness in stands and (ii) linkage disequilibrium between QTLs controlling traits of interest. Similarly, the selection gradient may also be estimated *in situ* by using parentage analysis. Finally, the interplay between directional selection and gene flow will be assembled in predictive equations. Theoretical predictions will be illustrated by results obtained for phenological traits and *in silico* data derived from simulations.

OA 01

Performance of seven tree breeding strategies under inbreeding depression

Harry Wu[1], Henrik Hallingbäck[1], Leopoldo Sánchez[2]
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Examination of breeding strategies for tree species is more relevant than for other crops due to the long generation times in tree breeding, and the impracticality of conducting multiple generation experiments. The seven breeding strategies (single breeding population with mass selection (SBPM), single breeding population within-family selection only (SBPW), subline strategy (SUBL), self and crossbreeding with single seed decent in lines (SELFL), selfing and crossbreeding in a single population (SELFP) and two slightly different nucleus strategies (NUC) were compared using a finite locus model for short (3-5), intermediate (5-15) and long-term (15-20 generations) breeding. 100 loci having additive, partially dominant or completely dominant allelic effects were simulated with intermediate, U-shaped allele frequencies, and with minor and major detrimental allelic effects.

Results indicated that genetic gain for the SELFP was the highest only during the first 4 to 5 generations and only for additive allele model. In contrast, both SELFL and SELFP showed considerable long term genetic losses under a complete dominance model. However, genetic gains in the production populations for SELFP were superior for the first 2, 3 and 4 generations for complete, partial dominance, and additive gene model, respectively, with one or two additional generations of superiority under a major locus model. For intermediate and long term breeding, NUC and SBPW strategies were superior to SUBL and SBPW. The fixation of unfavorable alleles and inbreeding accumulation were found to be a main issue for SELFL and other strategies, respectively. Reducing the fixation of recessive alleles and selection of both superior inbred lines and hybrids has potential for successfully implementing inbreeding and crossbreeding in short-term. For intermediate and long-term tree breeding, nucleus breeding or single breeding population would be the best choices given the results of this study.

OB 02

Genetic structure and diversity in two Norway spruce seed orchards of different size

Jørn Henrik Sønstebø, Arne Steffenrem,
Mari Mette Tollefsrud
Norwegian forest and landscape institute, Norway

The use of improved seeds from Norway spruce seed orchards in commercial plantings has recently reached an average of more than 75 % in Norway, and in the south-eastern part of Norway the proportion amounts to approximately 96 %. The current Norwegian seed orchards consist of large numbers of clones, mainly plus trees. To meet the demand for high and stable seed production as well as higher genetic gains, the future orchards will include fever and tested clones. With the present high proportion of seed orchard seeds in silviculture it is important to evaluate possible effects on the genetic diversity and structure of Norway spruce populations.

Here we have used microsatellite variation to study the effective population size, genetic diversity and genetic structure of seeds produced in two Norwegian seed orchards at contrasting seed years and compared these to stand seeds and natural forest within their seed zone. One orchard contains only 25 clones and is located in an area with no natural spruce forest. The other orchard has 60 clones and is located in an area with high density of natural spruce forest.

We found a reduction in genetic diversity of the seeds produced in the seed orchard with low number of cloned compared with the orchard with high number of clones. This effect was larger in years with poor seed production. The seed from both seed orchards were significantly different from the stand seeds. However, the differentiation was generally low. The implications of these findings for the breeding activities and forest management will be discussed.

OA 03

A comparison between two hybrid breeding strategies employed in the the production of Eucalyptus grandis x Eucalyptus urophylla in South Africa

Gert van den Berg[1], Steve Verryn[2], Paxie Chirwa[3], Francois van Deventer[1]
[1]Tree improvement research, Mondi limited, South Africa; [2]Creation Breeding, South Africa; [3]University of Pretoria, South Africa

In order to combine the good survival, disease tolerance and higher wood density of *E. urophylla* with the rapid early growth of *E. grandis*, hybrids of the two species have been produced in South Africa. Currently two main hybrid breeding strategies are used to test hybrid material. Both strategies make use of general combining ability (GCA) to select elite parent trees from pure species of main populations. These elite parent trees are then use for controlled pollinations to generate hybrid seed. The seed is then tested either with or without a hybrid seedling testing phase, i.e.:

- In a series of hybrid seedling progeny trials from which individuals are selected based on four year growth results and then tested as clones in clonal trials, or
- Established in hedges from which cuttings are produced and tested as clones in clonal trials (without going through a seedling testing phase).

The purpose of this study is to do a comparative study between these two strategies. The key focus areas of this study are to compare genetic parameters and phenotypic correlations between pure species parents, *E. grandis* x *E. urophylla* hybrid seedlings and *E. grandis* x *E. urophylla* hybrid clones that derived from these parents and some these results will be presented. Data from a total of seven *E. urophylla* and nine *E. grandis* main population trials, seven *E. grandis* x *E. urophylla* hybrid seedling progeny trials and 29 *E. grandis* x *E. urophylla* clonal trials are included in the analysis. Future work will utilise these results to structure and model an alternative theoretical strategy to optimise genetic gain and production.

OB 04

Effect of pruning and stand density on cone and pollen production in Pinus sylvestris seed orchards

Curt Almqvist
Skogforsk (The Forestry Research Institute of Sweden), Sweden

Seed orchards are the production population of a breeding programme and produces improved seed used for regeneration. Therefore knowledge about progression and annual variation in fertility of the seed orchard crop are important for seed orchard managers already at time of planning of the orchard. At the stage of planning decisions on stand density are taken. Later on managers have to decide at what height to start the shaping of the crown of the orchard trees.

Results from the 22 first years of an experimental seed orchard with in total 16 different combinations of stand density and targeted pruning height are presented.

Pollen production starts at the same orchard age for all studied combination of stand density and target height. However, pollen production level increases faster in treatments with higher stand density. Treatments with dense spacing will reach production levels worthy to harvest earlier than treatments with lower stand density. In dense spacing and low target height treatments cone and seed production reach the same levels for the whole study period as for treatments with lower stand density and higher target height. Seed production of the same magnitude can be obtained with quite varying combinations of stand density and target height, thus, giving possibilities for the SO owner/manager. A management strategy starting with dense spacing and low target height followed by thinning and increased target height will probably have the largest per hectare production over time.

OA 05

Acceleration of genetic progress in tree improvement programs with modest financial support

Milan Lstibůrek
Faculty of Forestry and Wood Sciences, Czech University of Life Sciences Prague, Czech Republic

Majority of tree improvement programs worldwide are based on relatively sparse financial platform. More recently, theoretical development of low-cost substitutive breeding strategies (Breeding without Breeding, BwB) capitalized on the availability of polymorphic genetic markers in forest trees, development of pedigree reconstruction methods, and advancements in genetic evaluation techniques. These strategies have been considered in some countries as alternatives to traditional tree improvement schemes that are economically demanding due to elaborate full-sib crosses and progeny trials.

Artificial regeneration in the Scots pine (commercially the 2nd most important forest tree species in the Czech Republic), is predominantly dependent on genetically improved seed from seed orchards. However, only the first generation orchards are currently operational in the Czech Republic. The pilot project was therefore conducted by the Czech University of Life Sciences Prague, the Forests of the Czech Republic, s. e., and the Ministry of Agriculture of the Czech Republic. Half-sib progeny trials (established to assess the genetic merit of original plus trees) were converted into full-sib trials. Next, the forward selection was applied to establish the second generation seed orchards in the Southern and Western part of the Czech Republic. Considerable cost and time savings were associated with this approach.

Practical lessons learned directly in this project will be confronted with theoretical expectations. Much wider use of the BwB strategies in different programs and countries can be recommended based on both theoretical predictions and operational feasibility.

OB 06

OPSEL – A tool for optimizing expected gain from clonal seed orchards while constraining relatedness and diversity among parents

Tim Mullin, Curt Almqvist, Torgny Persson
Skogforsk (The Forestry Research Institute of Sweden), Sweden

Tree breeders must often consider conservation of genetic diversity, while at the same time maximizing response to selection. In the case of seed orchards, the buyer of seed wants maximum performance, while satisfying a restriction, sometimes legislated, on the diversity deployed to the forest. “Group coancestry”, “status effective number” and “proportional gene diversity” are mathematical synonyms expressing average relatedness among individuals in a population using the concept of “identity-by-descent”. Optimal selection will not completely avoid kinship, but rather maximize gain while imposing a constraint on average relatedness. In the case of 1st-cycle orchards, where selected genotypes are unrelated, it has been shown that genotypes are best deployed so that their frequency is “linearly related to their genetic value”. Here we present the application of semidefinite programming (SDP) as a flexible approach to optimize the deployment of genotypes to a clonal seed orchard. We formulate the selection problem as an SDP, where average breeding value is to be maximized, while imposing constraints on relatednes s, as well as maximum and minimum contributions from each candidate. An open-source solver, SDPA, was embedded into a tool designed to make the optimization of seed orchards by SDP simple and flexible. A case study optimizing a Scots pine seed orchard illustrate how this flexibility can be used to impose additional constraints on the scion material available from some candidate genotypes, and optimize selection even when related candidates have varying degrees of coancestry among them. Additional situations where SDP can be employed are discussed.

OA 07

New models for the definition of Scots pine deployment areas in Sweden and Finland.

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Bengt Andersson Gull[3], Mats Berlin[3]
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The present deployment areas of Scots pine (*Pinus sylvestris* L.) in Finland are defined by models from earlier decades. These models are not based on extensive empirical field trial data. In these models transfer is expressed by the change in temperature sum from the seed source to the deployment site.

Cooperation was started between SkogForsk, Metla and the Swedish and Finnish Meteorological Institutes to produce new models for both growth and survival of Scots pine in Sweden and Finland. The models are based on the most recent small grid climatic data, and can react on various climate change scenarios. Field data of 152 trials from both countries were used, including provenance material with a wide geographical distribution and data on both growth and vitality.

The relevant parameters of the most significant survival model (r^2=0,595) are: year of establishment, age, annual mean temperature, altitude and latitudinal transfer. For the height model (r^2=0,844) they are: year of establishment, age, temperature sum and latitudinal transfer.

The main conclusions of the project are: 1) having a common model for both Sweden and Finland is well-founded and 2) a transfer effect driven by change in latitude has higher significance than when driven by change in temperature sum.

The effect of these models on the deployment areas of Finnish seed orchards will be demonstrated. Deployment areas may expand when compared to the present ones, especially southward. The models will be implemented in a decision making tool that allows selection of the optimal seed source for any specific location.

OB 08

Reduced background pollination using high tunnels

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Isolation tunnels were built in a Scots pine seed orchard "Västerhus" (63°18'N, 18°32'E) with the aim to reduce background pollination (BP). The idea was to create reproductive isolation from surrounding trees by inducing earlier flowering through increased temperature sum within the tunnels and to simultaneously form a mechanical barrier for background pollination.

The experiment comprised of two replicates of five treatments: tunnels (T_o), tunnels with fan (T_f), tunnels with supplemental pollination (T_{sp}) and two levels of unprotected controls adjacent to (C_a) and distant from (C_d) the tunnels. The tunnels were in place from mid-May to midsummer 2010, 2011 and 2012 and each unit covered 11-13 ramets.

All experimental units were established within a group of ten parents, of which four were represented in each unit. Supplemental pollen used in the T_{sp} treatment originated from five parents off the studied group. Seeds from one ramet of the four parents were analyzed in each unit using nuclear microsatellite markers to determine their paternal parentage (35 and 60 seeds in 2011 and 2012 crops, respectively).

Trees in tunnels flowered approximately one week earlier and had 10-25% higher seed weight in 2010-2013 crops than control trees. No BP was detected in any of the six tunnels in the two years whilst it was estimated to be 4.4% and 6.3% in controls in 2011 and 2012, respectively. In T_{sp}, supplemental pollen sired 60% and 48% of seeds in 2011 and 2012, respectively. Selfing was rather high in T_0 and T_f, collectively amounting to 25% and 17% in 2011 and 2012, respectively; in controls and T_{sp} it was only 1.4% and 8.5% in 2011 and 7.5% and 9.9% in 2012. Data from 2013 crop are being analyzed and will be included at the conference.

TUESDAY, AUGUST 26, 2014

Keynote Lecture
Clinal variation and the genetic basis of adaptive traits in trees

Martin Lascoux
Evolutionary Biology Centre, Uppsala University, Sweden

Identifying the loci underlying the variation in quantitative traits and detecting the selection acting on them remains, to this day, one of the main challenges in biology. Genome wide association studies (GWAS) have become the main approach to identify the genetic factors controlling complex traits. Limitations of GWAS have, however, started to become evident and different strategies have been offered to alleviate those. In particular, GWAS have limited power unless very large datasets are used. They therefore remain prohibitively expensive, and often not so informative, for non-model organisms with limited or nascent genome resources such as forest trees. So, at least in the short term, a more targeted strategy, combining common gardens, population genetics, physiological and expression studies of candidate genes remains a very fruitful alternative. We will illustrate this with recent studies of clinal variation in phenological traits in forest trees, with special focus on boreal conifers, oaks and poplars. Some general features emerge from these different studies: first, they confirm the presence of strong latitudinal clines in phenological traits such as growth cessation. Second, most of the studies highlight the importance of genes belonging to the photoperiodic pathway and the circadian clock in the control of growth cessation. For instance, in both Norway and Siberian spruces, FTL2, a homolog of one of the main integrators for flowering time in *A. thaliana*, exhibits evidence of local adaptation and significant latitudinal variation in expression. Finally, the study of parallel clines in phenological traits, offers a first glimpse at the importance of parallel or convergent evolution in forest trees. They thereby provide us with a bit of information on the genetic architecture of important adaptive traits.

Keynote Lecture
Community genetics of poplar as a keystone species for insect and fungal species

M.J.M. (René) Smulders
Plant Breeding, Wageningen UR, Netherlands

Community genetics aims to understand how genetic variation within and among populations of host species affects the composition of associated organisms (insects, fungi, and other organisms) interacting with the host species. Community genetics has been stimulated by pioneering work on poplars (Populus spp.), linking their genotypic and phenotypic variation to variation in associated communities. The future of community genetics will be to identify the genes that underlie the traits of hosts that affect the presence or abundance of associated organisms. This can be done by e.g. quantitative trait analysis (QTL) mapping and genome-wide association studies (GWAS). I will present some results from the Evoltree projects on community genetics in poplar and oak. In one of the experiments the F2 offspring of a hybrid poplar (Populus trichocarpa x P. deltoides) cross were assessed for seven categories of insect leaf damage at two time points, June and August. The seven forms of leaf damage were used as a proxy for seven functional groups of insect species. Significant variation in insect association was found among the hybrid offspring, including transgressive segregation of susceptibility to damage. QTL analyses identified 14 genomic regions across 9 linkage groups that correlated with specific insect association. The results provide insight into the genetic components involved in insect community structure in a fast-growing forest tree.

DeWoody J et al. (2013) Insight into the genetic components of community genetics: QTL mapping of insect association in a fast-growing forest tree. PLoS ONE 8: e79925. Gugerli F et al. (2013) Community genetics in the time of next generation molecular technologies. Molecular Ecology 22: 3198–3207.

Evoltree Lecture

Collecting tree phenotypes in the field – TreeType: a new, open-source project

Stephen Cavers[1], Santiago Gonzalez-Martinez[2], Members of the OSP working group EVOLTREE
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In the age of easy next-generation sequencing, a lack of good phenotypic data is a major bottleneck in tree genetic research. Although a lot of data is collected from experimental trials, much less is routinely collected from the very different conditions of forest populations in the field. Yet such data can provide important insights into the balance between local adaptation and phenotypic plasticity in tree populations. A new initiative from EVOLTREE will attempt to kick-start widespread collection of data on some simple phenotypic traits for European trees. The project will be open to participation by anyone with the enthusiasm and skills to record the data for the trees of their choice and data will be made openly available. We will create an online recording website, providing standard protocols and guidance, and making it easy to upload new records to a common database and track the progress of data collection for your species. A simple voting tool will allow the proposal of new species to record, with data recording initiated once a critical mass of recorders has been reached. The project will run for an initial trial period of two years, with a critical review at the end of that time – if the idea is taken up enthusiastically by the community, the project may continue and a valuable long term resource could be created!

Evoltree Lecture

New genomic tools to enhance discovery of functional diversity in natural populations of Populus nigra L.

Patricia Faivre Rampant[1], Giusi Zaina[2], Simone Scalabrin[3], Véronique Jorge[1], Vincent Segura[1], Frederica Cattonaro[3], Marie Christine Le Paslier[1], Gail Taylor[4], Michele Morgante[3], Catherine Bastien[1]
[1]INRA, France; [2]University of Udine, Italy; [3]IGA, Italy; [4]University of Southampton, UK

In a collaborative effort launched by Evoltree, INRA (F), Universities of Southampton (GB) and Udine and IGA (I), co mbined sampling of *Populus nigra* genetic resources at the European scale, high through put sequencing and SNP genotyping technologies, to characterize genetic diversity patterns and perform association genetics for adaptive and production traits. The Evoltree Plant Gene Repository Center's facilities in Tulln (AT), provided DNA for the accessions sampled and maintained in a clonal archive in France (INRA, ONF).

For SNP discovery, 51 individuals representative of the Western part of the European collection were resequenced with pair-end Illumina technologies. Four individuals were resequenced at high coverage ($\geq$20x) and 47 at lower coverage (2x-12x). Raw reads of the *P. nigra* clone 71077-308 were aligned on the *P. trichocarpa* genome sequence V2.0. Around 50% of the reads uniquely matched and 70% of the *P. trichocarpa* genome was covered. The resulting alignment was used as a framework reference genome of *P. nigra* to perform SNP calling after alignment of the 3 other high coverage genotype reads. SNP frequencies were then estimated after mapping of the 47 low coverage genotype reads. In the frame of FP7 projects Energy-poplar and NovelTree, over ten thousands SNP have been selectively filtered to target -15 known QTL regions -2916 expressional candidate genes related to rust resistance and wood properties -1007 genes spread out of the framework reference for the construction of a 12 K Infinium BeadChip array.

The results of admixture analyses based on 7896 SNP were consistent with geographical distribution of the 14 populations under study (721 individuals). Admixture remains an important feature in many populations except the Italian ones. High clonality rates were confirmed in endangered populations in Netherlands and Germany. The successful collaborative strategy developed here has fostered large scale genetic studies on a reference association population.

Evoltree Lecture

Divergence and selection on evolutionary and ecological timescales in closely related pine species from the Pinus mugo complex and P. sylvestris

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Centre for Ecology and Hydrology,NERC, UK

Efforts to detect genomic regions involved in adaptive variation in plants have mostly focussed on single species. Assuming though that intraspecific divergence may lead to speciation, comparisons of genetic variation within and among recently diverged taxa can help to locate genes under selection. In our research we propose to use an experimental system of closely related pines including three taxa from the *Pinus mugo* complex (Dwarth mountain pine (*P. mugo*), Peat-bog pine (*P. uliginosa*), Mountain pine (*P. uncinata*)) and Scots pine (*P. sylvestris*). As we demonstrate those species prove close genetic relationships but highly differ from each other in e.g. phenotype and ecology. To look at the patterns of genetic variation of the species, we measured phenology and growth rate traits in a progeny trial experiment and we evaluated the evolutionary relationships between species and genome wide patterns of nucleotide polymorphisms based on whole transctiptome sequencing. Finally we used coalescent and outlier detection methods to evaluate the role of selection on nucleotide polymorphisms in a set of 79 ortholog gene fragments (1680 SNPs) in 30 populations across the species distribution range. We found intra- and interspecific differences in adaptive traits and a large number of single nucleotide polymorphisms that constitute new genomic resources for downstream applications. A similar level of polymorphism was observed across species and in most individual populations that showed little evidence of population structure. The majority of loci showing significant within-species differentiation also showed significant between-species divergence suggesting selection has operated at these loci on both evolutionary and ecological timescales. With the fast progress of cost-effective genotyping methods, it is becoming possible to analyse many samples and SNPs and hence to provide higher resolution of adaptive and neutral genetic variation to advance forest management, conservation and breeding strategies in those pines and other forest trees.

Evoltree Lecture

Molecular signatures of climate adaptation and range expansions in Mediterranean conifers

Santiago Gonzalez-Martinez[1], Delphine Grivet[1], Rose Ruiz-Daniels[1], Juan Pablo Jaramillo-Correa[2], Francesca Bagnoli[3], Zaida Lorenzo[1], Katharina Budde[1], Isabel Rodríguez-Quilón[1], Ricardo Alía[1], Giovanni Vendramin[3]
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Understanding the interaction among environments, demography and evolution is essential in the face of impending climate change. Species from the Mediterranean Basin, inhabiting highly heterogeneous environments, are particularly at risk because of the predicted increase in aridity and recent land-use change. In this talk, we review our studies on population genomics and association genetics in maritime and Aleppo pines, two relevant components of Mediterranean landscapes. In maritime pine (*Pinus pinaster* Aiton), 17 SNPs (Single Nucleotide Polymorphisms) were found to be strongly correlated with climate, once population genetic structure was removed from environmental association models. The utility of these SNPs to predict climate maladaptation of forest stands was further tested in a common garden. Furthermore, some of these loci were correlated with both fire-related and drought traits using association genetic approaches. In Aleppo pine (*Pinus halepensis* Miller), we are studying population genetic signatures of range expansions, from refugia in Turkey and Greece towards the large western Mediterranean part of the distribution. This species showed signatures of selection in expanding populations based on drought-response candidate genes. However, new microsatellite and SNP data showed signals of recurrent bottlenecks in the colonized range and 'gene surfing' in the expanding wage of colonization appears now as a reasonable alternative explanation. These studies at large spatial scales are accompanied by research at local scales aiming at detecting the role of micro-environmental variation in creating and maintaining genetic diversity within populations. The combination of approaches and spatial scales provides an integrated view to understand the quantitative genetic and molecular mechanisms responsible for adaptation as well as the drivers of selection (both climatic and ecological) in Mediterranean conifers.

OA 09

New transfer models to predict spruces plantation productivity in Québec (Canada) under the influence of climate change

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[1]Québec government, Canada; [2]Canadian Forest Service, Canada; [3]Ouranos, Canada

The pace of climate change is expected to affect long-living organisms as forest trees. Planting prove nances better adapted to future climate could minimize drawbacks or maximize forest productivity. However these movements should be guided by sound science. New transfer models were recently developed in Québec for white and black spruce. Height of twenty year-old trees was measured in provenance tests representing species range in Canada and used to develop models predicting height gains or losses (%), compared to local populations, from "provenance-plantation site" climate differences Using reference climate data of 1940-1970 rather than 1971-2000 increased determination coefficient values of the models. Combining these transfer models to yield models allows future plantation productivity predictions.

Plantation productivity for white spruce should increase everywhere in Québec for the 2050 period, mainly in the northern part (+22%). However during the 2080 period, it should decrease in the southern part compared to now, while keeping increasing slightly in northern zones. Black spruce would loose 32% of its productivity during the first period south of the province, after which climate conditions would not be favourable at all to the species no matter where in Québec. While provenance transfers would compensate for losses and even improve productivity for white spruce, the decline would be overwhelming for black spruce.

OB 10

Genetic diversity and differentiation of Quercus petraea and Q. robur seed collection stands based on chloroplast microsatellites

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We have investigated genetic diversity and differentiation of 58 populations of *Quercus petraea* and 74 populations of *Q. robur*, being the certified seed collection stands utilized in reforestation and gene conservation programs in Poland. On average 19individuals were sampled per population and genotyped using 14 cpSSR genetic markers. Genetic diversity within populations ranged from 0 to 0.822 for *Q. petraea* and from 0 to 0.752 for *Q. robur*, with means equal to 0.309 and 0.260, respectively. While 14 cpSSR loci generated 27 distinct haplotypes for *Q. petraea* and 31 haplotypes for *Q. robur*, the number of haplotypes per population ranged from 1-7 with a mean of 2.33, and the effective number of haplotypes of 1.565. Effective number of haplotypes, haplotypic richness and genetic diversity measures appeared to be slightly higher for *Q. petraea* than for *Q. robur*.

The AMOVA indicated that only 10.8% of variation was attributed to differences between species, 63.4% to differentiation among populations within species, and the remaining 25.8% resulted from variation within populations. Genetic differentiation among populations within species was comparable (Gst = 0.692 for *Q.petraea* and Gst = 0.724 for *Q.robur*).

Spatial distribution of haplotypes over the entire country indicated aggregation of similar haplotypes in certain geographical regions. It is suggested that the geographic distribution of haplotypes reflects the recent migration history of *Quercus* populations and may be used as a tool for the management of forest gene resources, delineation of seed zones and genetic tracking of forest reproductive material.

OA 11

The phenotypic effects of selective breeding on climatic adaptation in lodgepole pine and interior spruce

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Climatic change is expected to disconnect locally adapted provenances from their adaptive optima, causing a lag in the productivity of Western Canada's forests that will have negative economic, ecological, and social impacts. The AdapTree Project is quantifying phenotypic and genomic architectures of local adaptation to climate in lodgepole pine (*Pinus contorta*) and interior spruce (*Picea glauca, P. engelmannii* and their hybrids) in Western Canada to inform climate-based seed transfer.

In Western Canada, reforestation using seedlots from selective breeding programs is increasing rapidly, however, the impacts of selective breeding on climatically relevant phenotypic traits in current and future climates remain unclear. To address this knowledge gap, within AdapTree we have established a large seedling common garden (n = 2880 individuals) for each species containing natural seedlots (pine n = 281, spruce n = 254) and selectively bred seedlots (pine n = 20, spruce n = 18) from provenances across British Columbia and Alberta. We have assessed phenological, growth and cold hardiness traits in relation to provenance climates. Our analyses compare trait means, climatic clines, trait – trait correlations and climatic transfer functions between natural and corresponding selectively bred seedlots. Here we present results for lodgepole pine only.

Phenotypes of both natural and selectively bred seedlots reflect provenance climates, but several phenotypic traits also show differences between these seedlot types within breeding zones. Growth rate appears to be the primary determinant of substantial differences in height between natural and selectively bred populations. Regressions of phenotypic traits against climatic variables suggest that selective breeding increases the strength of temperature-related clines for height, whilst artificial frost injury maintains similar strong clines in both natural and selectively bred populations.

Future research will integrate phenotypic data with approximately 50,000 candidate adaptive SNPs per seedling to determine whether selective breeding modifies the genomic architecture of reforestation seedlots.

OB 12

Early-season phenology impacts growth patterns in improved and wild sources of white spruce, Picea glauca (Moench) Voss

Carolyn Pike, Rebecca Montgomery
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Tree improvement programs have increased growth rates of white spruce (Picea glauca) substantially thro ugh selection and breeding. Differences in phenology (timing of bud-break and/or bud-set) have been associated with increased growth among improved genotypes of white spruce and other tree species. We tested the hypothesis that improved sources achieve additional wood production by extending their growing season. Phenology (bud-break time and weekly shoot elongation) was observed on seedlings from seven sources in a potted nursery at one location after two and three growing seasons. We also measured basal area increments on mature (25-year old) trees weekly from eight selected families (four high- and four poor-performing families) at two locations using dendrometer bands. Shoot elongation (seedlings) and basal area growth (mature trees) were fit with a non-linear Richards function, and parameters analyzed to calculate the upper asymptote, relative growth rate, and days to 10%, 50% and 90% of growth. A negative genetic correlation between heigh t and bud-break time was significant in seedlings. Bud-break time varied between years in the seedlings. Only the upper asymptote of basal area growth differed significantly among families. Differences between sites were significant for growth midpoint: North site reached 50% growth sooner than the south in 2010, but the opposite occurred in 2011. Both sites completed 90% of growth on approximately the same day in both years. Basal area increment of adult trees preceded bud-break of seedlings in 2011, extending the growing period. Growth patterns in white spruce seedlings and mature trees are driven largely by spring phenology. Improved white spruce trees are highly plastic and adapt their phenology to the site with no g x e interaction for basal area increment, but may be susceptible to growth reductions if conditions in the spring are not favorable for carbon fixation.

OA 13

Selecting Douglas fir provenances for future climates using universal response functions on basis of extensive provenance test series in Austria and Bavaria

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Douglas fir (*Pseudotsuga menziesii var.* (Mi rb.) Franco) has been introduced to Europe more than 100 years ago and is today an important coniferous tree in many countries. Moreover, due to the negative effects (e.g. drought, insects attacks, storm) of ongoing climate change on forests with native conifers (in particularly Norway spruce), Douglas fir is widely considered to be an alternative tree species also within a warmer climate. The present recommendations for reproductive material of Douglas fir are based on more than 100 years of experience with the introduced provenances and on manifold provenance trials which have been established on national but also on international (IUFRO) levels. However, the changing environmental conditions provoke a reassessment and reanalysis of existing trial data and the incorporation of the expected future climate into provenance recommendations. Within the joint project DougLAS, we utilize provenance trial data from about 68 test sites in Austria and Bavaria (Germany). The objective of o ur study is to understand the genetic variation of climate response. We use universal response and transfer functions to identify provenances that will allow stable and productive plantations also under future conditions. Moreover, our analysis will give general insights into the climate response of tree populations within a non-analogous climate.

OB 14

Crossing Between Breeding popular cultivations and wild relatives in Mediterranean Environment

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Most strategies for the genetic improvement and biotechnologies may be applied to the Populus spp. and their hybrids to increase productivity and adaptability. However, their weak reproductive barriers and spontaneous hybridization with natural populations may impact the sustainable deployment of new poplar cultivars. Deforestation and intensive management, with plantations of fast-growing tree species, may endanger tree species by the destruction or loss of their habitats, but also by loss of species integrity through hybridization and introgressive gene flow. Consequently, the implementation of conservation strategies requires the monitoring of gene flow in relation to habitat structure. For the purpose to establish the best management approaches for genetic diversity conservation, the objectives of this work were the characterization of spatial genetic structure in poplar stand, and the study of the potential breeding between natural and cultivated populations in the Mediterranean environment. Two study areas, near to a poplar plantation, were settled within a natural protected area, the Migliarino- San Rossore-Massaciuccoli Regional Park (Pisa, Italy). The spatial genetic structure showed that three clusters can be identified in the Area 1 and four clusters in the Area 2. The differences between the spatial genetic structures depended on the environmental features of the two habitats. The detection of hybridization (by paternity analysis) between poplars of Area 2 and the poplar plantation, suggested the occurrence of a possible genetic exchange among a natural stand and the plantation. Besides, the potential hazard due to hybridization between poplar cultivations and its wild relatives in the surrounding environments of the Regional Park was investigated. Based on the genetic information and using the spatial dataset available for the study area, it seems that gene flow could affect important habitats hosting naturally originated poplar population. The assessment of this potential hazard indicates that some management measures are required to mitigate the risk.

OA 15

Genetic architecture of local adaptation in widely-distributed, long-lived eastern white pine (Pinus strobus)

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Understanding the genetic architecture of local adaptation is critical in forest trees to assist tree breeding and sustainable management of genetic resources, especially in the context of climate change. Natural plant populations are often adapted to their local climate and environmental conditions. Forest tree populations exhibit this pattern, amply evident from their provenance tests. However, the knowledge of genetic architecture of local adaption remains largely unknown for forest trees. Very little research has focused on understanding relative contributions of single gene versus polygenic effects to the genetic architecture of local adaptation. We have examined this aspect in a long-lived, widely-distributed eastern white pine (*Pinus strobus*) by determining the genetic structure of range-wide 29 natural populations in relation to local climatic factors using both putatively neutral (SSRs) and putatively functional (SNPs in candidate genes) marke rs. Standard outlier analyses and a novel approach were used to identify loci showing signals of natural selection in response to local climate factors. Our study has demonstrated that the genetic architecture of adaptive traits is complex and that the polygenic interactions among functional loci primarily drive adaptation to local environments in eastern white pine. There is a need to take into account the multilocus effects in understanding the genetic architecture of local adaptation. I will discuss these results in context of tree breeding, sustainable management of genetic resources, and adaptation to climate change.

WEDNESDAY, AUGUST 27, 2014

Keynote Lecture
Opportunities for genomics in forestry

Bruce Tier
University of New England, Australia

It is now possible to test individuals of many species for hundreds of thousands of loci. This can be done directly or by imputing large numbers of loci from smaller, representative subsets. Tests for hundreds of thousands of single-nucleotide-polymorphisms (SNPs) on a single chip are available as are subsets. Also genotype s can be obtained by direct sequencing. Indeed, the cost of obtaining the genome sequence for new species has also become affordable. As a consequence considerable use of genomic information has been made by breeders of many species with tens of thousands of individuals being tested. The way that this type of information has been used varies with the industry, and depends upon the end product which range from inbred lines in plants through to livestock where cross breeding is necessarily ongoing. The problem for forestry breeders lies between these extremes, with goals in the long term, long times between making selection decisions and obtaining results and a variety of problems common to both plant and animal breeders. This paper examines how forestry breeding enterprises can best take advantage of the flood of genomic data that is becoming available. From the search for genes, through to genomic selection and the design of breeding programs for improvement and deployment, the use of genomic information is discussed with the goal of making better use of the available phenothypes to breed trees that are more productive in any environment.

Keynote Lecture
Impacts of Genome Sequences on Forest Tree Breeding

David B. Neale

Keynote Lecture

Alternative approaches to tree breeding for no-analog fiscal and physical environments

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[1]*USDA Forest Service, Southern Research Station, USA*
[2]*University of Kentucky, USA*

No-analog environments have probably been around forever, but they seem particularly evident now with respect to plant breeding and especially for forest and fruit tree breeding. On the physical side we have climate change (warmer, drier or wetter, and more extreme events) to account for and on the fiscal side we have ever-tightening budgets to complete the work for our current species as well as the need for addressing a wider-range of species. At the same time new technologies present opportunities for increasing the effectiveness and efficiency of tree breeding. We have certainly seen this from the availability of handheld computers for data entry to the increased computing power for calculating more complex genetic models. More recently the wide-scale development of genetic markers and reduced cost of genotyping combined with computing power offers innovative options for reducing workload while maintaining genetic gains and gene diversity; and m obile computing and social media facilitate new approaches including participatory breeding especially for increasing the range of species that can be addressed. Addressing the no-analog challenges will require a thoughtful consideration of breeding strategies for a wider-array of species. More species need to be conserved and improved for adaptive and economic traits and those species being improved need to be evaluated in a wider range of physical environments. Even though funding levels are tight the case must be made for additional resources for tree breeding all the while we develop more efficient ways to conserve these species and make the gains needed for a sustainable future.

OA 16

Estimation of number and distribution of QTL effects in forest tree traits

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The number of association studies and QTL analyses of forest trees published is probably just a foretaste of the number of studies expected to be pe rformed in the wake of recent and soon to be completed sequenced tree genomes. We wanted to examine what the status is in different trait types in trees and asses the possibility and the effort required to catch most of the genetic variation (80%) in these trait types. We examined traits studied in published association analyses which had found multiple significant QTLs and compared that to corresponding published QTL mapping experiments.

Estimated effect sizes from association studies were about 50% smaller than the effects from QTL studies. We estimated the numbers of underlying QTLs to be about five times higher for association studies than QTL studies for the same traits. The largest estimates of the number of underlying QTLs in association studies were for growth and the lowest estimates were for water use efficiency.

Using the estimated number and exponential distribution of allelic effects from published data for simulations reveal that QTL studies highly over-estimated the effects size and underestimated the number of QTLs, while the bias using association study decreased as number of detected QTL and population size is increased. For a particular effect size to be detected theory predicts the necessary mapping population size and in turn we can estimate the needed number of individuals to find most of the genetic variation (80%) of a trait given its allelic effect distribution and effect number. This population size turns out to be over 250 000 for growth traits and several thousand for water use efficiency and phenology traits. This will require novel uses of established and recent mapping and marker effect estimation methods to utilize genomic information in future forest breeding.

OB 17

Orthology of Sinnapyl Alcohol Dehydrogenase gene (Sad) in Cedrus deodara (Deodar)

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Deodar (*Cedrus deodara*) is an important timber tree belongs to family *Pinaceae*, due to the high lignin content. It grows in the high altitude with the elevation range of 1200m to 3050m. The sinnapyl alcohol dehydrogenase participates in the phenylpropanoid pathway of monolignols. This gene responsible for S type lignin biosynthesis. This gene contains the importance for timber production. There is no orthology and evolutionary relationship for *Sad* gene of deodar and the members of pinaceae had been reported. Here the orthology has been done through the BLAST search, phylogenetic comparison of deodar *Sad* gene against the *Pinus, Picea, Cathaya, Ketelleria, Abies, Populus* and *Eucalyptus* spp. *Sad* gene of deodar is close to *Picea* which has been concluded due to the 90% identity and 100% query coverage against it. The gene expression level has been validated through the tBLASTn analysis against the wood EST databases of *Pinus* and *Picea*. This analysis showed high expression as *Picea* EST with the bit score >900. This gene showed the orthology with the *Picea, Pinus* and *Abies. Sad* have very less conserve region with *Ketelleria, Cathaya, Populus and Eucalyptus.* In *Populus Sad* gene has significant similarity but there was no identity for functional annotation. The *Sad* of Cedrus is closely related to the cluster of the *Pinus armandii* and *Picea glauca* which have the bootstrap value 62%. The conclusion of these results showed that *Sad* gene of deodar is highly related to the genes *Picea sp.*

OA 18

Genome-wide analysis of heat shock protein 70 genes (Hsp70) in poplar

Mehmet Cengiz Baloglu, Esra Nurten Yer, Sezgin Ayan
Kastamonu University, Faculty of Engineering and Architecture, Turkey

Heat shock proteins (Hsps), also called stress proteins, are a group of proteins found in all living organisms. Hsps play key roles in regulating the stress response to salinity, drought, and extreme temperature changes. Hsps also act as molecular chaperones that provide favorable conditions for the correct folding of other proteins, thus preventing protein aggregation. In this study, we identified 34 *PtHsp70* genes in *Populus trichocarpa* and investigated their chromosomal location, exon-intron organization, phylogenetic analysis, conserved motifs analysis, gene ontology and 3D protein modeling. Firstly, *PtHsp70* gene members were physically mapped to 19 chromosomes of poplar. According to phylogenetic analysis, *PtHsp70* genes could be classified into 3 groups. Specific motifs, relatively conserved in each three groups were found in all predicted Hsp 70 proteins. Gene Ontology analysis showed that majority of the Hsp 70was predicted to function in response to abiotic/biotic stimulus and binding activity. In addition, predicted PtHsp 70 proteins were distributed among the cell, cytoplasm, organelle and membrane. The amino acid sequences of PtHsp 70 proteins were fed in Phyre2 for prediction of 3D protein structure. Among the 34 PtHsp 70 proteins, 28 of them was modelled at >90% confidence and the percentage residue varied from 90 to 100. These results provide characterization and functional information of PtHsp 70 proteins for poplar. This genome-wide identification will enable researcher to open new perspectives for further studies to improve stress tolerant forest trees.

OB 19

Molecular characterization of Corymbia torelliana, Corymbia citriodora and their recombinants by Molecular markers

Ajay Thakur, Santan Barthwal, Anugrah Tripathi
Forest Research Institute Dehradun, India

Hybridization may influence the evolution in tree improvement programme and make it enable for the development of new recombinants with the desired traits which could enhance the productivity. The species of genus *Corymbia* has tendency to produce spontaneous inter-specific hybrids and recombinants. Some of them are very productive. There is always a problem to identify these recombinants morphologically so molecular approach may be the way to identify these recombinants in early stages. Here, the molecular characterization of *Corymbia torreliana, Corymbia citriodora* and their recombinants have been done through the standardized protocol of DNA extraction and RAPD (Random Amplified Polymorphic DNA) markers analysis. In this study 8 Random Amplified Polymorphic DNA markers showed the polymorphism out of 16 markers which have been used. The *C. torelliana* and *C. citriodora* are quite different and they show similarity 36.99% while < em>C. torelliana showed 64.38%, 60.27%, 65.75%, and 53.42% with hybrid-2, hybrid-3, E1 and hybrid-1 respectively. The similarity index has also been calculated for E1 among the hybrid-2, hybrid-3, hybrid-1, *C.torelliana, C. citridora* and the result showed that 90.41%, 78.08%, 79.45%, 65.75% and 60.27% similarity respectively. The Dendrogram analysis which has been done through the statistical analysis software NTSYS, revealed that recombinants are closer to *Corymbia torelliana*.

OA 20

Genome-wide SNP discovery and sequence variability in Fraser fir

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Fraser fir (*Abies fraseri* Pursh. Poir.) is the only *Abies* species endemic to the southern Appalachian Mountains where it plays an impor tant ecological, aesthetic and economic role. Although globally threatened in its native range, it is grown in commercial Christmas trees plantations at lower elevations and is an important source of revenue for the region. The NCSU Christmas Tree Genetics Program is employing traditional tree improvement methods toward enhancing the growth, quality, and pest resistance of this species. However, the use of DNA markers and their association with important traits, as well as a better understanding of the species' genome, could dramatically increase the efficiency of this process.

A survey of genetic variation in expressed genes across existing populations of Fraser fir plants has been conducted using high-throughput DNA sequencing instruments. Roots and foliage samples from three seedlings, and pooled foliage samples from ten trees per provenance for each of six provenances, were used to prepare complementary DNA libraries. The root and foliage seedling libraries were sequenced using the Roche 454 technology, and one seedling foliage library and the provenance foliage pool libraries were sequenced using the Illumina technology. A total of 221 and 59 million base pairs of DNA sequence were obtained from the 454 and Illumina experiments respectively. These DNA sequences have been assembled into putative transcripts and thousands of candidate Single-Nucleotide Polymorphisms (SNPs) identified from the alignm ents. The recent releases of genome sequences for loblolly pine and Norway spruce also allow comparison of the putative fir transcripts to related species in the Pinaceae.

OB 21

Genetics of European ash versus the emerging invasive pathogen Hymenoscyphus pseudoalbidus

**Erik Dahl Kjaer, Lea Vig McKinney,
Lene Rostgaard Nielsen, David B. Collinge,
Albin Lobo, Lars Noergaard Hansen,
Jon Kehlet Hansen**
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It is assummed that *Hymenoscyphus pseudoalbidus* (the sexual form of *Chalara fraxinea*) was only recently introduc ed to Europe, but the fungus has already caused server damage across Europe on the European ash (*Fraxinus excelsior*). The introduction history of *H. pseudoalbidus* to Europe remains unclear, but the fungus is most likely associated to one or more Asian *Fraxinus* species as a harmless endophyte.

The disease is now threatening the future of *F. excelsior* in European forestry.However, observation from progeny and clonal trials have revealed important genetic variation among *F. excelsior* trees in their ability to cope with infection of *H. pseudoalbidus,* and breeding programmes are being developed in several European counties. In this presentation, we provide an overview of published knowledge of the quantitative genetics of the host resistance, how the genetic variation seems to be distributed within and between populations, the degree of genotype by environment interactions and the expected gain from selection at different ages and based on different methods. We discuss correlation between resistance and other important fitness related traits, the likely biological background of these correlations, and their implications on selection and deplyoment strategies. We discuss future prospects of using marker-aided selection and other genomic techniques to tackle the disease problem, and outline potential genetic deployment strategies at the landscape level in a dynamic gene conservation context.

OA 22

Mapping Phytophthora Root Rot Resistance in Fir Species through Genotyping by Sequencing

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Root rot disease, primarily caused by the Oomycete *Phytophthora cinnamomi* Rands, is a large problem for the Christmas tree industry in North Carolina, leading to more than $US 6 million in losses annually. Fraser fir (*Abies fraseri* [Pursch] Poir.), one of the most desirable Christmas tree species in the United States, has no known innate resistance to this disease while some exotic fir species, such as Trojan (*Abies bornmuelleriana* Mattf.) and Turkish (*Abies equi-trojani* Aschers. et Sint) fir, display varying amounts of resistance.

A large Phytophthora resistance screening study was completed using 1600 seedlings from 12 Turkish and Trojan fir families with Fraser and momi fir (*A. firma* Sieb. et Zucc.) included as susceptible and resistant controls, respectively. DNA was extracted from one Trojan fir family (n=161) with an overall mortality of 71%. Libraries were prepared for Genotyping by Sequencing to identify genetic marker loci. A small subset of individuals from other Turkish, Trojan, Fraser, and momi fir families screened in the study will also be genotyped for comparison. Using the genetic markers that are present in the individuals that exhibited resistance, and not present in susceptible individuals, we hope to identify the genetic basis of the disease resistant phenotype. The markers associated with disease resistance in the large Trojan fir family can then be compared to the markers identified in the other fir species and families to look for consistent association of specific markers with disease resistance. The understanding of the genetic basis of Phytophthora root rot resistance obtained from this study will guide future breeding efforts to develop resistant planting stock suitable for planting on Phytophthora-infested land.

OB 23

Characterization of gene-based SSR markers polymorphisms of the Chinese fir and transferability in comparing with six conifers and model plant

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Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook is one of the most important timber production species in southern China. The molecular breeding strategies, such as MAS, GWAS, and GS variation selection have been included in the advanced generation breeding programs for multi-traits genetic improvement.

Here, there were 6,092 perfect SSRs associated with 5,000 genetic loci to be identified.Trinucleotide motifs are the most common (56.5%), followed by tetranucleotide (20.0%) and hexanucleotide motifs (13.0%). The AG/CT motif (50.6%) is the most abundant dinucleotide repeat, whereas AAG/CTT (22.0%) is the most common trinucleotide repeat. From the 588 Class-I SSR motifs, 354 primer pairs were designed. One hundred of these primer pairs were then used to detect polymorphisms among Chinese fir provenance samples. Thirty-four of these primer pairs were shown DNA polymorphism. The pattern of diversity with UPGMA dendrogram is closer to fit the diversity patterns generated by morphological descriptors and RAPD markers. Functional categorization of EST sequences that contain SSRs revealed that, 55.9% of these expressed sequence tags (ESTs) encode proteins with characterized homologs. There were 34 polymorphic EST-SSRs appeared in Chinese fir, and showed the transferability amplif ication at an average of 22.7% in other reported conifers, two *Picea spp.*, *Pinus massoniana*, *P. taiwanensis*, *P. taeda* and *Cryptomeria japonica*, and one model species, *Arabidopsis thaliana.*

In conclusion that, there was lower transferability on gene-based SSRs for Chinese fir with other six conifers and model plant, interestingly, even comparing with *C. japonica*, which was in same family with Chinese Fir(Taxodiaceae). Such a trend was also found in geographic distribution patterns supported by conventional provenances testing. IT was more like in trends of random on Chinese fir, in other word, more genetic variation might be appeared among stands nearby. The bio-information from Chinese fir EST sequences may provide a valuable reference for us to develop/modifying genetic improvement strategies in advanced generation.

OA 24

Genomic Selection for Embryogenic Clones Deployment on the Arauco's Radiata pine Program in Chile

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Bioforest S.A. – Arauco, Chile

Arauco's Radiata pine operational clonal plantations started in year 2007 with embryogenic clones, phenotypically selec ted on field clonal trials established on different sites. The company decided to evaluate the use of genomic aided selection to speed up the process and increase gain.

The goal of the project is to evaluate the extra gain per year that could be achieved by early molecular screening of thousands of embryogenic clones produced every year before the field evaluations. In addition to larger selection intensity, genomic selection could reduce the field testing time due to avoiding the need for two-stage field testing (large number of clones tested with low accuracy in the first stage followed by a small subset validated in a second stage).

Genetic simulation was used to compare phenotypic selection (control) and combined selection, which included an early selection of clones at the tissue culture laboratory, based on their genomic value, followed by a field testing of the winners. Before engaging in finding a set of SNP markers in the population and testing them for prediction accuracy (r), two alternative possible genomic accuracies ($r = 0.5$ and $r = 0.7$) were evaluated using genomic screening of 4,000 clones a year, followed by field testing of the best 300 clones. The marginal benefit over the control scenario was estimated during a 25 years cycle calculating Net Present Value and Internal Rate of Return.

A positive marginal gain for combined selection was found, due to the genomic screening effect on selection intensity and time saving by doing only one field test, especially when the correlation between genomic value and true genetic total value of the clones was set to $r = 0.7$. The economic feasibility of the proposed scheme needs to be confirmed by experimental prediction accuracy estimation.

OB 25

Cloning and analyzing of CAD and CCoAOMT genes from Pinus massoniana

Kongshu Ji, Fengkai Zhang
Nanjing Forestry University, China

Pinus massoniana Lamb. covers about 2,000,000 km^2 land in south China. More than 20,000,000 m^3 *P. massoniana* wood is used for timber and paper use each year for the whole country. CAD and CCoAOMT are two important enzymes in the lignin synthesization pathway. We used CODEHOP to design the degenerate primers online, the CAD and CCoAOMT genes of *P. massoniana*(GenBank accession number:KF419291 and KF419292) were cloned through RT-PCR and SMART RACE RT-PCR. The CAD of *P. massoniana*, whose length is 1450bp, contained an open reading frame of 1074bp which encoded a polypeptide of 357 amino acids with predicted molecular mass of 38945.1u and PI of 5.8. The CCoAOMT of *P. massoniana*, whose length is 1126bp, contained an open reading frame of 780bp which encoded a polypeptide of 259 amino acids with predicted molecular mass of 29068.3u and PI of 5.12. Tissue specific expression showed that CAD and CCoAOMT genes expressed in stem was much higher than in leaf, root and shoot from *P. massoniana.* pBI121-antiCAD and pBI121-antiCCoAOMT plant expression vectors were established. The expression vectors were then introduced into *Agrobacterium tumefaciens* strain EHA105 by freeze-thaw method and proved by PCR detection. The antisense genes were transferred into the tobacco using leaf-disc cultivated method also proved by PCR detection. The lignin content was measured by using the method of Klason. The average lignin content of transgenic plants of pBI121-antiCAD was found to be less reduced, and transgenic plants of pBI121-antiCCoAOMT reduced 6.6% comparing with the control plants after 1 month of transfer. Utilizing vector pET-28a to construct expression vector of PmCAD gene and we optimize the inducement conditions for expression vector in BL21(DE3). The result was, its expression products were mostly composed of inclusion body, less inclusion body was formed under the condition of low temperature, and the protein molecular of this expressive gene was 39kDa.

OA 26

Chloroplast genome assembly from NGS data, with applications to forest trees

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Chloroplast genomes are haploid uniparentally-inherited molecules, useful for estimating divergence and tracking gene flow. Although chloroplast genome structure is highly stable across the majority of angiosperms, gymnosperm plastomes are labile, with extensive gene losses and rearrangements, typically associated with a reduction of the inverted repeat. This poses small challenges for assemblies, with experimental validation typically required for contig ordering, gap filling, and isoform confirmation. To assess plastome structure within a broad phylogenetic framework, chloroplast genomes were sequenced, assembled and validated from *Abies sibirica*, *Gnetum gnemon*, *Juniperus communis*, *Picea abies* (5 individuals), *Picea glauca*, *Picea obovata*, *Pinus sylvestris*, and *Taxus baccata*. All *ndh* genes were fully retained in *J. communis* and *T. baccata*, completely excised in *G. gnemon*, and progressively pseudogenized in *P. sylvestris* and *A. sibirica*, suggesting convergent losses in the Pinaceae and Gnetales. Similarly, the inverted repeat appears to be independently reduced in Pinaceae and Cupressophyte clades. While there was high structural conservation between the plastomes of *A. sibirica*, *P. sylvestris*, and *G. gnemon* with their closest relatives, rampant rearrangements were detected within *Picea* and the Cupressophytes. Two major inversions and two translocations were detected between *Juniperus* and relatives, including a major 36 kbp *Juniperus*-specific inversion, flanked by two copies of *trn*Q and AT-rich breakpoints. At least four inversions have occurred after the divergence of *Taxus baccata* and *Cephalotaxus wilsoniana*, the largest being 20 kbp. A deep phylogenetic split was detected within *Picea abies*, and polyphyly of the group confirmed with sequence data that spans the mitochondrial genome. We will highlight applications of chloroplast genomes to our understanding of divergence and gene flow within conifers.

THURSDAY, AUGUST 28, 2014

Keynote Lecture
Genetic evaluation in New Zealand: A history of compromises

Luis Apiolaza
University of Canterbury, New Zealand

This presentation will discuss 20 years of evolution of the genetic evaluation system for the New Zealand radiata pine breeding program. Some of the changes reflect advances of quantitative genetics methodologies (e.g. selection indices to BLP to univariate BLUP to multivariate BLUP), while others represent philosophical changes to the management of the breeding and deployment populations (e.g. our changing interest in Genotype by Environment interaction).

Beyond discussing the evolution and successes of the breeding program I would like to cover some of the challenges. For example, the legacy of poor connectedness between selection series, which has affected our ability to model GxE interaction. Despite a major overhaul to our genetic evaluation system, including modeling of multivariate reduced animal models with Factor Analytic covariance structures, we are still unable to exploit changing performance due to lack of data. Another example, this time in the deployment area, is the distinction between predicted breeding values and the system used to market genetic material (GFplus), which affects the stability of the reported superiority of commercial material, reducing the trust industry can put in some of our results.

Overall, the choices made on genetic evaluation reflect compromises between computational feasibility, data availability and our openness to challenge the assumptions embedded in operational breeding programs.

Keynote Lecture
Massively multivariate genetic value prediction in tree breeding – experience from a variety of species

Gregory Dutkowski[1], Richard Kerr[1], Tony McRae[1], Bruce Tier[2]
[1]PlantPlan Genetics, Australia; [2]Animal Genetics and Breeding Unit, Australia

Massively multivariate prediction of genetic values using Best Linear Unbiased Prediction (MM-BLUP) and the additive (or numerator) relationship matrix (NRM) offers the optimal way of predicting breeding values in large multigenerational breeding programs. It can use all historic data and deal with different measurement traits, different ages of measurement, and different site types (genotype by environment interaction). The NRM allows prediction of breeding values for provenances, parents and offspring, regardless of generation and location, to maximise gain. The framework can deal with cloning, non-additive genetic variation, and phenotypic and genomic data.

There are many challenges in MM-BLUP. A comprehensive data management system is needed for the data itself, metadata, trial designs, pedigree and genetic parameters. An efficient mixed model solver is required to allow all of the data to be used. Recovery of data and pedigree from previous decades an be problematic, as can its quality. Bias in the breeding values due to missing selection data can be overcome for known pedigrees and selection intensities by creating binary selection traits that are correlated with the traits under selection. Data needs to be standardised to deal with variance heterogeneity across trials, based on high quality within site and trait analyses. Data needs to be put into classes of traits, ages and site types to reduce the number of equations that need to be solved and the number of genetic parameters to be estimated. While estimation of additive correlations between traits and ages is relatively straight forward, identification of site ty pes can be problematic if differences in provenance performance or other historic program subdivisions mean that there is poor linkage between sites with different environmental parameters. Non-additive genetic parameter estimates can be difficult to obtain due to limited historic crossing programs and shallow pedigrees. Hybrid breeding programs further complicate this issue.

OA 27

Inheritance of ring width, density, microfibril angle, and modulus of elasticity in wood of Norway spruce at two locations in south of Sweden

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[1]Swedish University of Agricultural Sciences, Sweden
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Increment cores were sampled at breast height from 5618 trees in 524 open-pollinated families in two 21-year-old Norway spruce progeny trials in southern Sweden to quantify genetic variation, age-age genetic correla tion, efficiency of early selection, and multiple traits selection. Wood quality traits measurements were performed on 12 mm increment cores using SiliviScan. Heritability was moderate (~0.4-0.5) for wood density and modulus of elasticity (MOE), but low (~0.2) for microfibril angle (MFA). Genetic correlations among growth, wood density, MFA, and MOE increased to a considerable high value from pith to bark with very high adverse genetic correlations (-0.6) between growth and wood quality traits. Age-age genetic correlations reached 0.9 after ring four for DBH, wood density, MFA, and MOE traits. Early selections at ring ten, six, and seven reached similar effectiveness as selection conducted at reference age 15 for diameter, MFA, and MOE, respectively. Selection at ring six reached 89% efficiency for wood density. Selection in diameter alone produced 19.01% genetic gain in diameter, while produced 4.80% and 9.44% decreases and 8.01% increase in wood density, MOE and MFA, respectively. Index selection imposing the restriction in wood density, MOE and MFA produced a relatively lower genetic gain in diameter (16.35%, 12.19%, and 14.13%, respectively), indicating such index selection was efficient. But index selection using economic weights was recommended for maximum economic efficiency.

OB 28

Phenotyping Castanea hybrids from controlled crosses for resistance to Phytophthora cinnamomi

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Castanea sativa is susceptible to *Phytophthora* spp., a serious root pathogen causing root rot, while *C. crenata* and *C. mollissima* show resistance to infection. Inter-specific controlled crosses were established between *C sativa* and *C crenata* and *C sativa* x *C mollissima* for introgression of resistance genes from the resistant species into the susceptible *C. sativa* and two mapping populations were produced. These crosses had two purposes: the first to create mapping populations to identify Quantitative Trait Loci (QTL), genetic factors that control ink disease resistance, the main threat that affects *Castanea sativa* still nowadays, the second to select resistant hybrid genotypes for rootstocks for propagation and delivery to the productive sector which presents a serious deficit of improved plant material for plantation.

Phytophthora cinnamomi susceptibility of each progeny was evaluated by root and excised shoot inoculation tests. For each test multiple copies of each genotype were used. The number of days of survival after root inoculation was the best discriminator of resistance to *P. cinnamomi* while the percentage of shoot with internal lesion was the most explanatory symptom associated with survival. The lesion progression rate in the excised shoot inoculation test was strongly and positively correlated with the survival in the root inoculation test. The excised shoot inoculation test appears to be a reliable and expedite method for screening large numbers of genotypes regarding resistance to *P. cinnamomi,* which is very useful for mapping purposes for example. The most resistant genotypes selected from this study are being tested for stock-scion compatibility with Portuguese fruit varieties for production and the results will be presented.

OA 29

Quantitative Genetic Survey of Scots Pine

Zhou Hong, Harry Wu, Anders Fries
Swedish University of Agricultural Sciences, Sweden

The development of multiple trait selection indices for solid (structure) wood production in the Scots pine (*Pinus sylvestris L.*) breeding program requires genetic variances and covariances among growth and wood quality traits including stiffness. Heritabilities and phenotypic and genetic correlations a mong growth and wood quality traits and age-age genetic correlations were estimated using a Scots pine full-sib family trial. Wood quality traits from 778 trees in 179 families were measured using SilviScan in a 40 years-old progeny trial. Heritability ranged from 0.15 to 0.31 for growth, and earlywood, transition wood, and latewood proportion traits, and from 0.26 to 0.52 for fibre dimension, wood density, MFA, and stiffness traits. The highly unfavorable genetic correlation between diameter, and whole core density, and clearwood stiffness, and dynamic acoustic stiffness were observed in this study. Age-age genetic correlations were very high for wood quality traits and growth traits. The genetic correlation reached 0.8 after age 5 for all wood traits. High genetic correlation and moderate heritability at early ages indicated that early selection should be very effective for Scots pine. Selection at age 10 had at least 80% efficiency for wood quality traits. It was recommended th at early selection at age 10 would be effective for growth and wood quality traits for Scots pine.

OB 30

Phenotypic and genetic correlations among tree volume, wood specific gravity and foliar traits in white spruce, Picea glauca (Moench) Voss and implications for selection

Carolyn Pike, Rebecca Montgomery
University of Minnesota, USA

White spruce is highly valued by the forest products industry in North America. Through tree improvement efforts, genetic gains in volume growth approach 30% for selected genotypes. Negative correlations between growth and wood specific gravity have been established, but other morphological differences between high and low performing genotypes are less understood. We sampled five trees from each of 30 families at each of two locations (North and South) in a 25-year old progeny test in Minnesota. One wood core was collected from each tree to sample wood specific gravity (WSG), along with a branch collected from the upper crown to compare foliar traits. We confirmed negative correlations between stem volume and WSG, but several families combined high wood volume with only small reductions in WSG. Foliar nitrogen was lower in larger trees, following stoichiometric expectations. Specific leaf area, leaf area ratio, and leaf mass ratio were all positively, genetically correlated with volume growth. Genotypes with high capacity for wood production may improve carbon fixation through small improvements in foliar morphology an d/or photosynthetic apparatus. Selection for top-performing genotypes should be preceded by assessment of wood specific gravity prior to placement into seed orchards and to inform advanced-generation breeding efforts. No genotype by environment interaction was observed for improved families in Minnesota, facilitating broad deployment for commercial reforestation.

OA 31

The utility of GBLUP in forest genetic testing: examples from different genealogical complexities

Jaroslav Klápště, Yousry El-Kassaby
University of British Columbia, Canada

Breeding values are the main criteria for parents and offspring genetic ranking, thus the integrity of the selection process is dependent on their accuracies. Breeding value are commonly estimated using mixed linear models and phenotypic and pedigree information. The pedigree provides the information needed to construct the average numerator relationship matrix (**A**) to account for relatedness. The mixed model theory assumes an error free covariance matrix (**A**); however, the precision of the estimated breeding values depends on how well the **A** matrix reflects the segregation in functional variants. The development of high throughput genotyping technologies made it possible to genotype individuals for large number of markers which, in turn, are used to construct the realized relationship matrix (**G**), thus more precisely accounting for the Mendelian sampling term and genome heterogeneity then those estimate derived from classical pedigree (i.e., **A** matrix). Habier et al. (Genetics 194:597-607, 2013) identified three factors contributing to the precision of genomic breeding values: 1) additive genetic relationship, 2) co-segregation, and 3) linkage disequilibrium between markers and causal variants (QTLs). While additive genetic relationship and co-segregation are present in related population, only linkage disequilibrium between markers and QTLs is present in unrelated population. Here we present two GBLUP analyses on unstructured black cottonwood provenance trial and structured open-pollinated interior spruce progeny test using two genotyping platforms; namely, transcriptome and genotyping-by-sequencing, respectively. The GBLUP approach allowed estimating breeding values, heritabilities, and genetic correlations and aided in the simultaneous management of genetic gain and diversity during the selection process. Generally, the genomic relationship matrix provides higher accuracy of breeding values especially in populations with shallow pedigree, a typical scenario for most forest tree breeding programs.

OB 32

Testing progeny of beech plus trees in terms of height stability (preliminary study from northern Poland

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Since 2006, research have been carried out in Poland related to the testing of progeny of forest trees from the categories "selected" and "qualified". The first tested species was the European beech. The tests are conducted in order to select families and populations with the best adaptation, qualitative and quantitative properties. After five years of growing of the progenies of plus trees within 4 experimental plots, we made an attempt to analyse the height stability in the selected families. The test covered the progenies of 66 plus trees from northern Poland and it was carried out within a system of random blocks. In order to determine the genotype and environmental interactions (G × E) and to indicate the stable genotypes in relation to environment (experimental plots) we used the GEE biplot graphic method for an additive model. To study the impact of the experimental plot on the height of the tested progenies of plus trees we used the GREG multiplicative model. Obtained results allowed for the indication of families characterised by the strongest height stability. Progenies of plus trees from Elbląg and Kolbudy Forest Districts (EBG8098, EBG8105 and EBG8006 as well as KBY8432 and KBY8433 – respectively) showed the strongest stability of the analysed trait in three out of four experimental plots (Kolbudy, Miłomłyn, Wejherowo). Growth conditions within the Wipsowo experimental plot were significantly different than in the other plots, what was confirmed by the biplot analysis. The strongest differentiation in height stability was determined for the groups of progenies of plus trees from Kartuzy and Wejherowo Forest Districts. The progenies of trees from one stand may be different in terms of stability of quantitative traits. Those result should, however, be treated as preliminary and requiring further verification in the course of planned measurements in the following five-year cycles.

OA 33

Genetic parameter estimation for growth and form traits in *Eucalyptus regnans* progenies tested on two sites in New Zealand using spatial analysis

Mari Suontama, Charlie Low, Yongjun Li,
Heidi Dungey
Scion, New Zealand

Eucalyptus regnans is the world's tallest hardwood tree able to grow at a rate of up to three metres per year. In New Zealand, *E. regnans* was widely planted until the 1990's, mainly for short-fibre pulp production. In the 1990's, planting was reduced due to issues with forest health. Breeding for growth, form, and health, however, has continued, using a simple single-population breeding population with open-pollination and forward selection. From two trials comprising the third generation located in the central North Island of New Zealand, variance components and heritabilities were estimated using linear mixed tree models with heterogeneous additive genetic and residual variances. An autoregressive spatial component related to rows and columns in the experimental design was fitted to partition residual variances into a component reflecting the exponential distance based correlation and an independent error. Tree height, and a subjective score for malformation were assessed at the age of three from both sites. An additional assessment of diameter-at-breast-height (DBH) and a subjective score for stem forking was available from only one site. Heritability estimates for height and DBH were low to moderate (0.15 to 0.27). Using an autoregressive spatial structure in the tree model increased heritability estimates for height and DBH. Scores for malformation and forking had low heritability estimates (0.04 to 0.05). Genetic correlations for height and malformation between two sites were estimated as an indication of the level of genotype by environment interaction. The genetic correlation estimated for height between sites was significantly different from one (0.54), indicating high genotype by environment interaction and suggesting that different genotypes should be selected for each site type to maximize genetic gains in growth.

OB 34

Results of Scots pine provenance trial research in Siberia

Sergey Kuzmin, Nina Kuzmina
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The object of the work is Scots pine provenance trial established in 1976-1977 in south-taiga in Central Siberia. Now it exists on two plots with different soil conditions (58°22'-58°24'N 97°11'-97°13'E). 84 provenances (also called as climatypes) representing wide climatic conditi ons of Scots pine areal – from Kola Peninsula to the Sea of Okhotsk are tested here.

Long-term observations showed that height and radial growth and resistance to pathogens of tested pine climatypes strongly depend on plot conditions in the place of trial. On loam soil average tree height is in 2-4 times higher than it on sandy soil. High occurrence of pathogens: needle cast (*Lophodermium pinastri* Chev.), snow blight (*Phacidium infestans* Karst.), cenangium dieback (*Cenangium abietis* (Pers.) Rehm) was observed on sandy soil. Phytopathologic monitoring of pine on sandy soil revealed clymatypes with different resistance to pathogens. High genetic resistance to pathogens was revealed for clymatypes from northern latitudes. These provenances are from European North of Russia and from middle and southern taiga of Siberian regions. Low resistance to pathogens was observed for climatypes from western part of pine areal and southern regions of Siberia.

The features of assimilation apparatus and wood anatomy of resistant and nonresistant to pathogens climatypes were revealed. Resistant to fungal pathogens climatypes have short and wide needles with smaller stomatal density, more long duration of needle life and bigger lumen area of tracheids in early wood in contrast to nonresistant ones. The presence of different anomalies of xylem ("false" ring formation, cessation of growth, "frost" rings) and features of wood anatomy allowed revealing different reaction of provenances to weather condition and other external factors. Best climatypes in height and radial growth, stem productivity and resistance to pathogens were selected.

OA 35

Towards clever breeding: use of hyperspectral imaging for rapid assessment of field trials in genetic diversity

Darius Danusevicius, Gintautas Mozgeris, Gediminas Masaitis
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A typical high-input tree breeding programme contains many field trials with hundreds of thousands of trees. Often time consuming repeated measurements are required. Owing to the large-scale of work and time limitations, measurements are usually done once per decade sacrificing some precision in the BV estimates and introducing a risk for identification errors in older trials. We suggest sacrificing some precision too but employing modern remote sensing based on hyperspectral imaging in the field assessments. This approach may well exploit all the seemingly immense IT developments and may allow trial measurements within a few seconds, with some error of course. Straightforward traits for such assessments are growth rhythm, condition, survival, lignification, first hardness and may be even height. As the first step, we studied the spectral reflectance properties of detached shoots of several distant Scots pine provenances (northern and continental Russia, western Ukraine and Lithuania) with a hyperspectral camera. Shoots were sampled over a two week intervals during active growth and fall. There were significant provenance differences in the reflectance strength at most of the sampling occasions, allowing reliable identification of provenances. However, the significance of the provenance effect varied depending on the wavelength and sampling occasion. The near infrared spectral part was most efficient in detecting the provenance variation after growth cessation during development of frost hardiness. The most informative wavelengths were determined for each occasion and related to accumulated temperature sums.

OB 36

Evaluation of characteristics of the cultivars with resistance to snow-damage of Japanese cedar

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In regions with heavy snowfall, trees occasionally show “basal sweep” due to snow pressure damage and experience irregular growth. In Japan, from 1970 tree breeding program on resistance to snow-damage of Japanese cedar (*Cryptomeria japonica*) was conducted by Forest Tree Breeding Center and two cultivars with resistance to snow-damage named “Dewanoyuki-1” and “Dewanoyuki-2” were selected in 1996. The objective of this study is to evaluate the characteristics, especially wood property of these cultivars. Wood properties (wood density (WD), microfibril angle of S2 layer of latewood tracheid (MFA) tracheid length of latewood (TL) and stress-wave velocity (VS)), growth and tree form traits (diameter at breast height (DBH), tree height (TH) and basal bending (BB)) were investigated in four plus trees including “Dewanoyuki-1” and “Dewanoyuki-2” which were 16-18 years old and planted in three different progeny trial stands. In order to evaluate the wood properties with a high degree of accuracy, all individuals were identified using genotyping. Growth traits are not On the other hand, wood properties of “Dewanoyuki-1” and “Dewanoyuki-2” are different from other cultivars, for instance WD, and MFA which are significantly related to stiffness of stem were observed significant different between snow-damage resistant cultivars and others especially near the pith. Furthermore VS of “Dewanoyuki-1” and “Dewanoyuki-2” is faster than others. These results suggested that high stem stiffness at the young age could be closely related to the resistance to snow-damage.

OA 37

Matching Radiata Pine Genotypes to Current and Future Production Environments

Milos Ivkovich[1], **Washington Gapare**[1], **Harry Wu**[1], **Greg Dutkowski**[2], **Paul Jefferson**[3]
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Current radiata pine (*Pinus radiata* D. Don) breeding and deployment in Australia and New Zealand is largely based on state and regional boundaries rather than on patterns of genotype by environment interaction (G×E). Therefore the current genotype deployment cannot deliver optimal genetic gains across all plantation areas. To further improve radiata pine plantation productivity and to maximize realised genetic gain from breeding and deployment, it is necessary to: (*i*) delineate breeding and deployment zones based on site and climatic factors, and (*ii*) match genotypes with current and future production environments considering multiple objectives, such as maximizing growth, and improving form, branching and wood quality.

Genetic information from progeny trials was combined with soil and climatic data for more than 300 test sites to pinpoint environmental factors affecting the response of radiata pine to environmental variation, and to provide a better understanding of the factors contributing to the observed G×E. Analyses of G×E was performed using multivariate analytical techniques and mapped using geographic information system tools.

For trials in Australia, we created a database of genetic parameters as a basis for the modelling of genetic correlations. Classification of sites based on genetic correlations involved grouping conditional on certain goodness of fit criteria to minimise G×E within site types. Genetic evaluations using the TREEPLAN system incorporating the new site types demonstrated the genetic gain resulting from the improved site classification.

For trials in New Zealand, we used several analytical techniques, including Conditional Inference Trees and Multiple Regression Trees, to explain Factor Analytic clustering of sites based on geo-climatic variables. The environmental variables determining the G×E patterns were identified at different geographical scales. Potential genetic gain was evaluated that could be achieved by regionalisation of breeding values relative to using a single set of breeding values across all plantation areas.

OB 38

The rotation-aged performance of Chinese fir provenance trial in the northern Fujian

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Rotation-aged growth and wood quality traits data were analysed from a provenance trial of Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook) planted in the northern Fujian, China. A total of 81 provenances were collected range-wide from 14 pr ovinces in 1980. The provenance variation on growth and wood quality traits were associated with environmental components of geographic source. The information on provenance performance is important to select adaptive population for local breeding program to maximise genetic gain. Our analyses provided a biological and geo-climatic basis for the selection of breeding and deployment population in northern Fujian for Chinese fir. All provenances were divided into eight groups with different characters in terms of the results of principal components analysis and cluster analysis. The canonical correlation analysis show that there exists significant correlation between the biological traits and geographic coordinates (longitude and latitude), and significant geographical variation trends were obtained for traits. Seed transfer guidelines were updated according to the rotation-aged provenance testing results. Seed planning zones were delineated using current climatic data and predic ted climate changes to minimize negative impacts associated with predicted climate change over the rotation. In addition, the best genetic material from the trial will be infused into the advanced generation breeding program of Chinese fir for enhancing genetic gain.

OA 39

Genetic evaluation – the breeders' perspective

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Tree improvement programs aim to breed, select and deploy genetic material with improved biological characteristics for traits of commercial importance. Advanced generation breeding programs are complex: data is generated for numerous traits measured on many trees at different times, at different locat ions, and for different reasons. The purpose of data analysis and genetic evaluation is to summarise all performance data amassed in many trials over time, and use the results to make predictions about the potential of different genotypes for use in breeding and deployment.

The tree breeder has many decisions to make. Does the economic model differ among companies? Does the potential of different genotypes vary according to site type and production system? What genotypes to select and graft into breeding arboreta and seed orchards? What genotypes to use as parents and in what combinations throughout the breeding season? What progeny to put in trials, how many sibs in each trial, and what trial designs to use? When to assess trees in trials? Which traits are measured on which genotypes? Where and how is the data and information stored and analysed? Who can access the data and results? Are predictions meeting expectations? Are gains over time monitored? Can genetic material from different sources be compared, in biological and economic terms? How is relatedness managed in populations over time? How do we incorporate new theories and the latest research findings?

Decision making is dynamic as information available to the breeder is continually changing on a daily, monthly and yearly basis with rolling front programs. The breeder needs ready access to flexible tools and systems that can assist with various tasks if optimal decisions are to be made. Progress will be discussed in the context of cooperative tree improvement programs in Australia.

OB 40

Assessment of Arbutus unedo L. clonal plants in a field clonal trial

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[3]*GREENCLON, LDA, Portugal*

Arbutus unedo L. grows spontaneously in several Mediterranean countries. The edible fruits are used to make a spirit which represents the main income for owners. Adult plants were selected according to their fruit production and quality and then were micropropagated. Clonal trials were established in different conditions to test: traits heritability; clonal production in different environments; to compare clonal productivity and quality to seedlings; to evaluate the interaction between the genotype and environment and, finally, to identify the elite clones and their clonal allocation.

In this work, fruit production results are presented after 5 years of a clonal trial establishment, where clonal plants and seedlings were tested using 2 fertilization levels (compared to control). The experiment consisted of four blocks with 5 plants per treatment in a total of 120 plants (2 plant material x 3 fertilization x 5 plants x 4 blocks; 1920 m^2 at 4x4m^2). The clonal production was significantly higher (557.5±5.8 Kg/ha) compared to seedlings (62.6±1.2 Kg/ha). The addition of fertilizer significantly improved the production, compared to control (4.03 times; regardless of the fertilizer). Significant differences between blocks were observed. The best results were observed with clonal plants when fertilized (746.9±18.9 Kg/ha).The clone tested proved to be stable, as even in the worst block/site had higher production than seedlings (respectively 160.0±9.3 and 2.1±0.4 Kg/ha). The physico-chemical fruits characterization was performed on 2 successive harvests. The fertilization improved significantly the total sugar content (23.5±2.35 ºBrix) compared to non-fertilized plants (20.8±1.4 ºBrix). Clonal plants produced 8.9 times more than seedlings but showed inferior ºBrix content. This suggest that the use of adult selected clonal plants and properly fertilized can lead to achieve a greater fruit productivity and quality. Currently other clones are being evaluated in different environments.

OA 41

Use of prediction error co-variances for determining connectedness among test series

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Establishing connectedness across multiple test series is an important component of forest tree breeding programs. It is well recognized that the genetic means of each series can change especially if series comprise different sub-populations (genetic groups). Simultaneously estimating genetic group and environmental effects is made more accurate if there are genetic connections between the series. Even if genetic group effects are absent genetic connectedness is important for improving the accuracy of a contrast between genotypes from different series.

The accuracy of a genetic value contrast is the correlation between the predicted and the true difference of genetic values and is derived using prediction error co-variances (PEC) and the additive relationships between the individuals in question. The PEC are obtained by inverting the coefficient matrix of the mixed model equations. The aim of our study was to determine the best strategy to connect multiple test series, where best is defined as improving the average accuracy of a genetic value contrast while minimizing resources needed to achieve connection. Connection is achieved by sharing common reference material. The source and amount of reference material defined each strategy. The percentage of reference material relative to test material ranged between 0% (total disconnection) to 100% (full connection). It was shown that use of over-replicated families as the source of reference material was the most efficient strategy. Test families for a series will have variable numbers of seedlings. Some will have sufficient numbers to plant as replicated treatments in other series. If few as 10% of the families in each series are tested across all series, the accuracy of comparing two genotypes is the same regardless of whether they are from the same series or not. Implementing such a strategy also improves the accuracy of individual genetic values, even for those progeny in families not over-replicated.

OA 42

Results from a joined Polish–French network of progeny trials with European larch

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Open pollinated seed from selected trees were jointly collected by IBL and INRA in native stands of *Larix decidua* ‘*polonica*’ in Swietokrzyskie Mont. in 1987. From this collection a network of progeny trials was set up. Altogether 157 progenies were collected and shared among France, Poland and Belgium. Eight progeny trials (4 in France, 2 in Poland and 2 in Belgium) were established in the nineties. The aim of the experiment was to investigate genetic variability at the stand and progeny levels and the behaviour of the half sib families in various environmental conditions. Network aim was also to study the possibilities of long-distance seed transfer in ecologically contrasting environments.

The growth and architecture of trees (stem form and branch angle) were recorded ten years after planting in the field. Polish sites were better in growth than the Western European sites. In contrast, stem form was on average slightly worse in Polish conditions compared to French and Belgian sites. In France, *polonica* larch suffered on some sites from several defects connected to growth (drought cracks), sensitivity to wind storm and to *Meria laricina*. Compared to sudetan larch and hybrid larch controls from seed orchards, *polonica* larch looked in Belgium on average less good for both growth and stem form. In France, polish larch competed well with other European larch origins (sudetan, alpine) or species (Japanese and hybrid larch) except with some of the best commercial varieties of hybrid larch. It is shown that a high genetic variability exists in network trials for all traits, offering large possibilities for genetic improvement and high genetic gains. This study indicates seed transfer possibilities across Europe, but GxE interaction exists. A detailed study will be required to take into account ecological data of the trial sites.

OA 43

Evaluation of Near Infrared (NIR) Spectroscopy to Predict Post-Harvest Needle Retention in Fraser Fir

John Frampton, Gary Hodge, Robert Jetton
North Carolina State University, USA

Cultivation of Fraser fir (*Abies fraseri* [Pursch] Poir.) as Christmas trees has developed into a significant industry in the Southern Appalachian region of the United States producing a revenue exceeding $US 100 million annually. National surveys indicate that needle loss or "messiness" is a major complaint of real Christmas tree consumers. Therefore, post-harvest needle retention is being incorporated into efforts to genetically improve Fraser fir for the Christmas tree industry. Although they change during the harvest season as chilling hours accumulate, heritability and variation for post-harvest needle retention estimated utilizing a detached branch technique are sufficient to select and breed for improvement. Because NIR spectroscopy may offer a faster less costly method to assess this characteristic in Christmas t ree genetic improvement programs, a study was carried out to evaluate its effectiveness.

During each of three sampling periods (16-17 Oct, 30-31 Oct and 13-14 Nov 2013), two branches (one from the north side and one from the south side) from each of 105 grafted clones established in Ashe Country, NC, were collected for assessment of post-harvest needle retention via the detached branch technique. In the field, the adaxial and abaxial side of each branch were NIR-scanned with a mircoPHAZIR handheld unit (100 wavelengths from 1600 to 2400 nm). Additionally, needles from each tree were placed into plastic bags and scanned in the field with the handheld unit as well as later in the lab with a Foss NIR Systems 6500 desktop machine (1050 wavelengths from 400 to 2498 nm). Models were developed to predict post-harvest needle retention as well as needle and branch mo isture content using various preprocessing transformations and partial least squares regression techniques with cross-validation.

FRIDAY, AUGUST 29, 2014

Keynote Lecture
Today's Successes and Future Challenges for the NC State University Cooperative Tree Improvement Program

Steve McKeand, Graham Ford, Jarrod Morrice, Austin Heine, Ross Whetten, Tori Brooks, J.B. Jett, Fikret Isik
North Carolina State University, USA

The economic impact from almost 60 years of tree improvement in the southern United States has been staggering. Over 300,000 hectares are planted each year with seedlings from the breeding efforts of members and staff of the North Carolina State University Cooperative Tree Improvement Program. The present value of continued genetic gains from tree improvement is estimated to be $1.9 billion USD to landowners and citizens in the southern US. A significant challenge for tree breeding programs is the dwindling capacity to conduct breeding, testing, and selection, so all tree improvement activities must be as efficient as possible in future breeding cycles. In preparation for the Cooperative's fourth breeding cycle, pedigree analysis tools were used to provide insights for our population management options. Pedigree analysis showed minimal inbreeding in any of the third-cycle breeding populations of *Pinus taeda*. In 2012, we implemented the Differential Evolution (Kinghorn 2011. GSE 43:4) algorithm developed for animal breeding programs to develop a mating design for the fourth cycle. The objective was to increase genetic gain while maintaining long-term genetic diversity so gain can continue for multiple generations. The plan is to test 1,600 crosses for the fourth cycle in the Coastal, Piedmont, and Northern populations. Cooperative tests will employ an alpha cyclic incomplete row-column design and rolling front progeny testing approach to evaluate large numbers of trees in field trials. Such a strategy will allow better connection between progeny tests across years and also reduce the total testing effort. The Cooperative aims to finish crossing for the fourth breeding cycle by 2016 and progeny testing and selection by 2024. Research from the NCSU Tree Improvement Program seeks to strengthen the efficiency of traditional breeding while enhancing resources for its members. We will discuss genomic selection and climate change research projects.

Keynote Lecture
Tree Breeding in B.C. in 2014; new tools, variable targets, and uncertain times

Alvin Yanchuk
BC Ministry of Forests, Lands and Natural Resource Operations, Canada

Tree breeding in British Columbia goes back now over 60 years, and has seen tremendous changes in the technologies and approaches we have used, as well as the level of support going into forest genetics research and tree improvement. Over this time, we have moved a number of impor tant tree species into advanced generation level breeding, as well as sorted through many challenging and important forest genetic resource management questions (e.g., genetic conservation, genetic diversity management, disease and pest resistance breeding, seed transfer in changing climates, to name a few) that have given us a social and economic license to continue. Although we have seen a large increase in the technology available to us in both quantitative and molecular genetics over the past 15 years or so, there has also been a large reduction in the importance of forestry in the Province.

In this presentation I will review the status of some of our major breeding programs, the current traits of interest, and how we are attempting in integrate some of these new technologies available to us. While it is an exciting time in tree breeding, with all these extraordinary genetic tools we have in front of us, not to mention decades now of developing intimate biological familiarity with the species we work with, the biggest decisions now in front of us may be what to invest our time and resources on with the expectation of continual reductions in support and overall capacity in tree breeding.

OA 44

Towards a European Douglas-fir breeding population for long term breeding

Daniel Michaud[1], Jean Charles Bastien[2],
Léopoldo Sanchez[2]
[1]FCBA, France; [2]INRA, France

Since the Second World War, Douglas-fir has become a major reforestation species in Western Europe. In France, after Maritime Pine, it is the most widely planted conifer, currently accounting for 400,000 ha. Presently Douglas-fir seed used in French reforestation is harvested in eight clonal seed orchards.

The future of the Douglas fir breeding program in France is based on the evaluation of a vast progeny test network, involving 1,000 open progenies collected in 1985 within the coastal range of the species at low elevation in Washington and Oregon states.

The present paper describes the way this material was collected, then planted over a period of 10 years by six western European countries with the financial support of the European Community. The progeny test network today covers a total area of 280 ha, more than two-thirds of which is planted in France.

A description of the structuration of the genetic diversity (between & within population), observed in the French part of the progeny test network, is also given for adaptive, growth and form traits.

A forward selection of superior trees for generating the future Douglas-fir breeding population is also proposed with preliminary indication on expected genetic gain for the next generation.

This paper finally discuss the perspective offered by this progeny test network to start a European joint Douglas-fir breeding program, taking into account the global warming challenge.

OA 45

Management of the gene resources of broad-leaved species in Sweden

Lars-Göran Stener[1], Ola Rosvall[2]
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The demand of renewable and sustainable resources is expected to increase. At the same time the climate change scenarios creates uncertainty regarding futur e growth conditions in the Swedish forests. Thus it is essential to make the genetic adaptation to a new climate easier. Skogforsk has now proposed a strategy for a fast and effective adaptation of the broad-leaved gene resources. Totally 13 species are of main interest and maybe another 4 exotic species will be included.

The basic plan is to harvest open-pollinated seed from totally 500 trees for each species within the Swedish distribution area. Such a population will with high probability (95 %) and in long term (50 generations) preserve alleles existing in a frequency down to 0.02.

The 500 selected trees will be distributed on 1-2 degree latitude belts, creating 5-10 subpopulations each including 50-100 of the selected trees. For each subpopulation three gene resource stands, each including 50 plants per selected tree, will be established within and north of the selection region. These stands are managed to favour seed production to rapidly create new generations of gene resource stands to be established more or less further to the north of the existing ones. The general idea is to use open pollinated seed and establish plants without knowledge of family identities. A future option is to use DNA technique for pedigree construction if for instance breeding activities is of concern.

OB 46

Genetic improvement of Acacia senegal for gum arabic production: a question of ploidy level

Adja Diallo, Erik Dahl Kjær, Lene Rootsgaard Nielsen, Jon Kehlet Hansen
University of Copenhagen, Denmark

Acacia senegal (L) (Willd.) is a multipurpose tree, native to semi-desert regions of Sub-saharan Africa, Pakistan and Northwestern India and represents the main species producing gum arabic, a commodity of the international trade. It also provides multi ple services such as soil fertility improvement in agroforestry systems and fodder for livestock in dry season. Estimates of genetic parameters for gum production based on an effective progeny test and reliable information about gum yield and yield trends are missing. We therefore studied gum quality and yield in an 18 years old progeny trial consisting of 15 families from each of four different populations in Senegal. Growth traits such as basal area, height, biomass and crown diameter and gum quality regarded as nitrogen and carbon contents were assessed in 2012 while gum yield was assessed both in 2012 and 2013. The estimation of the coefficient of relatedness within family from microsatellites markers indicated that families are true half sibs. However, based on markers we also found that the trees represented two levels of ploidy, and that the average performance of the trees differs between these two ploidy levels. In addition, the estimates of additive genetic variance and heritability were higher for polyploid families compared to diploid ones for growing traits as well as gum yield, predicting high genetic gain from selection. These results suggest that a breeding program for gum productivity based on polyploid families can lead to significant improvement in gains. Implication for breeding of *Acacia senegal* is discussed.

OA 47

Progeny testing noble fir in Oregon and Washington for traits important to Christmas tree producers

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Noble fir (*Abies procera* Rehd.) is the leading Christmas tree produced in the states of Oregon and Washington (USA) with yearly plantings of over 4 million trees. Progeny testing in commercial plantations (primarily ½ sibling families) with evaluations for traits important to Christmas tree producers in the region has gone on since mid 1970's. Testing since 1996 has included 215 additional families from across the natural range of noble fir along with selected families from seed imported from Denmark.

Some of the emerging observations in a regional scale are the following:

- The ½ sibling sources from the coastal mountains in Oregon, consistently are among the top for Oregon and Washington producers based on rapid growth, tree value, bud development and branch structure.
- The ½ sibling families tested from the Oregon and Washington Cascade mountains consistently rank low in grower selections for value.
- Sources from the southern limit of noble fir in the Cascades are consistently slower growing with an open growth habit and share traits with Shasta fir (*Abies magnifica* var. *shastensis)*
- Average tree values range from near $16 (USD) for the higher ranked families to a low of $5.5 (USD).
- Some isolated coastal mountain sources in Oregon, consistently rank in the top groups of tested progeny for value.
- The tested Danish sources share many traits with the Cascade mountain sources with consistently high evaluations for superior color.

OB 48

Genetic improvement and breeding of Pinus massoniana in China

Kongshu Ji
Nanjing Forestry University, China

Pinus massoniana Lamb. is a very important forestation species in China. Genetic improvement and breeding of *P. massoniana* was begun in 1950s, and was widely developed since 1980s when genetic improvement and breeding of main forestation trees was arranged in the sixth National Five-Year Plan. Its genetic improvement and breeding has been experienced five periods. First is the provenance tests focused on the growth traits to draw up the strategy of allotting seeds and to get the most adaptable seeds for a special site. The population variation model was also studied and the first generation seed orchards were established. Second period, mainly studies were on the techniques for seed orchard with more than 5,500 plus trees were selected during the seventh National Five-Year Plan. Totally, more than 1,000 hectare of clonal seed orchards was established. Third period was mainly focus on the wood quality improvement and seed yield increase in the seed orchards. Clonal forestry began to be set foot in *P. massoniana.* The fourth period was from 1995, since the ninth National Five-Year Plan, with the necessary for forest of industrial material, the genetic improvement and breeding was focused on the directional breeding. *P. massoniana* was directed on the improvement for material of pulp and paper use. The seed orchards established in the past were transformed into the directional seed orchards. From 2005, the construction on the second cycle of the seed orchard began, and more than 600 hectare of second cycle clonal seed orchards has been established by now. Moreover, techniques of cell engineering began to be studied. And the transcriptome sequencing was done. This has helped the genetic mapping and molecular markers assisted breeding. The functional genomics and associated analysis will be studied in the future and this will make progress on the study of genetic improvement and breeding.

OA 49

Tree improvement of birch and alder for Ireland based on progeny testing

Ellen O’ Connor
Teagasc, Ireland

A tree improvement programme to increase the choice of species and quality for broadleaf forestry in Ireland has included three native pioneer species.

Two species of birch are native to Ireland; *Betula pubescens* Ehrh (downy birch) and *Betula pendula* Roth (silver birch). Unlike most other countries, where the emphasis has been on silver birch improvement, the two species of birch were included in an improvement programme that began in 1998. Poor stem form was a feature of birch growing in Ireland. Seed from plus-trees was used to establish progeny/provenance trials for family comparisons. ANOVA analysis has shown significant differences in growth values between families (and provenance). Stem form was also associated with family. At age 10, about 5% of the progeny trees displayed exceptionally good stem form. Re-selection within the progeny trials, based on quality and growth characteristics, has been used to develop an indoor seed orchard. The downy birch programme has reached a stage where it is ready for scale-up to commercial production. Progress with silver birch has been slow due a scarcit y of native plus-trees at the very start of the programme.

The improvement of *Alnus glutinosa* (alder) was initiated in 2005. Seed from these plus-trees has been used to establish progeny trials for family comparisons. Family variation in the characters of early height growth, crown damage index in response to pest and disease and leaf flushing have been determined in the progeny trials. The early results indicated that, although the original distribution of the plus-trees was restricted, family variation existed on which to base an improvement programme. Backward selection is planned for the alder breeding.

The presentation will present the results from the progeny/provenance trials.

OB 50

European Larch breeding programme in Poland

Jan Kowalczyk, Jan Matras, Marek Rzońca
Instytut Badawczy Leśnictwa – IBL, Poland

European Larch is one of the main forest tree species in Poland. It is growing in all Poland in scattered distribution. Traditionally larch is recognized as important part of stands in Skarżysko and Bliżyn region (Góry Świętokrzyskie) and in Sudetan. In the north Poland larch stands heve considerable contamination of Japanese larch and their hybrids.

Larch breeding in Poland was started after second word war together with Scots pine and Norway spruce. In the beginning two direction of breeding activities was launched. One was plus tree selection and based on phenotypically selected material seed orchard and seedling seed orchard was created. Second one selected seed stands was appointed and utilized as for the seed collection. Basically until now this two directions are running. Because it is difficult to collect cones from the adult high trees about 70% of reforestation material are coming from seed orchards. All material is first generation of seed orchard coming from phenotypic selection.

Breeding activities are supported by research concentrated mostly on progeny material coming from Świętokrzyskie Góry region and from Sudeten. This part of investigation is realized with cooperation with INRA Orleans. In last year also all seedling seed orchard treated in Poland also as progeny tests was measured. On the base of the result of the analyzed progeny test new breeding population was created. One simple outcome will be backward selection the best 40 genotypes and creation 1.5 generation of seed orchard. Estimated mean genetic gain expressed in percentage in relation to studied tests will be 12 % for DBH, 8% for height and 4% for stem straightness. For the research propose we intended to start controlled crossing between some of the selected clones.

OA 51

Tree improvement through breeding seed orchard; strategy and current situation in Nepal

Youba Raj Pokharel
District Forest Office, Nepal

Breeding Seed Orchard (BSO)s are a robust, efficient means of implementing seed delivery oriented tree improvement activities in Nepal. Improvements in the quality of the trees being planted, and secured supplies of seed at district level can be achieved only by the establishment and management of BSO. It is an important alternative to conventional breeding strategies, which is based on recurrent selection from a single breeding population. In their simplest form, BSO can be established to provide a secure supply of improved seed in a relatively short term and the design is characterized by planting at a close initial spacing followed by progressive and highly selective thinning, using available genetic information. BSOs are established principally as *ex- situ* gene conservation resources.

A strategy for the improvement of *Dalbergia sissoo* in southern Nepal, based on the establishment of a series of BSOs was developed by tree improvement program under Department of Forests. Using seed from the base breeding population, intermediate level BSOs (the design is a randomized complete block, with a plot of each family allocated randomly within each block) were established to prepare first generation BSOs between 1994 and 1996. In the mean time, a total of 43 seed orchards of 27 multipurpose and commonly demanded species have been established in 18 districts of all 5 development regions. Out of them one progeny trail, one infusion trail, one provenance trail, 30 intermediate level and 10 simple level seed orchards have been established and managed. This paper deals with the current status of those different level of seed orchards in different ecological zones of Nepal.

OA 52

Review on advanced-generation genetic improvement of Chinese fir in China

Renhua Zheng[1], Liming Bian[2], Shunde Su[1], Jinhui Chen[2], Jisen Shi[2], Harry Wu[3]
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Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook) is one of the most widely planted tree species in southern China, and the plantation area was occupied around 6.6 million hectares, which is around 15% of total plantation area of China. The Chinese fir breeding program st arted in the 1950s, and the initial efforts were mainly focusing on growth traits and a little bit attention on needle diseases. Breeding for growth rate and resistance in the first three generations (1957-2003) has increased substantially by ~12% (by volume) within each breeding cycle, with a soundly benefits from the increased wood production per harvested unit. Meanwhile, the genetic gain on wood basic density was by 5% in each breeding cycle, which is largely due to the weaker genetic correlation between growth rate and wood density on Chinese fir. With this large genetic improvement and better silviculture treatments, the harvesting rotation of the commercial plantations planted with genetic improved stock has been substantially reduced from 25~35 years for previous rotations to around age 18 or less. As we put forward the Chinese fir breeding program advancing to the fourth breeding cycle, there would be more traits need to be considered for wood quality in the genetic improvement program, especially on the formation mechanism for higher proportion of the heartwood with dark brown color at harvesting.

POSTER PRESENTATIONS

P 01

Forecast of Phenotypic Parameters for Growth Traits in a Clonal Seed Orchard of Scotch Pine (Pinus sylvestris L.) in the Erzurum Region in Turkey

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Genetic and phenotypic parameters for height, diameter at breast height (dbh), and volume were estimated for Scotch pine (*Pinus sylvestris* L.) clonal seed orchard in Erzurum using an ASReml program, fitting an individual tree model. The data were from 30 clones assessed at 5, 10, 15, 20 and 23 years of age. Heritability estimates for height, dbh, and volume were moderate to high ranging from 0.25 to 0.63, from 0.18 to 0.61, and from 0.26 to 0.65, respectively, suggesting a strong genetic control of the traits at the individual level, among families, and within families. The genetic and phenotypic correlations between the growth traits were significantly high and ranged from 0.72 to 0.98 and from 0.76 to 0.97, respectively. This suggests the possibility of indirect selection in trait with direct selection in another trait. The predicted genetic gains showed that the optimal rotational age of the Scotch pine clonal seed orchard is 20 years. The genetic gains were high for all studied traits. These results suggest that the growth improvement through individual selection in the orchard or a combined selection among and within families is possible. The results show that the genetic gains increased with an increased age up to the age of 30 years and decreased with an increased age after the age of 20 years for all the traits. This indicates that the rotational age of the Scotch pine clonal seed orchard is 20 years of age.

P 02

Flowering variation as to years in Scots pine young clonal seed orchard

Sezgin Ayan, Temel Sariyildiz, Esra Nurten Yer
Kastamonu university, Faculty of Forestry, Turkey

In this study, in “young” clonal seed orchards (YCSO) of flowering among years depending on the clone and ramets variation exerts how the change has been attempted. The hypothesis of the study; in YCSO thanks to aging, flowering variation among years, clones and ramets was assumed to decrease. The measurements were made on five ramets for each of 30 clones in four years on Scots pine seed orchard. The orchard was established in 1995 by using 2 years-old grafts in Kastamonu, northern Turkey. The examined characters were number of male (NMF), female flowers (NFF).

When the four-year of data from Scots pine YCSO was evaluated, mean values from NMF and NFF among the clones were significant and among the ramets the variation was high. The coefficient of variation (Cv) from the NMF of the clones gradually decreased with the subsequent years (Cv2006=%147,54, Cv2010=%59,04), whereas the Cv in the NFF did not show any decrease with the same years. There were significant differences among the years as to the NMF, NFF. Over the four years, the NMF was significantly lower than that of the NFF, and even in some years the NFF was doubled compared with the NMF (NMF2006=65,9; NMF2007=314,29; NMF2008=427,85; NMF2010=115,73 & NFF2006=123,80; NFF2007=604,68; NFF2008=394,62; NFF2010=196,72). Abundant flowering periods seen in natural stands were also observed in the male and female flowers of the clonal seed orchards.

For the studied seed orchard high variation both among the clones and the ramets indicates the high selection capacity in the breeding programs. The bigger variation among the ramets confirm that the genotypes have responded against the heterogeneity of growing area in seed orchard or the ramets have not reached the optimum flowering period. These results have shown the importance of the practices which increase the flowering yield and effectiveness of fertilization in YCSO.

P 03

Review and Outlook on hybridization breeding of the Genus Liriodendron in China

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Nanjing Forestry University, China

Hybridization in breeding of tulip tree (*Liriodendron* L.) in last 50 years was reviewed. The history since the beginning of hybridization carried out by the late professor Ye Peizhong could be divided into three stages with researches focusing on: test of hybridization compatibility; investigation concerning heterosis of hybrids on growth and adaptability; promotion the use of hybrid varieties. The results of past hybridization experiments have shown that Interspecific crosses involving *Liriodendron* chinense × *L. tulipifera* & *L. tulipifera* × *L. chinense* had good compatibility. Cross-pollination surprisingly increased the percentage of filled seeds per aggregate samaras compared with open- pollination. Interspecific hybrids were successfully obtained from reciprocal crosses between *Liriodendron chinense* × *L. tulipifera* &*L. tulipifera* × *L. chinense* . Growth and adaptability of the hybrids had the more vigorous compared to their parents. More than 300,000 of Liriodendron hybrids have been planted for landscaping, and also used in the mountains of southern plantations of about 10,000 hectares in China. The genus hybrid is a separate species, having its distinct morphological & biological characteristics and being widely cultivated and used in China. According to the“International Code of Botanical Nomenclature”on hybrid naming rules, the name of the hybrid is newly revised. Now the new name is *Liriodendron sino- americanum*P.C.Yieh ex Shsng et Z.R.Wang. The paper also presented an outlook for the breeding of the tulip tree.

P 04

Degree of hybridization in Pinus engelmanni in eight locations in the State of Durango Mexico

Israel Avila, Cristhian Wehenkel, Jose Ciro Hernandez
Juarez University of the State of Durango, Mexico

Pinus engelmannii Carr is a species native to northern Mexico, which is used in the conservation and restoration programs currently being implemented in the country. Therefore it is required; inter alia, accurate recognition of genetic variations of species, which will introduce genotypically suitable individuals. This way it may have a tool to reduce risk by introducing species and unsuitable backgrounds to a particular site, plus may be at perform better management and conservation programs of the forest resource. Methods based on variable polymerase chain reaction (PCR) amplification such as amplified fragment length polymorphisms (AFLPs) can provide a rapid and affordable approach to collecting polymorphism data on a genomic scale Using the software Structure version 2.3.4 (http://pritch.bsd.uchicago.edu/structure.html) a simple method was applied to study the degree of hybrids in eight populations of *Pinus engelmannii* Carr. in the state of Durango Mexico. The study was conducted with AFLP for detecting hybrid individuals in these populations. In total, 286 individuals were typed at 204 loci. One clustering based method was applied to classify and identify the individuals into three categories: pure individuals, other species and, hybrids. Individuals identified as *P. engelmannii* were clearly separated from the hybrids in the stands, finding a total of 28 individuals hybrids. The results of the study demonstrate a minimum degree of hybridism in the population studied.

P 05

Application of the Minimum Inbreeding (MI) seed orchard design in the Czech Republic

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[1]Czech University of Life Sciences Prague, Czech Republic; [2]Military Forests and Estates of the Czech republic, s.e., Czech Republic

In the past, seed orchards layouts in the Czech Republic have been solved by simple randomization, despite that several modifications of permutation designs were available based on the avoidance of direct neighborhood of the same or related clones and these were implemented into the forestry practice in other countries. With continuous shift to advanced generation seed orchards, it became necessary to outline new, globally optimum seed orchard layouts, such as the Minimum Inbreeding seed orchard design by Lstibůrek and El-Kassaby, that can optimally accommodate many operational constraints. In line with the theoretical development of the MI design, we extended the problem complexity and applied the algorithm to optimize clonal allocation in new seed orchards (Scots pine and Norway spruce) established by the Czech State Forests, s.e. and the Military Forests and Estates, s.e. (two biggest forest owners in the Czech Republic). Our approach to the MI design utilizes custom made software built around the Quadratic Assignment Problem (QAP) with additional routines for large data support. One of the most efficient heuristics for the QAP has been implemented with further refinements to facilitate specific optimization problem types encountered in seed orchard optimization. Number of the first and the second generation seed orchards in the Czech Republic have been established using this method with several options, including added relatedness, unequal clonal deployment, preferential mating, and/or variable spatial configuration.

P 06

Establishment of seed and planting zones of teak (Tectona grandis Linn) in Myanmar based on Bayesian genetic structuring with nuclear microsatellites

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Teak is one of the most precious tropical timber species due to its versatile qualities and indigenous to only four countries: India, Myanmar, Laos and Thailand in South and South East Asia. Teak plantations are established in 36 countries in the world and ca 9% is in Myanmar. The establishment of plantations using seeds sources without genetic information would result in genetic disturbance of teak. Myanmar has nearly 60% of total natural teak forest ca. 27 million ha, however Myanmar teak has never been investigated using molecular markers. To provide the genetic information for breeding program through understanding genetic structure of Myanmar teak and to fill the gap in genetic information of natural teak, we investigated 480 individuals representing 20 natural populations from five regions of Myanmar using 10 microsatellite markers. The genetic diversity parameters such as expected heterozygosity (HE: 0.530 min, 0.763 max) and allelic richness (R: 3.09 min, 8.29 max) displayed high genetic diversity and moderate level of genetic differentiation among populations (FST = 0.079) of Myanmar teak. The highest genetic diversity was detected in population from lower region while the lowest diversity in population from southern region. Bayesian structuring method showed four genetic clusters of teak generally corresponding to the different regions. Cluster I was dominant in the lower and central regions, cluster II in the upper and eastern regions, cluster III in two populations which are geographically close, and cluster IV mainly in the southern region. To retain the natural genetic resource of teak from Myanmar and to formulate the sound breeding program, basically four zones would be designated as planting and seed zones.

P 07

Estimation of fertility variation by strobili and cone productions in Taurus cedar (Cedrus libani A. Rich.) populations

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[1]Suleyman Demirel University, Turkey; [2]Seoul National University, Korea

Taurus cedar or also calledLebanon cedar (*Cedrus libani* A. Rich) was classified as one of the economically important species for Turkish forestry and the “National Tree Breeding and Seed Production Programme”. The study was carried out onto the estimation of fertility based on the strobili and cone productions in two natural stands of Taurus cedar (*Cedrus libani* A. Rich.). Fertility variations were investigated as the proportion of the numbers of strobili and cones counted from individuals in the populations. The averages of female strobili and cone production were similar between two stands, but the average of male strobilus production was much higher in Population-1 than Population-2. The coefficient of variation (*CV*) of strobili and cone production showed that there was a small difference among reproductive characters and between populations. However, there was a large variation of individual fertility in both stands. The effective population size estimated by female fertility variation was highly correlated with that by cone fertility variation. Our results showed that about 64% of individuals in the Popualtion-1 seemed to act as they were under the ideal population. The effective population size, equivalent to the ideal populations, was 15% larger in Population-2 than Popualtion-1, based on the fertility variation of individuals in the studied populations.

P 08

Mating System in open-pollinated progenies of a natural population of Genipa americana L., by analysis of microsatellite Loci

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The mating system is fundamental in the determining the genetic structure of natural populations. However, anthropogenic changes such as the fragmentation and degradation of continuous forests can affect the population in the density of individuals reproductive and behavior of pollinators may result in increased rates of selfing and correlated mating. In this study investigated the mating system in a small population of the tropical tree species dioecious pollinated by bees *Genipa americana*, using eight microsatellite loci and mixed-mating model and model of correlated mating. Open-pollinated seeds were collected from 13 trees located in a small natural population (7.2 ha) located in the Mata da Figueira, in the Ecological Station of Mogi-Guaçú in São Paulo State. The population of *G. americana* presented mixed mating system, with higher prevalence of crossings ($t_m = 0.91 \pm 0.03$). The correlation of selfing was significantly different from zero in the progenies and the outcrossing rate ranged from 0.79 to 1.00 in the trees of the population. The estimate of multilocus crossing rate different of unit (1.0) confirms the presence of selfing. The difference between the multilocus crossing rate and unilocos, which measures the outcrossing rate between relatives (t_m-t_s = 0.37 ± 0.07) was low (up to 0.50). The low rate of outcrossing between relatives observed suggests intrapopulation spatial genetic structure in the population. However, the highest proportion of inbreeding in the progenies of the population studied was due to the outcrossing between relatives. The paternity multilocus correlation between and within fruits ($r_{p(m)} = 0.47$) was significantly higher, suggesting that most of the trees were effectively fertilized by few pollen donor (N_{ep}), average of two pollen donor, indicating that great part of the offspring are full-sibs.

P 09

Concept of intra-population analysis of genetic structure within populations of wild cherry (Prunus avium L.) in influenced urban area and selected superior trees originated from natural forest stands

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Insights into the within-population spacial genetic structure of two distinctive groups of wild cherry population enhance understanding of pollen dispersal processes and provide information valuable in breeding and forest management.

Urban-influenced population of wild cherry is represented by 140 individuals selected within area of interest nearby campus of Czech University of Life Sciences Prague. Population of phenotypically superior individuals originated from natural forest stands is represented by 61 grafted plants (clones) in the wild cherry seed orchard.

Wild cherry belongs to the group of species which employ gametophytic self-incompatibility. This phenomenon prevents self-fertilization and thus encourages outcrossing. We plant to investigate the genetic diversity and the spatial genetic structure within a *Prunus avium* population at two contrasting gene loci: nuclear microsatellites (10-12 loci) and S-locus. We believe that the assessed genetic structure at the SSRs and S-locus help to specify possible mating patterns in the seed orchard and elucidate the origin of individuals belonging to the urban influenced population.

Generated genomic data will be analyzed and discussed at three levels (evaluation of the genetic structure of individuals with the assumption of high anthropogenic influence, evaluation of individuals from indigenous forest stands and mutual comparison between these two types of habitats). Agro – landscape aspect of the project is to elucidate principles of dissemination of genetic information between cultural varieties of wild cherry populations and the impact of this phenomenon on the overall genetic variation at these man-influenced habitats. In the seed orchard the relationship coefficient will be estimate and the effective population size will be determine. Comparison of parameters from two types of sites will lead to draw general conclusions about the population structure and pollination dynamics of this important tree species. Findings will be applied in an operational way which helps to improve the management of forest seed orchard.

P 10

Evolutionary and functional analyses of LysM-contained genes in Populus revealed two potential chitin receptors

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Plant diseases are the important cause of the reduction of plant production, and quite a number of diseases are caused by fungi. During evolution, complicated interactions were formed between plants and fungi. The most common form is infection and defence. Perception of a pathogenic fungus at the cell surface is an important step in plant immunity.

A conserved domain called LysM plays a central role in this process. *Populus* is not only an important plantation tree, but also a model system for plant biology. In this study, we identified and characterized nine LysM-containing receptor-like kinase (LYK) genes, and two chitin elicitor binding protein genes in *Populus*. Based on the genome database of the black cottonwood, the distribution characteristics and genetic evolutionary relationship of these genes were studied. A correlation between the exon-intron structure and amino acids sequence was revealed by phylogenetic analysis. All these genes were mapped on 10 chromosomes (1,2,4,5,7,8,9,11,14 and 15). Among them, two LYK genes (LYK3 and LYK8) and two CEBiP genes (CEBiP1 and CEBiP2) were direct segmental duplication. Two LYK genes (LYK3 and LYK7) and one CEBiP gene (PcCEBiP1) were selected for cellular localization observation. All of them were located on the cell membrane. Functional analysis showed that LYK8 and CEBiP2 were the potential chitin receptors in *Populus*, both of them could be induced by chitin and might play a key role in immune responses to the pathogenic fungus *Marssonina brunnea*.

P 11

Validation of the Cryptomeria japonica seed and seedling transfer system by progeny tests analysis in conjunction with environmental factors

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Forest trees often exhibit diminished ability in traits such as growth when they are transplanted at different environments from original environments. Therefore, the transfer of seeds or seedlings of forest trees for afforestation are regulated in a confined area. Japanese cedar, *Cryptomeria japonica*, which is distributed throughout the Japanese Archipelago and a major tree species for afforestation, have been set up the seed and seedling distribution zone based on meteorological conditions and information of natural vegetation.

Seeds and seedling transfer system should be studied and set up not only based on environmental factors but also provenance tests. Unfortunately, there is no nation-wide provenance test in *C.japonica* although tree breeding zones have been delineated and local-scale provenance test have been established. Therefore, the study on seeds and seedlings movement was not sufficiently conducted until now.

In recent years, GIS technique has progressed and become common, and it enables to investigate progeny test data in conjunction with environmental factors.

The objective of this study is to validate the current seed and seedling transfer system using the accumulating progeny test data with environmental factors.

Nation-wide environmental condition was characterized by principal component analysis using climatic conditions (precipitation, temperature, snow depth, solar radiation, sunshine duration), and classified into five environmental clusters using x-means method. These environmental clusters well coincide with current seed and seedling transfer zones.

We conducted the comparison analysis using 10-year progeny test data at two breeding regions (Tohoku (northern Honshu) and Kansai (western Honshu)). In Tohoku, growth of plus-trees derived from southern provenance is suppressed in northern area, implying the current regulation of seed transfer is valid. In Kansai, growth of southern provenance is not suppressed in northern area, implying the current regulation is not necessarily proper, and further investigations are necessary.

P 12

Origins of the present day Portuguese Scots pine populations assessed by molecular markers

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The southwestern limit of Scots Pine (*Pinus sylvestris* L.) distribution lies in Portugal. Most of the exemplars currently present in Portugal resulted from the plantation of allochthonous material during afforestation efforts developed in the beginning of the XX century. Scarce populations of Scots Pine located at 'Biduissa' (Bid) and 'Ribeira das Negras' (R.N.) ('Serra do Gerês'; NW, Portugal) are believed to be native. Variability within the internal transcribed spacer of ribosomal DNA (ITS rDNA) can be studied using the PCR-RFLP technique in amplified ITS products. The co-dominant molecular markers ITS PCR-RFLP were used in the current study in order to extrapolate the origins of the present day Portuguese Scots pine populations. We analyzed 11 populations (260 individuals) of Scots pine – seven from Portugal and four natural populations of Spain, Germany and Sweden. After testing several restriction enzymes, only the *Sau*96I and *Taq*I revealed polymorphic patterns indicating a total of 130 ITS length variants. The Principal Coordinate Analysis (PCoA) suggested that (i) both 'Biduissa' and 'Ribeira das Negras' are in fact native Portuguese populations and may be remnants of a glacial refugium formed during the last glacial age, (ii) whereas the remaining populations located in Portugal derived from plant material with a German or Central Europe origin. The results obtained will contribute for the deployment and development of appropriate conservation strategies of these areas, once this genetic material may constitute an important source of reproductive material to other countries that will face in a nearby future, as a result of the global climate changes, similar situations to those that are occurring nowadays in Portugal.

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P 13

Source to cold resistance in Eucalyptus breeding progrmams

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The global area forested with Eucalypt has exceeded 20 million hectares, distributed in more than 90 countries with the most diverse climates. About 50% of these surfaces are located in low temperature countries with frequent frost. This is risky for the production and can cause death in juvenile trees; and also combined with other damages like fungal or insects reducing its growth. The correct species and provenance selection for cold resistance phenotypes is a key factor before starting the tree improvement program. The comparison of native original climates with the exotic regions can be inferred with climate mapping software, associated with genetics studies and field tests. A wide range of genetic variability of Eucalyptus genus ensures the availability of species and germplasm resistance. The species *E. globulus, E. dunnii, E. nitens, E. viminalis, E. benthamii* and *E. pauciflora*, are originated from the coldest region of Australia. These species have good frost resistance and optimal growth. In the case of *E. Pauciflora* is distinguished by its extreme cold resistance, representing optimal model specie for the physiological basis to cold resistance researches. These species were used in different countries and continents, producing different results on each of the experiences. The aim of this article is to review different strategies and results obtained from cold resistant breeding around the world.

P 14

Altitudinal adaptation of Scots pine (Pinus sylvestris L.) in northern Finland

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Survival and height growth was studied in four 10-years old field trials at altitudes 180 – 340 metres above sea level in western Finnish Lapland (latitude 67 °N). The material in the trials consisted of a) altitudinal provenances collected from stands located at different elevations (120 – 380m) on two hills in western Finnish Lapland, b) commercial seed lots from different altitudinal levels and c) latitudinal series ranging from central Finland to northern Lapland (63 – 69 °N). The aim was to study how the pine populations have been adapted to lowering temperature with increasing altitude on the hills. Altitudinal transfer from lower to higher elevation decreased survival by 5 % units/ 100 m. Height of the seedlings instead increased in similar altitudinal transfer by 9 cm (ca. 6% of average height)/100 m. When the altitudinal change was expressed in degree days, the change in survival in upward transfer was 5.5 % units/ 100 dd. In latitudinal transfer the response in survival was 8.2 % units/100 dd. From this difference in adaptive response it can be deduced that the adaptation to the climatic deterioration is not as effective in altitudinal cline as in latitudinal cline probably due to stronger pollen migration in hill slope compared to longer distance lowland stands. Based on these results, the use of seeds from lower elevations in forest regeneration on higher elevations is not recommended due to the decrease in survival of the seedlings. In conclusion, taking the altitudinal origin of the seed into account is important in practical forestry in northern Finland.

P 15

Application of biophysical factors and molecular markers to explain spatial genetic structure in strawberry tree using GIS tools

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The strawberry tree (A*rbutus unedo* L.) is a widely distributed native species in Portugal, water stress and low fertility soils tolerant and actively resistant to wildfires. The fruit is used in the spirit production, the main source of income. Red fruits, with antioxidant potential, also represent a new market opportunity. The geographical isolation and extinction-recolonization dynamics are two factors causing strong genetic structure in metapopulations. We investigated how history, demography, and geoclimatic factors have affected population genetic structure, local adaptation, and, ultimately, its landscape genetics. We genotyped nuclear microsatellites and cpDNA markers in populations from across the species range. Under the project ARBUTUS (PTDC/AGR-FOR/3746/2012, *Arbutus unedo* plants and products quality improvement for the agro-forestry sector) 30 trees were selected, georeferenced, and leaves sampled, in 15 natural populations distributed throughout the country. The stands were ecologically characterized, at a local scale, using lithology, topography, soil type, climatic and landscape metrics, and wildfires records. The spatial data was stored in a GIS database and geo-processed in order to generate different Local Landscape Units (LLU) associated with each population. The populations were further clustered using large scale biogeographic and successional units' information at the country range. This *a priori* hierarchy, together with the genetic structure information, was used to explain the species landscape genetics. We aimed at identifying the most likely scenarios to explain the current patterns of genetic structure and diversity unfold for the species. The obtained information will be used in the species improvement, management and design of conservation programs.

P 16

Assessment of Genetic Diversity in Wild and Managed Stands of Norway Spruce

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Norway spruce (*Picea abies*) is late successional species capable of persisting in the understory for decades. In the absence of human activities, *Picea* regeneration is facilitated by small-scale gap dynamics driven by wind, insects, and fungal pathogens. In contrast, industrial *Picea* forests throughout much of Fennoscandia are managed under even-aged regimes. Because the seed production of Norway spruce is sporadic, logged forests are replanted with seedlings germinated from locally collected seeds, or increasingly often, with improved genotypes from seed orchards. Extensive use of artificial generation could result in a loss of genetic diversity if germplasm sources are poorly selected. However, artificially regenerated forests may also harbor more genetic diversity than natural populations due to the potentially higher number of effective breeders in a seed orchard. In Fennoscandia, empirical evidence for either scenario is lacking.

To address this knowledge-gap, we are using a panel of 300 SNPs to characterize genetic variation in four types of spruce stands: 1) pristine and undisturbed, 2) naturally regenerated after a clear-cut, 3) replanted with locally-collected seed, and 4) artificially regenerated with improved genotypes from seed orchards. Our first results suggest that primeval sites, those clear-cut and regenerated naturally, and sites reforested with locally-collected seed all have high and nearly equal levels of genetic diversity (mean $H_e = 0.31$, SD = 0.003; mean $N_a = 1.87$, SD = 0.005). In addition, no significant evidence for inbreeding was found in stands regenerated under any of these three treatments (mean $F_{is} = 0.005$, SD = 0.008). Collections in stands planted with seed orchard material are ongoing. Together, these data will be used to assess whether the dominant management strategy for Norway spruce maintains high genetic diversity and avoids imposing inbreeding and genetic drift.

P 17

Genetic divergence among progenies of Pinus elliotii Engelm. var. elliottii in a subtropical region from Brazil

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Despite of a lower volume production compared to other pine species, the *P. elliottii* shows characteristics of extreme importance for the forestry sector, as its physical and mechanical wood quality properties widely used in packaging, building, wood working as well in resin production. Breeding programs has been established to identify genotypes with high yield potential for use in commercial plantations and development of interspecific hybrids. The aim of this study was to estimate the genetic divergence among progenies of *P. elliottii* from silvicultural traits (DBH, height, and volume) evaluated from the first to the fourth age. A trial established in Ponta Grossa, Paraná, Brazil, in 2009. This experiment was carried out in a randomized complete block experimental design with 25 treatments (progenies) 32 blocks, and one plant per plot at a spacing of 3.0 x 3.0 meters. The divergence between progenies was performed by distance matrix of Mahalanobis. Based on these values, progenies were grouped into clusters using Tocher’s method. Estimative of genetic divergence, using a multivariate analysis, showed a pattern of nine groups. The majority of progenies was grouped in the first one (I) (54.17%) followed by groups II, III, IV (8.33%) and V, VI, VII, VIII, IX (4.17%). The most productive individuals of genetically divergent groups they can be used to controlled crossings to produce highly productive individuals.

P 18

Genetic divergence in Pinus caribaea var. hondurensis progeny in Brazil

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Pinus caribaea var. *hondurensis* has been planted successfully in Brazil especially in warm regions, free of frost and drought. With a fast growth, good adaptability and stem form besides a high resin production. It is originated from Central America and Mexico, at altitudes ranging from sea level to 500 m altitude, and exceptionally at 1000 m altitude. The genetic divergence was estimated by distance between pairs of *P. caribaea* var.*hondurensis* progenies for quantitative traits. The progeny trial was established p on June 1986, in Selviria, in Mato Grosso do Sul State, Brazil. Experimental design was 10x10 triple lattice design, with 100 treatments (96 progenies from a clonal seed orchard of *P. caribaea* var. *hondurensis* and four commercial controls), with linear plots of ten plants. The spacing between plants was 3 mx 3 m. Analyses of genetic divergence were performed according to REML/BLUP procedure. Dissimilarity measures between pairs of *Pinus caribaea* var. *hondurensis* progenies for silvicultural traits were estimated through generalized distance of Mahalanobis (D^2). The maximum distance ($D^2 = 65.51$) was observed among progenies 42 and 14, and the minimum (D2 = 0.15) among the progenies 33 and 22, and 93 and 38, respectively. The pattern of phenotypic structure of 96 progenies of *P. caribaea* var. *hondurensis* resulted in the formation of four groups. One constituted the majority of progenies (96,9%) and others aggregately by (1,05%). Despite crosses between some genotypes with high estimates of divergence, it will not ensure high heterosis due to the necessity of dominance and epistatic interactions. There is a greater probability to obtain more promising combinations when divergent genotypes are crossed.

P 19

Variation of the timing of bud burst phenology in an old-growth forest stand composed of Fagus sylvatica and Quercus robur

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Phenology of tree development seems to be one of the most important adaptive traits affected by climate changes. Although, the variation of bud burst timing in forest trees has been well documented based on common garden translocation experiments, our knowledge on the extend of bud burst dynamics within natural populations seems still insufficient for understanding the potential of adaptation of natural temperate forests.

We have investigated the timing of bud burst in a natural old growth forest stand (215 yrs old) composed of *Fagus sylvatica* and *Quercus robur*. The development of buds/leaves was assessed on 346 beech and 165 oak trees over the period of 37 days in Spring 2013. On average, beech trees appeared to flush 5 days earlier than oak trees. The difference between the earliest and the latest flushing trees was about 12 days for beech, and 14 days for oak. However, the time interval from the beginning of bud flushing until the full leaf development ranged from 8 to 19 days for beech, and from 5 to 29 days for oak trees. In beech, several phenology indices were negatively correlated with tree size/age, indicating that the largest beech trees had the tendency to start flushing earlier, which was not observed for oaks.

The timing of bud burst development appeared to be strongly spatially clustered in beech (up to 60-90 meters depending on phenology index, based on Moran's I). In oak, the phenology development was also spatially aggregated but to a lesser extent. However, the spatial distribution of clusters of trees with similar phenology phases did not overlap between the two species, suggesting that other than local-site factors (eg. temperature, moisture, soil type) could be responsible for nonrandom distribution of bud burst phenology. The study will continue in the following seasons for validation/verification of the results.

P 20

Genetic differences in Scots pine resistance to needle cast (Lophodermium seditiosum Minter, Staley & Millar): possible impact of climate ganges

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Needlecast may in future become a more frequent and severe problem in young Scots pine plantations due to changes in climate: tests of 64 provenances in two climatic regions in Latvia, differing by 1.8°C reveals, that warmer climatic conditions significantly increases the needle cast damage grade. Data from 3-5 year old open-pollinated progenies of Scots pine plus trees suggest, that degree of needle cast damages has a significant (α=0.001) effect on annual height increment and survival. Therefore it is important to assess the genetic differences in damage level and presumably recommend the resistance as one of selection criteria.

Study reveals, that degree of resistance has a genetic component (h^2=0.13-0.24), that is relative stable over two vegetation periods (family mean correlation r_{fam}=0.77). Needle cast strengthen correlation between the family average three height in the beginning of vegetation period and height increment (first season r_{fam}=0.49, second – r_{fam}=0.89) suggesting a cumulative effect, when susceptible families fall behind in height growth.

Genotype x environment interaction influences the resistance to the same extent as height growth: family mean correlation between two sites for tree height and for needle cast damage score r_{fam}=0.43. Families belonging to particular populations have significantly (α=0.05) less needlecast damages then families belonging to other. Degree of needlecast damage has no significant effect on survival and height increment of trees from more resistant populations, suggesting that population differences are not only related to susceptibility of this disease, but also to recovery rate after the damages.

P 21

Genetic differentiation and Phenotypic plasticity of Maritime pine in D13C: adaptive signifiance

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Phenotyic plasticity and standing genetic variation is of fundamental importance in evolutionary, population, conservation, and global-change biology. Maritime pine is a conifer species with high differences in plasticity and genetic variation at different levels (populations, families and individuals) and among traits. However, it is still necessary to understand the adaptive significance or the plasticity, and also the tradeoffs among plasticity and other traits. We therefore checked the extent of standing variation and phenotypic plasticity at three different levels (population, families and individuals) in d13C (a WUE related trait), growth, and reproductive output (estimated through the number of female cones). We sampled ca. 200 trees in two contrasting experimental sites, and for two consecutive years. The sampling scheme included 12 populations covering the distribution range of the species, 119 families and 8 trees/family. The results showed a large genetic variation in the isotopic composition (d13C) of the material assayed (h^2: 0.31-067 depending of the site and year), with an important GE interaction, and phenotypic plasticity (1 order of magnitude among families for max-min value of the different environments). We explored the adaptive significance of such differences by using reproductive output and growth as proxies to fitness, and by regressing the values with environmental variables of the sites of origin (R^2: 0.13-0.40,with Mean Annual Temperature and Rainfall).

P 22

Scots pine adaptation depending on the reaction norm of geographical populations on the conditions of the West Transbaikalie

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The increasing of productivity and resistance of the forests on the genetic and selection basis is tightly associated with the studying and using of genetic potential of the woody plants.

The satisfactory survival in the age of 32 was shown by the progenies of the Pine populations from the regions of the East Siberia including insular pine forests located near the South bounds of the areal. The Scots pine progenies from the Far East regions are characterized by the high adaptation potential; their survival in the new conditions is satisfactory.

The progenies of the Scots pine growing in Siberia near the northern bounds of the areal are less resistant because of the significant differences in natural-climatic conditions of the origin and the place of growing.

Especially irresistible in the conditions of the West Transbaikalie the progenies of the Scots pine from South-East and South European origins are. They were totally eliminated in the first years of growing; on the contrary, the Scots pine from the Northern areas of the European part of Russia is more resistible and has satisfactory survival.

The highest characteristics of intrapopulation variability of linear growth (23–29%) are peculiar to the progenies, representing Northern European and North-Eastern Asian regions of the Scots Pine areal. The lowest variability (10–17%) of growth in height characterizes the progenies of the maternal plantings from the central areas of the European part of Russia with the smaller hereditary reaction norm and as a result of severe selection in the new conditions.

The progenies of the maternal plantings, mainly from the natural-climatic conditions close to the place of growing, are characterized by the highest productivity, which is the complex index of linear and radial growth and survival.

P 23

Hydric stress tolerance of Arbutus unedo L. selected trees

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Arbutus unedo L. is typical of Mediterranean sclerophyllous and laurel vegetation, where either frost or summer dryness are not very intense. It is an underestimated fruit tree, with different possible commercial usages from processed to fresh fruit production.

Micropropagated clones, from selected adult trees were used to establish new orchards. It is expected, due to natural selection, that genotypes from provenances characterized by a hydric stress show greater tolerance than genotypes from more humid and cooler regions.

In this work the hydric stress tolerance of seven *A. unedo* clones from four provenances was evaluated *in vitro* conditions. The Anderson basal media with sucrose (0.18; 0.29M) and mannitol (0.14; 0.49; 0.69 M) were tested and compared to the control (0.09M sucrose). Five subcultures were accomplished (14 days were used as subculture period; 3 months/total). The height increase, the survival rate and the proliferation were recorded. The *in vitro* shoots per clone and tested condition were observed to evaluate their morphological differences probably connected with the defense mechanisms to hydric stress.

The hydric stress led to different behaviors in each clone, the clone from a drier and warmer zone (HP) showed the highest survival rate (100%) and morphological changes (increased number of trichomas and a reduced height growth), while clones from a wet and shadowed zone, sloughed completely when mannitol was tested at 0.49 and 0,69 M. The less tolerant clone to hydric stress (IM6) showed a 13% survival rate, after 5 subcultures in the same medium. Moreover a clone tested in a clonal trial, showed good fruit production after 5 years in a dry site, compared to seedlings. These results suggest that it is possible to predict the adaptability of a genotype to drought, considering their tolerance to the hydric stress, applied *in vitro* conditions within a short time.

P 24

The verification of the correctness of clone grafts planting in the seed orchard of Scots pine (Pinus sylvestris L.) in North-eastern Poland

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The verification of the correctness of clone grafts planting in the seed orchard of Scots pine (*Pinus sylvestris* L.) in Forest District Susz were aim of the studies. Performed identification enabled to confirm occurrence of planting errors in the analysed seed orchard and their impact on the parameters of the genetic variation of tested object. Measurements of DBH of progeny grafts of tested seed orchard were carried out also as part of the work. Analysis of measurements allowed to assess the impact of the homogeneity of mother generation genotype to the variability of DBH in progeny.

Laboratory analysis were performed on the basis of plant material collected from Polish north-eastern archives of clones trees and Scots pine seed orchard from Susz Forest District. Measurements were performed of progeny of tested trial for this seed orchard. The correctness of planting of individuals was investigated on the basis of DNA microsatellite four loci analysis (Spac. 12,5 Spac 11.4, Spag 7.14, RPtEST 11) as well as thirteen isoezyme protein.

Tested plots from the seed orchard contain 58% of the inappropriate grafts comparing to mother tree genotypes, which should be planted during establishment of the this plantation. Among the incorrectly growing grafts of clones 57% of individuals have been assigned to be another genotype of the mother trees, which were planted within the plantations. This allowed to reduce the planting error up to 25%. Described incorrect genotypes had an impact on the parameters of genetic variability and there were mainly observed in the frequency of alleles. Changes in the frequency of alleles occurrence significantly influenced the effective number of alleles, which was increased up to 10% while did not have any impact on the level of Hardy-Weinberg equilibrium. In studies been proven no effect of genotype homogeneity on the quality of the progeny generation.

P 25

Shoot growth responded differently to imposed drought among half-sib families of Norway spruce

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The objective of this study was to examine within-species variability in response to drought and in traits associated with drought resistance in Norway spruce. In a greenhouse experiment involving drought (D), intermittent drought (ID), and control (C) treatments we measured growth of current year leader shoots in seedlings of seven half-sib families and two populations (provenance level) of Norway spruce during their third growing season from seed. Mid-season drought was imposed in early July, and was broken in the ID treatment in early September, based on measurements of xylem water potential.

Current year leader length differed by family, whereas treatment did not affect that trait. However, there was a tendency for seedlings in the C treatment to have longer leader shoots than in two drought treatments. Significant variation was found among families and treatments in duration of period between the onset of drought and the completion of 95% of leader shoot length, which was considered the end of elongation growth. That period varied among families from 33 to 48 days in the control treatment, and from 1 to 18 days in both drought treatments. Thus, in reaction to drought seedlings completed elongation of their leader shoots, but duration of that response differed by 2.5 weeks in our set of half-sib families. This indicates that the observed response to drought might be general for Norway spruce, however, within-species genetic variation may affect its timing.

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P 26

Fertility variation and its effect on the effective number of parents at two seed stands of tropical species

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The fertility variation among individuals was evaluated in the seed stands of Tamarindus indica and Azadirachta. Based on the fertility variation, parental balance and femaleness index were determined. The effective number was drawn from the fertility variation, and the effective number was also estimated under the option of equal seed collect among trees. There was a large variation of growth and flowering characteristics among individual trees in seed stands of T. indica and A indica in southern India. The percentage of fertile trees was higher in 2007 than in 2006. The good male flowering individuals also showed good female fruit production. Highly significant correlations among growth characters, male flowering and fruit production were found for both species. There was a great deviation of parental contribution from expectation; 20% of individuals contributed about 70% male flower and female fruit production in both species. Femaleness index showed that contribution between female and male in the good flowering year was more balanced, compared to the poor flowering year. The coefficients of variations of male flower and female fruit production were higher at the poor flowering year than at the good one. This was coupled with the fertility variation among individuals and the effective number of parent. In T. indica, the contribution of female parent was close to more equal than that of male parent. In A. india, however, the female fertility variation was larger than the male fertility variation among individuals. The relative effective numbers of male and female parents were low under the two consecutive years. When combined both male and female fertility variation, the relative effective number of parent was increased.

P 27

Genetic imprints and environment effects in a common garden of 500 Picea abies families from 160 sites throughout the Alps

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Norway spruce is one of the economically and ecologically most important tree species in the Alps as well as in Europe. Given current climate change scenarios, a full understanding of the function of complex traits and their genetic base appears to be the only way to cope with effects that may be a menace to the performance and the survival of this species. In 2013 we established a common garden nearby Trento, Italy, using seeds from a mapping population constituted by the half-sib progeny of 518 families from 160 sites in the Alps. The phenotypic traits assessed were: germination time, bud-set, bud-burst, height and biomass parameters. Preliminary results show significant differences in germination and bud-set, and Northern families generally grew more in the common garden conditions than Southern ones. Parallel to the phenotypic assessment, genotyping was carried out on needle samples from the mother trees, and a candidate gene-based approach was used to search for association between single-nucleotide polymorphisms (SNP) detected in genes putatively involved in the control of the measured adaptive traits and the observed phenotypic variation. The discovery of polymorphisms underlying adaptive phenotypic traits is a fundamental goal of molecular genetics. Elucidation of the genetic components for ecologically relevant traits through association mapping at markers in candidate genes has been achieved for a variety of adaptive phenotypes in other tree species, and it has important applications ranging from marker assisted breeding to gene conservation in the face of climate change.

P 28

Adaptation of forest trees to climate change in the Alps: a study on ecologically different families of Pinus cembra and Picea abys

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This research project investigates the climate-change related adaptive potential of *Pinus cembra* L.and *Picea abies* L., two keyspecies of the subalpine and treeline ecotones in the Alps. The microclimate of these ecotones is known to be particularly sensitive to climate change, thus a full understanding of the function of given complex traits and their genetic base seems to be important to safeguard survival of the species. For both trees, there is some evidence that genetic variation may be associated to environment and in particular to precipitation, showing the importance of water availability. Another major cause of the species decline is the fungus *Phacidium infestans*, which attacks *P. cembra* at the juvenile stage.

For each species, four to six families from ecologically contrasting environments were chosen for an experiment on drought stress and phenology. The experiment was carried out in a growth chamber during spring 2014 and several phenotypic and physiological parameters were measured. Needle samples were collected to assess changes in gene expression among seedling provenances. For *P. cembra*, the potential differences in susceptibility to the pathogen infection was tested among seedling of different provenances. A gene expression study, intended as a first step towards landscape genomics and longer term adaptation studies, will allow the discovery of suites of genes involved in these adaptive responses. Moreover, a reciprocal trasplanting experiment across core-perypherical gradients will confirm the environmental effects on species survival and could provide important information for species genetic conservation and its forestry management.

P 29

Accuracy of genomic selection methods in Eucalyptus: a tentative exploration

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Genomic selection (GS) is a form of marker-assisted selection that is extended to a genome-wide scale by using effects of a large number of markers to predict breeding values for any kind of complex trait. Breeding values are estimated using a training population which is both genotyped and phenotyped where as the prediction population is only genotypes. The benefits of GS are greatest when complex traits show 'missing heritability' due to the failure of quantitative trait locus and association mapping to identify all causal loci affecting a trait of interest. Multiple methods and models that differ with respect to assumptions regarding distribution of marker effects have been proposed and may perform differently for traits with distinct genetic properties. In order to explore the accuracy of these methods, a comparison was carried out across 5 traits, including height, circumference at breast height, basic density, pulp yield and volume with distinct heritabilities and genetic architectures from 339 interspecific crosses between *Eucalyptus urophylla* and *E. grandis.* A training population consisting of 937 individuals was genotyped with approximately 46k single-nucleotide polymorphisms (SNPs) using a newly developed SNP-chip specific for Eucalyptus. After comparing the overall performance of the GS models across traits will give us a better understanding of the characteristics among these models and how to apply them to improving the accuracy of predicting abilities for future *Eucalyptus* breeding.

P 30

Joint use of phenotypic, pedigree and genomic information in genetic evaluation: An example in Eucalyptus grandis

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The availability of informative DNA markers has created a quantitative genetics paradigm shift where the classical pedigree-based relationship ***A*** matrix is replaced by the ***H*** matrix that combines both pedigree and genomic information in genetic trails' evaluation. Simply, the ***H*** matrix combines two types of genetic information; namely, 1) pedigree information (***A*** matrix) for a group of non-genotyped individuals and 2) marked-based relationship (***G*** matrix) for an additional group of genotyped individuals. This approach is dubbed as the "unified approach" is appealing as it produces more accurate genetic parameters than that of the pedigree-based counterpart. Here we compared the accuracy of estimated genetic parameters of diameter (DBH) and height (HT) in a 5-year-old *Eucalyptus grandis* open-pollinated progeny tests obtained from the traditional pedigree- and ***H*** matrix-based methods. Comparisons are made for parents and genotyped and non-genotyped offspring for single and bivariate models using offspring (N=1,637) derived from 130 families with 168 trees genotyped for 15 SSRs. The ***H*** matrix produced, on average, higher heritability estimates than the ***A*** matrix (DBH: 0.186 vs. 0.157 and HT: 0.144 vs. 0.088) and better BVs accuracy across traits and models for parents (from 23.4 to 11.3%) and genotyped (from 92.6 to 53.7%) and non-genotyped (from 32.7 to 11.5%) offspring. Averaged across parents and all offspring, HT showed higher incremental improvement in accuracies than DBH for the two models, demonstrating ***H*** matrix beneficial effects with low heritability traits. Spearman-rank correlations between predicted BVs from ***A*** and ***H*** matrices were high for parent (0.96) and genotyped (0.81) and non-genotyped (0.97) offspring across the two traits and models analysed. This study demonstrated the merit of combining the pedigree and genomic information in genetic evaluation.

P 31

Protocol: fast and high-performance Eucalyptus DNA extraction in 96-well plate method

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Forestry genetics and genomics require a great number of samples to produce accurate results. This is a simple method with high-throughput DNA extraction that combined CTAB protocol with fast rupture tissue and centrifuge steps in plates to obtain good yield, quality and low price. Approximately 576 samples can be processed for day. The obtained DNA is suitable for use in PCR-based study like Microsatellites. This method employs 96 Racked Collection Microtubes (Qiagen). *Eucalyptus* tissue 15mg was placed in each well together with a tungsten bead and 420ul of CTAB2X buffer distributed with a multichannel pipette, the samples were disrupted to full power in a TissueLyser (Qiagen) for 3min. Then we added 360ul of CIA24:1 to each tube and both racks were centrifuged at 4000 RPM for 20min (ThermoScientific SorvallST16). Afterwards the upper phases were transferred carefully to clean rack-wells and 150ul of isopropanol were added to each well and centrifuged at 4000RPM for 20min. The liquid phases were removed and the pellets were washed twice with ethanol 70% and centrifuged at 4000RPM for 5min. Finally the pellets were resuspended in 100ul of RNAse and incubated at 37°C for 60min. To evaluate the yield and purity, this prococol was compared against results obtained with four commercial extraction kits: DNeasy Plant Handbook® (Qiagen), Power Plant DNA® Isolation Kit (MOBIO), Wizard® Genomic DNA Purification Kit (Promega), ZR Plant / Seed DNA MiniPrep® (Zymo). The procedure of rupture was the same for all protocols and in all cases racks of 96 wells were used. The data of absorption spectrum indicate an average concentration of 400ng/ul for CTAB protocol, Qiagen 150ng/ul, Wizard 165ng/ul MOBIO 85ng/ul for Zymo. Comparing this method to the commercial is demostrated that the final concentration superior, the time os processing is lower, also it is considerable cheaper.

P 32

Temperature driven changes in transcriptomes and smallRNAomes during formation of an epigenetic memory in somatic embryos of Norway spruce

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Epigenetic memory in Norway spruce permanently affects the timing of vitally important adaptive traits, such as dormancy, bud burst and bud set. Epigenetic memory marks are established in response to the temperature conditions prevailing during early seed formation.

We continue identification and characterization of molecular mechanisms during formation of epigenetic memory in somatic embryos of Norway spruce, which closely mimics the natural processes in seeds. We prepared nine transcriptomic and nine smallRNA libraries from embryogenic tissues at three stages of maturation at three culturing temperatures (18, 23 and 28°C). Obtained libraries were sequenced on the PGM™ (Ion Torrent™) system and analyzed using CLC genomic workbench software.

Extensive characterization of small RNAs in Norway spruce embryos during different stages of embryogenesis under different temperature treatments allowed identifying at least three different types of small RNAs: micro RNAs(miRNAs), *trans*-acting small interfering RNAs(ta-siRs) and 31-nt length sRNAs, similar to animal's PIWI-interacting RNAs(piRNAs). In embryonic tissues, the most prevailing were 24-nt siRs, followed by 21-nt sRNAs with separate large fraction of sRNAs with length around 31-nt. In total, we defined around 3000 ta-siRs, originated from 50 TAS loci. We report the identification of 438 novel putative miRNAs in Norway spruce.

We studied expression patterns of sRNAs together with mRNAs during different stages of SE in dependence on the epitype inducing temperature prevailing during SE growth and leading to establishing of epigenetic marks. We showed that Norway spruce poses a variety of sRNAs with distinct temperature dependent expression patterns, and these sRNAs target large amount of spruce genes with a wide range of functions, including also genes involved in epigenetic regulation. Unknown mechanisms provide fine-tuning of sRNAs pool content participating in the epigenetic memory formation.

P 33

Genomic selection of Eucalyptus breeding by SNP analysis in northern Brazil

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Eucalyptus species is the main hardwood used in intensively managed forest plantation in the world. Their woods are used as a raw material for the production of cellulose or energy source. Eucalyptus species have fast growth rates and the ability to adapt to a broad range of geographic locations and are most important commercial temperate hardwoods for the pulp and paper industries because of its wood properties and pulp production characteristics. Woody plant cell wall is mainly composed cellulose, hemicellulose and lignin. For kraft pulp making, wood prefers higher contents of cellulose and hemicellulose. On the other hand, for the purpose of combustible energy production, higher lignin content is ideal, because lignin has relatively high-calorie as compared with polysaccharides. In this study, we apply genomic selection into conventional breeding of Eucalyptus in northern Brazil of tropical area. Generally, traditional Eucalyptus breeding takes over 12 years to select plus trees from seeds. Hence, genomic selection using single nucleotide polymorphisms (SNPs) analysis would be useful breeding tool to shorten terms for plus tree selection. We analyzed correlation between 60k SNPs of Eucalyptus genome and phenotypes, such as basic density, holocellulose, alpha-cellulose, hemicellulose and insoluble lignin content of 930 trees. Those trees have 38-59% of alpha-cellulose contents and 20-35% of insoluble lignin contents. There are no relations between growth rates and alpha-cellulose or lignin content. We selected 29k SNPs that showed genetic diversities. Pre-models for expecting the phenotypes by BLUP estimation have been established.

P 34

Use of DNA markers in forest tree breeding programs

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Plant breeding is a dynamic area of applied science, which relies on genetic variation and selection and leads to improved plants for traits and characteristics that are of interest for the user. Tree improvement is based on traditional breeding techniques, that is selection of superior trees for volume and stem straightness, grafting these into breeding orchards and establishement of seed orchards. The crucial problem referred to traditional approaches in tree breeding is the long growth cycles, which make this process very time-consuming. Many traits of interest, such as wood properties, change during growth and maturation. Another trouble is a low heritability for many traits of practical interest in forest trees. Moreover, selection for one chosen feature may lead to a change of another one. In the context of these problems, any other tool which can accelerate the selection process and enhance the productivity of the traditional approach is of significant value. Dominant and co-dominant molecular markers are routinely used in plant genetic research. For that reason, the development of a high-density DArT genome profiling resource is reported to demonstrate its potential for genome-wide diversity analysis of Scots pine. DArT is the name of the evolving technology of genotyping to detect all kinds of DNA variation. This technology provides a good alternative to currently available marker techniques including RFLP, AFLP and SSR in terms of speed and amount of data generation. Scots pine progeny representing 67 plus trees was analysed. We identified 4086 SNP markers and 7086 DArT markers. Preliminary results indicate the usefulness of DArT marker – system in the identification of valuable genotypes.

P 35

Double digested RAD tags in Norway spruce, a test of different restriction enzyme combinations

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Double digested RAD tags is an inexpensive and fast method of SNP discovery and genotyping of large number of markers. The protocol requires a single combined restriction digestion-adaptor ligation reaction, a fragment size selection and a small pre-amplification step. Furthermore this system is tunable, particularly in relation to the numbers of markers obtained and the number of individuals profiled, allowing us to effectually scale our analysis. By combing restriction enzymes with longer recognition sites and selection of a narrow size fraction it is possible to reduce the number of fragments that are sequenced, which is particularly important for species with large genomes such as Norway spruce. Here we have tested several different combinations of enzymes and sequenced RAD tags from samples of Norway spruce. The main objective is to find a method that can be used for genotyping of samples for both population genetic analyses and in breeding (e.g. genomic selection). We have sequenced the RAD tags on a Ion Torrent sequencer that we recently have aquired at the Norwegian forest and landscape institute. After sequencing, the reads have been mapped to the Norway spruce genome using different aligners. These results are examined in the context of the power, efficiency and broad range of possibilities that RAD tags and the Ion Torrent system offer. Here we report the number of fragments sequenced for the different enzyme combination and also the variation in which regions they target.

P 36

Genetic analysis of survival and growth traits in Pinus radiata D. Don seedlings submitted to water restriction

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Understanding the survival capacity of Pinus radiata -the world's most extensively planted exotic softwood with over four million ha of planted worldwide- to periods of severe water stress could improve knowledge of its adaptive potential under future climatic scenarios. We performed a water stress experiment (well watered vs. water stress) to test for survival and growth of a wide range family collection of radiata pine in Chile. Genetic parameters for survival, growth and biomass were estimated from measurements from 5 month old seedlings. The trial comprised 98 open and control-pollinated families from first to third breeding generations, covering collections from interior and coastal populations in Central Chile. Families differed significantly in most of traits, with individual tree heritabilities ranging from 0.14 for survival, to 0.63 for diameter in the well watered treatment. Families from the interior showed the highest heritability for diameter and dry masses of shoots, roots and total when grown in the water stress treatment. The genetic correlations between treatments were medium to high and suggest the presence of a genotype by watering regime interaction. Most traits were strongly correlated (genetic correlations often exceeded 0.40). Thus, potential exists for identifying drought-hardy families at the seedling stage, with families from the interior sites having potential to produce a more drought-resistant breed with satisfactory growth rates and yields in dry environments. The present study can be considered as a complement to the breeding program for P. radiata, which is nowadays of great importance in Chile.

P 37

How representative are plots in provenance trials? – An example from beech (Fagus sylvatica L.) genotyped with SSRs

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Provenance trials are very important to answer questions in practical forestry as well in forest science, i. e., to estimate the adaptation potentials of tree species to climate change. Experimental plots in field trials have to correspond to the characteristic variation of phenotypical traits under the given environmental conditions and should be representative of the respective seed origin. For that reason, seed lots for provenance trials should represent the genetic variation of the particular populations.

Samples from six selected beech (*Fagus sylvatica* L.) provenances from an international field trial (two provenances from Germany, one each from Austria, Czech Republic, Romania and Spain) were genotyped at 14 nuclear microsatellite loci (total sample size n = 315, 50... 55 individuals each). Several parameters characterising the within-population genetic diversity show high levels in the Romanian, in the Austrian and in the Czech samples, and low levels in the two German samples and the Spain sample.

Additional information was derived from the reconstruction of parental generation from offspring multilocus genotypes by an efficient likelihood approach in according to WANG (2012). Large halfsib families with 10 resp. 15 offspring individuals were ascertained in the samples from one of the German and from the Austrian provenance. These large halfsibs were confirmed in different runs of the procedure. The family sizes are four to five times larger than the average expected number of offspring per mother estimated from beechnut harvesting documents. The effective population sizes in these two provenances were estimated at 45 – 49 in comparison to 82 – 115 for offspring samples from other provenances with smaller halfsib families. The above-expected degree of relationship within offspring populations is discussed regarding a biased seed collection in parental populations.

P 38

Estimates of genetic parameters in progeny of G. americana test

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Forest fragmentation becomes vulnerable natural populations to environmental, demographic and genetic events. The understanding of the genetic parameters of a species contributes in establishing strategies for conservation *in situ* and *ex situ*. Genipap (*Genipa americana*) is a tree species widespread from Tropical America, very important ecologically, indicated for use in programs of reclamation degraded areas, construction and folk medicine. The objective of this study was to evaluate the genetic variation between individuals of *G. americana* through silvicultural characters in an Active Germplasm Bank (BAG) installed in November 2000 as a progeny test in the municipality of Rosana / SP, Brazil. To 13.4 years after planting was evaluated the diameter at breast height (DBH) and survival (SOB). The randomized block designed was used with 30 progenies, three replications and eight plants per plot in linear form. The average for DAP (12.22 cm) and SOB (88.19%) showed good development and adaptation of progenies of *G. americana* for the region. There was variation among progenies detected by the likelihood ratio test (LRT) for DAP (4.61 *) and SOB (6.75 **). The coefficient of genetic variation at the level of individual (CV_{gi}= 12,07%), heritability ($h^2_{a=}$ 0,13±0,08) and accuracy (r_{aa}=66,26%) indicate that the progenies of *G. americana* have high potential for use in conservation and breeding programs from this population.

P 39

Genetic variation in Dipteryx alata progenies in Brazil

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Dipteryx alata is a species highly potential for silvopastoral systems due to its canopy structure and abundant fruit production used in cattle feed. This species can also be used in landscaping, as timber in shipbuilding, building and its seeds used as food by humans in its natural occurrence region particularly in Central Brazil. The aim of this study was to estimate genetic parameters in a progeny trial of *Dipteryx alata* established in municipality of Perdeneiras, Brazil (Estaç?o experimental do Instituto Florestal de S?o Paulo). This trial was established in 1986 in a completely randomized design with 2–8 replications. Plots were fitted linearly, comprising 5 plants in a spacing of 3.0 × 3.0 m. Data of the diameter at breast height (dbh) were analyzed using the REML/BLUP statistical procedures. At 27 years old progenies showed an average dbh of 16.8 cm. Analysis of deviance revealed significant differences among progenies at 1% probability level for diameter at breast height. The coefficient of variation among progenies for dbh was 10.54 % and individual narrow-sense heritability was 12.33%. The variability in the present trait may be exploited in genetic conservation and pre-breeding programs in order to support the seedlings production to supply programs for environmental restoration and production systems.

P 40

Genetic variation for quantitative traits in progenies of Myracrodruon urundeuva Fr. Allem

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Information about genetic variation for quantitative traits in forest tree species needs are met through progeny test studies. These skills are especially important for native arboreal species with economic value, like*Myracrodruon urundeuva* (aroeira), because it is possible to indicate the potentiality and choose the appropriate genotype to economic exploitation and breeding, and even the production of seeds with broad genetic base for environmental recovery. Thus, this study was to evaluate the genetic variation for diameter at breast height (DBH) and survival of *M. urundeuva* in an open-pollinated progeny, from Seridó- RN, Brazil, after 17 years from planting. The progeny test was set up on July 23, 1997, in data from randomized complete block design with 12 progenies, six replicates and ten plants per plot in linear form, in the Teaching, Research and Extension Farm, from Engineer University of Ilha Solteira located in Selvíria-MS, Brazil. The results were based in REML/BLUP (restricted maximum likelihood/best linear unbiased prediction) procedure; it suggests that the *M. urundeuva* population has a good adaptation to environmental condition of Selvíria, due to the high survival rate (73 %). There was significant variation among progenies detected by the likelihood ratio test (11,09**) to DBH. Estimates of high magnitude were also observed to DBH in relation to the coefficients of individual genetic variation (23,73 %), individual heritability (0,34 +/- 0,14) and accuracy (88,10 %), indicating that these progenies can be used in conservation and breeding programs, as well as for environmental recovery.

P 41

Optimization of plant quantity for one family in Picea abies L. progeny tests

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Efficiency of test design in progeny testing depends on number of trees for each plus tree (family). Large quantity of trees for each family allows to obtain reasonable precision of the progeny testing. But this approach increases the cost of the test. Optimization of plant quantity for one family in progeny test gives good test design. Aim of our study was to evaluate the influence of the number of plants for one family on the efficiency of progeny tests. Objects for study: Picea abies progeny test (North-West Russia, age varies from 20 to 45 years old).

For determining of necessary tree quantity for one family were generated sampling (random and regular) within each of the families. Volume of the samplings varies from 3 to 50% of initial quantity of plants for one family. The procedure of sampling repeated minimum 10 times for each fixed percentage of sampling. The basic purpose of study is estimation of correspondence between source population and sampling.

Estimation criteria:

1) correlation between original family set means and random (regular) sample set means;

2) significance level of differences between the families (estimated by F- criterion, one-way ANOVA);

As a result of the studies recommended minimum number of plants for one family in the Picea abies progeny test was obtained. Average minimum number of plants obtained by the «correlation criteria» is 65 plants for one family (varies from 30 to 135 pcs). The minimum number of plants determined by the « significance criteria» is 80 plants/family (varies from 30 to 170 pcs). If we take into account some essential reserve number of plants for considering plant mortality thus initial plot size in progeny test equal to 170 plants per family. This number provides a balance of reasonable precision and the moderate cost for the progeny tests.

P 42

Relationship between microfibril angle of S2 layer and mechanical properties in 10 open-pollinated families of Picea jezoensis

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Microfibril angle (MFA) of the S2 layer is a key trait for determining quality of timber in many species. The present study aimed to clarify the among-family variation of MFA, and relationships between MFA and bending properties. In addition, possibility for early evaluation of superior mechanical properties by MFA in *Picea jezoensis* (Carr.) was also discussed. The trees from 10 open-pollinated families of *P. jezoensis* were harvested at a 43-year-old progeny test site in Hokkaido, Japan. Dynamic Young's modulus of logs (DMOElog) collected from 1.3-2.3 m above the ground were measured by the tapping method. In addition, 40-cm-length logs were collected to determine MFA and bending properties, i.e., modulus of elasticity (MOE), and modulus of rupture (MOR). MFA was measured at every 5 annual ring positions from the pith. DMOElog ranged from 8.54 to 10.60 GPa in 10 families, and mean value of DMOElog was 9.57 ± 0.69 GPa. MFA at near the pith showed higher values than other radial positions. MFA decreased up to the 15th annual ring from the pith and then became almost constant toward the bark. Significant differences among families were found in MFA at all radial positions, suggesting existence of contribution of the genetic factor to MFA from the pith toward bark. Significant negative correlation coefficient was obtained between DMOElog and MFA at the 5th annual ring from the pith. Therefore, it is possible to evaluate the value of DMOElog by MFA at the 5th annual ring from the pith.

P 43

Genetic variation in dieback resistance in Fraxinus excelsior confirmed by progeny inoculation assai

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Ash dieback caused by the pathogenic fungus *Hymenoscyphus pseudoalbidus* is a widespread problem in Europe. Here we assess crown damage from natural infection and necrosis development following artificial controlled inoculations on progenies from Danish *Fraxinus excelsior* clones with contrasting and well characterized levels of susceptibility to the disease. The inoculation assay was performed on a total of 123 progenies, and necrosis development monitored over two years. Microsatellite analysis on these 123 progenies from two clonal seed orchards revealed that five clones among the thirteen clones present in the seed orchards have mothered all these progenies. The offspring from low susceptible mother clones had significantly less crown damage and also developed significantly smaller necrosis when compared to offspring from susceptible clones. The correlation coefficient between average crown damages of mother clones and the average of their progenies (natural infections) was 0.85, while the correlation between crown damage of mother clones and the average necrosis development in their progeny after controlled inoculation was 0.73. The correlation between resistance of parent trees and crown damage/necrosis development on their off-spring confirms the presence of heritable resistance, and supports that a bioassay based on controlled inoculations has the potential of becoming a fast and cost effective tool for estimation of dieback susceptibility in breeding programmes for healthy ash trees.

P 44

Variability of biometric seed traits of Polish black locust (Robinia pseudoacacia L.) provenances

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Black locust is an alien species in the Polish dendroflora. However, due to the properties of wood and a wide ecological amplitude, the attempts to introduce this species into the Polish flora by afforestation and growing plantations have been made for the last 200 years. The study included nine populations of black locust from various regions of the country selected by straightness of the trunk. The seeds collected from the best quality populations of this species may be used for creating fast-growing trees plantations. The purpose of the study was to determine variability of seeds of selected populations of black locust and to analyse potential possibilities of generative reproduction. During the study performance of seeds from pods was determined, followed by the mass of 1,000 seeds, length and width of the seed, surface area of a single seed and the curvature of the seed. The analyses were carried out using the WinSEEDLE (Regent Instrument) software. Moreover, efficiency of several seed scarification methods was tested. The best results (more than 90% of germination capacity) was obtained at alternate temperature: between -80°C to +90°C for the period of ca. 10 minutes. Average performance of seeds from pods reached 24.7%. Other analysed traits showed minor variability within one stand, but considerable differences were observed between different stands. Obtained results allow for an assumption that the variability of seed traits is affected by the origin of the stand, rather than the geographic location. It may indicate that seeds from which stands with straight trunks were grown had different origins. Crucial are also other environmental variables (such as the habitat, availability of pollinators) as well as the traits of particular stands (such as the age, spacing), which considerably affect the abundance of seeds.

P 45

Studies of adaptive features of Siberian pine and Korean pine from provenance trial in the Asian part of Russia

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Provenance trials *of Pinus sibirica* Du Tour and *Pinus koraiensis* Siebold et Zucc. (hereafter "studied species") were studied. The plantations of the studied species were created according to the same protocol in Krasnoyarsk region in 1983 and in Khabarovsk region in 1977. In our study we assessed the growth parameters as well as anatomical and morphological parameters of the studied species corresponding to different provenances. We determined that the growth rate of trees corresponding to different provenances is determined not only by the inherited characteristics, but also by the adaptation. At both experimental regions the offspring of trees corresponding to local provenance are clearly better adapted. Nevertheless, at Krasnoyarsk region provenance trials we found that the phenotypic indicators and degree of preservation of the offspring of two Korean pine corresponding to Obluchensky and Chuguevsky provenances are at the same level as for the local Siberian pine. In Khabarovsk region elimination of the Siberian pine corresponding to different provenances is observed. Tree rings widths have been measured for Siberian pine corresponding to different provenances at both plantations. We conclude that 1) at the Ermakovskoe plantation there is a positive impact of the environmental conditions on tree-ring width for Korean pine corresponding to different provenances, 2) in Khabarovsk region there is a negative impact of the environmental conditions on tree-ring width for Siberian pine corresponding to different provenances, 3) adaptions to the new conditions are reflected in phenotypic, anatomical and morphological, as well as phytopathological characteristics at the experimental plantations.

P 46

Genetic gains in the selection in progenies of Copaifera langsdorffii (FABACEAE)

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Copaifera langsdorffii is a tropical tree species that occurs throughout Brazil, mainly in the Cerrado biome. In a forest breeding program is necessary the pursuit of quality of selected trees, which should gather among other characteristics, the genetic merit transmitted on to following generations. Moreover, the choice of species to be improved is very important, because it has to present interesting qualities to the market. The present study aimed selects the best progenies of *C. langsdorffii*, aimed at the genetic improvement of the specie installed in the form of progenies test. At 48 months, the characters were measured plant height (ALT, m) and stem diameter (DCO, cm). The progenies test was installed on 23 November 2009 under tree a population of rubber installed in 1991. The experimental design was random blocks, with 35 progenies of open pollination, six repetitions, with five of plants per plot, with spacing 3.0 x 5.0 m. Estimates of variance components and genetic parameters were obtained based in the restricted maximum likelihood procedure and best untainted prediction (REML/BLUP). From these estimates was made a selection of 50% of the best individuals, selected 344 plants. The average for the ALT and DCO of individuals analyzed before selection was 1.50 m 10.75 cm, respectively. However, with the selection of 50% of the best genotypes for ALT and DCO, these estimates passed to 1.63 m and 11.88 cm, i.e., a gain of 8.66% and 10.51%, respectively. The selection, based in the ALT and DCO, at 48 months of age in the progeny test valued, may result in considerable genetic gains, besides keeping the population with genetic basis sufficient for new selections to be practiced in the future.

P 47

Genetic variation for growth characters in progenies of Hymenaea stigonocarpa under Myracrodruon urundeuva in Selvíria, Brazil

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The loss of biodiversity due the reduction of areas with native vegetation and the increase of remaining caused by fragmentation habitats, led to the isolation of natural populations of tree species occurring in the Brazilian Cerrado, reduced gene flow between them. The establishment of progeny tests contribute to the ex situ genetic conservation, and provide a base population for breeding programs. *Hymenaea stigonocarpa* has a wide dispersion in the Brazilian Cerrado, and the economic importance of the species is associated with the use of wood, bark and fruits. The objective of this study was to estimate genetic variation for growth characters in progenies of *H. stigonocarpa* under a planting Myracrodruon urundeuvaque with 25 years old. The progeny test of *H. stigonocarpa* was installed on 05/25/2013 and the experimental design was a randomized complete block, with 37 treatments (progenies), 40 replications and one plant per plot, spaced 1.5 x 6.0 m, being planted between the lines of planting of *M. urundeuva*. Variance components were estimated by REML/BLUP procedure. Data were obtained stem diameter (SD), height (H) and survival (SOB) at ten months old. Mean values for SD, H and SOB were 0.56 cm, 32.97 cm and 63%, respectively. The coefficient of genetic variation (CVg) for H (11.61%) and SD (6.59%) indicates the variability in this population of *H. stigonocarpa*. Another indication of this variability was obtained based on the Mahalanobis Distance with formation of seven divergent progeny groups, according to the method of grouping Tocher, involving the H and SD characters. Thus, the system of planting *H. stigonocarpa* under *M. urundeuva* is promising and the study population expressed genetic variation for growth traits.

P 48

Genetic variation in Pinus caribaea var. hondurensis progenies for resin production

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Pinus caribea var. *hondurensis* is the most important species of the genus *Pinus* planted in tropical regions. Its wood and resin are used widely for different purposes in forest sector. However, few private companies and public institutions perform the supply of seeds with high genetic quality. The main purpose of this work was to estimate genetic parameters and gain of *P.* caribea var. *hondurensis* progenies for resin production. The progeny trial was established on June 1986 in municipality of Selviria, Mato Grosso do Sul state, Brazil. The trial was established in a 10 x 10 triple lattice design, with 100 treatments (96 progenies from a clonal seed orchard of *P. caribaea* var. *hondurensis* and four commercial control), with linear plots of ten plants in spacing 3 x 3 m. Twelve years after planting was applied thinning based on silvicultural traits and leaving six plants per plot. At 27 years old resin production of the remaining individuals was measured. Analysis of deviance and estimates of genetic parameters were performed according to REML/BLUP procedure. Significant difference was observed between and within progenies at 1% probability level for resin production and dbh. This result confirms the existence of significant variation phenotypic. Additive genetic effects may explain the most of this variation. Furthermore, significant genetic gains can be achieved with different selection strategies. Individual genetic variance coefficient was 26.52% and 9.17% for resin production and dbh, respectively. The Individual narrow-sense heritability was 0.30 and 0.26 for resin production and dbh, respectively. Genetic correlation between dbh and resin production traits was positive, but not significant. Therefore, different selection methods should be proposed separately for both traits. *P. caribaea* var. *hondurensis* progeny trial showed good growth performance and resin production, and sufficient genetic variation to attend the next generations of breeding and commercial plantations.

P 49

Estimation of genetic parameters for conservation and genetic improvement in progeny from Dipteryx alata Vogel in agroforestry system

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The natural vegetation, especially the Cerrado, is highly fragmented due to large anthropogenic pressures, causing the loss of genetic variability and concomitant risk of extinction of remaining populations in these fragments. *Dipteryx alata* is a tree species occurring in the Brazilian Cerrado and has been threatened by fragmentation of this biome, the value of timber, fruits and seeds confirm the species as of potential interest for use in agroforestry system, silvopastoral and pasture recovery. The present study aimed to estimate the genetic variability of *D. alata* of a natural population of Paulo de Faria, SP, installed in the form of a progeny test in a silvopastoral system for conservation and breeding. Thus, from seeds collected from this population installed on 06/10/13 progeny test in a silvopastoral system with 43 treatments (progenies), 17 repetitions and one plant per plot in Selvíria-MS. The spacing used was a double line of 3.0 x 1.5 x 4.5 m, being that between lines were seeded guandu (*Cajanus cajan*). The data stem diameter (DAC), height (ALT) and survival (SOB) were obtained at seven months of age. Statistical analyzes were based on the procedure REML / BLUP. The average value for the DAC, ALT and SOB were 1.00 cm, 48.33 cm and 59 %, respectively. Genetic variation (CV_{gi}) for height (ALT) and stem height diameter (DAC) was considerable, allowing the use of the test in *ex situ* conservation programs and plant breeding from this population of *D. alata*. Genetic divergence among the progeny provided the formation of six divergent groups, the method of Tocher, for characters ALT and DAC. The installation of a progeny test in a silvopastoral system allowed the expression of genetic variation for characters ALT and DAC and consisted of a system of planting promising for *D. alata*.

P 50

Estimation of genetic parameters in Pinus tecunumanii

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Pinus tecunumanii occurs naturally in regions from southern Mexico to Nicaragua. It has high growth potential, especially mechanical wood. The genetic variability of population phenotypic characters represents an important technique in plant breeding programs as it provides useful information for conservation. The main purpose of this work was to estimate genetic parameters of *Pinus tecunumanii* for silvicultural growth traits. The trial was established in November 1991. The test area was located in the municipality of Assis, S?o Paulo State, Brazil. Seeds from two provenances of *Pinus tecunumanii* of Guatemala (Bosque San Jerónimo, Salamá, Baja-Verapaz and San Vicente, Salamá, Baja-Verapaz). The experiment used randomized complete blocks, spaced 3 × 2.5 m, with two treatments (two origins), four replications (blocks) and 49 plants per plot. The diameters at breast height, height, and volume data were available. The analysis of deviance and the estimates of genetic parameters were performed according to established REML/BLUP procedures. The diameter at breast height and height and volume averages were 25.85 cm, 18.42 m and 1.10 m^3, respectively. Significant difference was observed between provenances for the character height (5.67*). This result confirms the existence of a significant variation phenotypic characteristics only in height. Furthermore, this trait should be considered in breeding programs. The individual heritability for height was 0.10. The individual results of the San Vicente, Salamá, Baja-Verapaz origin showed more genetic values than the Bosque San Jerónimo, Salamá, Baja-Verapaz. This indicates provenances should be prioritized for future breeding program.

P 51

Selection of European White Elm plus-trees based on half-sib variability in nursery progeny test

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Objective: European White Elm (*Ulmus effusa* Wild.) is one of species which are rare and endangered. Draining of wetlands for poplar cultivation or agricultural purposes has led to dramatic changes in ecosystems where European White Elm is present. Fragmentation of European White Elm populations in small populations, groups of trees and individual trees, leads to the problem of genetic drift, ecological instability and slowed extinction of this species.

Methods: In order to determine genetic potential of European White Elm in the area of Great War Island (Belgrade, Serbia), selection of 13 maternal trees was performed and progeny test was formed. At the level of seedlings, one- and two year old plants and 13 half-sib lines, analysis of several morphometric characteristics was made. Descriptive statistic (mean and range of variation) was performed for all analyzed traits. The significance of differences between mean values of the analyzed parameters was tested by One-way Analysis of Variance (One-Way ANOVA). Statistical significance between groups as well as the homogeneity of the groups were tested by post hoc test- Tukey HSD test. In order to grouping test trees, based on analyzed morphometric characteristics, Cluster analysis was used.

Results: The results indicate a high between and within population variability in observed juvenile development stages.

Conclusions: The observed differentiation between the analyzed maternal trees is the starting point for breeding of species and the mass production of seedlings for conservation of available genetic resources.

P 52

Tree height estimation in progeny trials using UAS fotogrammetry

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Traditional methods for measuring tree heights in progeny trials are time consuming and expensive. Particularly measurements in stands taller than 10 m are often very expensive as the tree tops become difficult to see from ground. We therefore wanted to study the accuracy of canopy height models (CHM), derived by subtracting a LiDAR based digital terrain model from digital surface models. The digital surface model was obtained by unmanned aerial system (UAS) photogrammetry.

The test site is a progeny trial established in 1992 with 74 poly-cross families. The site is variable in terms of topography and growth conditions. Average tree height when measured with the traditional method (H_trad) using a Vertex in 2013 was 7.7 m.

The aerial images were acquired in November 2013 by Norwegian contractor Asker Oppmaling, using a Aibotix X6 hexacopter with a Olympus E-PL5 camera. Images were acquired from the UAS flying at approximately 80 m above ground level. Tree heights (H_uas) for 1344 trees were derived by subtracting the CHM from the digital terrain model.

The regression between phenotypic values obtained by the two methods show an R^2 of 0.66 and RMSE of 0.95 m (12 %). The error connected with H_uas is relatively larger for smaller trees. The h^2 estimated for H_trad and H_uas were 0.15 (±0.06) and 0.07 (±0.05), respectively. The additive genetic correlation between the two methods was 0.97 (±0.22) when estimated from individual tree data and 0.66 when estimated as the Pearson correlation between breeding values (BLU-predictions).

The method based on UAS and digital terrain models are promising in terms of more efficient data collection in progeny trials. The method also opens up for large scale screening of tree heights in regular plantations of seed orchard materials and cost reductions for the phenotyping in BWB and GBLUP selection strategies.

P 53

Future and present testing of the progeny of basic forest material in Poland

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The main aim of the program is testing the progeny of basic forest material which is used in practical silviculture in Polish forests. There are reserved seed stands and plus trees. The progeny testing program in State Forest started in 2005, determining the genetic value and silvicultural components of the forest basic material. The material is being tested in mixture progeny tests by planting seedlings which come from seeds collected from listed objects. Material is tested using the principles of rational use of the seed base by determining the area of possible transfers according to the adopted seed regionalization norms. The results of the test will help the optimalization of tasks realized in the State Forests with regard to forest permanence, improvement of forest management with the purpose of the basic of conserving forest genetic resources.

The detailed aims of progeny testing are:

Determination of the genetic and silvicultural value of reserved seed stands, plus trees

Qualification of basic forest material of reserved seed stands, plus trees to the category of tested unit

Verification of the current borders of regions of origin and of the rules for the transfer of forest reproductive material

Development of a database concerning the genetic characteristics of basic forest material registered in the Country-wide Register of Basic Forest Material under this program of testing

To the current day, on the territory of Poland is testing four species, scots pine, norway spruce, silver fir, common beech from reserved seed stands and trees. Total number of objects on the progeny testing programs are 2069 which is 41% part of the total number of reserved seed stands and trees in Polish forests. From this material 149 progeny tests were planted. More information and analyses about the results and practice principles about the program will be shown on the poster presentation.

P 54

Biomass productivity and distribution in half-sib progeny of pedunculate oak

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We investigated biomass productivity of individual trees at age 14 years in the experiment with 8 provenances and 186 half-sib families of pedunculate oak (*Quercus robur* L.). The aim of the study was to develop allometric equations and estimate heritability of tree biomass. We sampled 28 model trees and measured dry mass of their components, including roots (on 14 trees). An increasing share of aboveground biomass was allocated to stems, and a decreasing share to branches and foliage with increasing tree size. Also a decreasing share of total tree biomass was found in roots with increasing tree dimensions. Allometric equations involving diameter at breast height in combination with tree height were developed to successfully model biomass of all components and whole tree. We used those equations to estimate tree biomass on the subset of research area, involving all 8 provenances and 180 half-sib families. Heritability coefficients for tree biomass were greatest for provenances (0.84), average for families (0.58) and the lowest for individual trees (0.18). However, estimates of family heritability varied by provenance from 0.18 to 0.67. These findings suggest that the most efficient strategy for improvement of productivity in pedunculate oak would be a selection of provenances and families within provenances.

P 55

Estimates of genetic parameters for wood production in natural populations of rubber from the Brazilian Amazon

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Although the main objective is to produce latex, the rubber plantations also have good prospects as suppliers, with different purposes, for the segment of solid wood. The estimated production of rubber wood for the 2010-2015 period is a worldwide total of 48.491 million m³ annually, with 12.849 million m³ of logs for use in the logging industry. At the end of the cycle to decline in latex production, preventing the activity, targeting to logging, is promising, considering the quality of rubber wood. The objective of this study was to estimate parameters for logging in adult seedlings rubber tree. The experiment was conducted in the Teaching, Research and Extension Farm/Engineering College at Ilha Solteira/UNESP, located in Selvíria-MS, Brazil. A natural population of *H. brasiliensis* coming from Rio Branco –AC, Brazil was evaluated. The progeny trial was installed in 1991 at a spacing of 5 mx 3 m, following a randomized block design in 37 progenies distributed in three replications unbalanced manner with a maximum of ten plants per progeny. The following characters were evaluated at 23 years after planting: a) commercial height (H) (m), measured by hypsometer vertex b) commercial volume (V) (m³). Estimates of variance components and genetic parameters were obtained by restricted maximum likelihood method and best linear unbiased prediction (REML / BLUP). The CVgi% showed up with a good genetic variation for (H) and (V) characteristics of 24% and 33%, respectively. The (H^2_a) present with moderate magnitudes with 22%-25% for the characters analyzed. The accuracies, showed up with high values of magnitudes 78% and 76%, for both characteristics, i.e., the estimated values are close to the actual breeding values. The (C^2_p) showed low magnitude, which demonstrated the good experimental design. The estimates proved to be excellent prospects for improvement program designed to logging.

P 56

Finnish Elite Seed Orchard Programme: A Progress Report

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Genetically improved seed has an important role in the Finnish forestry. Over the past 35 years, nearly 1.3 million hectares of forest land have been reforested with open-pollinated seed from 3300 hectares of Scots pine and Norway spruce seed orchards. Most of these orchards established in the late 1960's have already bypassed their production peaks. Already in late 1980's, the Finnish government commenced a programme to replace the first-generation orchards with 1.5 generation (a.k.a. elite) seed orchards to secure the long-term supply of genetically improved seed and to ensure maximum use of the progress made in long-term tree breeding programs.

The programme has progressed steadily, resulting in 541 hectares of elite seed orchards (Scots pine: 410 ha, Norway spruce: 131 ha) established since 1997. The goal, 822 hectares of new seed orchards, will be reached by 2025. The orchards are placed in fertile soils and warm microclimate to ensure favorable conditions for seed production. In full production, the annual seed crops of elite seed orchards are expected to exceed 5000 kg. Such crops will suffice to cultivation of about 120 000 hectares of forest land by planting and direct seeding. So far the crops have been small and the share of elite seed in nursery use is currently only about 15% (in Scots pine).

The elite seed orchard units are 10-30 hectares in size and comprise grafts of 25–35 superior first-generation plus trees selected on progeny test results. The primary selection criteria comprise growth, overall stem quality, vitality and propensity for female flowering (assessed in first-generation orchards). The seed of tested parents is anticipated to yield approximately 25% genetic gains in stem volume, as well as some improvements in branch traits compared to woods-run seedlots.

P 57

Estimation of growth performance of Cryptomeria japonica clones in various environments based on the physiological process model

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In forest tree improvement, evaluation of growth performance of trees under various environments is important for successful forestation and for the adaptation to the probable shift of suitable planting site for each genotype caused by future climate change. It is necessary for the estimation of the growth performance of each genotype to characterize genotype by environment interaction. For these objectives, we constructed the physiological process model composed of key physiological processes as functions of meteorological elements, such as radiation, temperature and vapour pressure deficit (Vpd). We used common 10-clone set of *Cryptomeria japonica* planted in many test sites located in various environments. The adopted model consisted of photosynthesis part and temperature- and Vpd-limiting parts. Biomass growth of each clone estimated from the periodically measured growth data (height and DBH) were the response variables, and the random clonal effect, fixed effects and meteorological elements per month were used as the explanatory variables in our model. In the result of the simulation, the parameters of photosynthesis part showed clonal characteristic irrespective of different environmental sites. This suggests that this model could separate growth potential (photosynthetic ability) of each genotype from environmental limiting. Furthermore, this model allow the estimation of growth performance in a certain range of the environment.

P 58

Genetic preservation ex situ of Myracrodruon urundeuva population in a germplasm bank

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Myracrodruon urundeuva, popularly known as aroeira, is a tree species of recognized economic value, due to the extraordinary durability and resistance of its wood, and its pharmacological properties. Thus, the use of *M. urundeuva* as a natural population is important to establish conservation and breeding programs, aim to produce better seeds with genetic quality for the recovery of anthropogenic degraded areas. Thus, this study was to evaluate the variation and to estimate genetic parameters for quantitative traits in a population of *M. urundeuva* belonging to the Active Germplasm Bank (BAG) from an Electrical Company of São Paulo (CESP), installed on November, 2000, in Rosana city São Paulo, Brazil. After 14 years from planting, the diameter at breast height (DBH) and survival was evaluated. In data from randomized block design with 30 progenies, three repetitions and eight plants per plot in linear form, spaced 3.0 x 1.5 m. The estimation of variance components was based on REM/BLUP (restricted maximum likelihood/best linear unbiased prediction) procedure. The development of *M. urundeuva* progeny was 11.56 cm for average DBH with a survival of 84.17%. The individual heritability estimation (4.6 %), genetic variation coefficient (9.3 %) and accuracy (46.5 %) for DBH provides grants to conduct a genetic conservation "*ex situ*" program with this *M. urundeuva* population.

P 59

The potential of breeding for Phytophthora alni subsp. alni resistance in black alder

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Black alder is a typical riparian tree that plays a range of important economic and ecological roles in riparian stands. *Phytophthora alni* ssp. *alni* (PAA), an oomycete pathogen that causes a devastating disease of black alder, changes the environment of riparian and alder carr ecosystems. One of the main approaches to finding a solution to this problem is to identify naturally resistant genotypes of black alder using a series of artificial inoculation experiments, and to use them in resistance breeding programmes. For this reason ninety black alder genotypes from different regions of the Czech Republic and two isolates of the PAA pathogen were selected for use in *in vitro* infectivity trials. Subsequently the resistance/susceptibility levels of ten of the best-performing black alder genotypes and one with a high level of susceptibility were tested again in a second round of tests using five other PAA isolates.

Host susceptibility varied significantly and widely among individuals. The observed differences in the black alder populations were also found to be dependent on the particular isolate used, altitude and geographical origin of the genotypes. A significant positive correlation between the pathogenicity of both tested isolates was also observed in the first round of infection tests. In the second round of tests a significant variation in host tree resistance and the isolate pathogenicity was also observed. The observed resistance in individual genotypes differed with the particular isolate used, so it is always necessary to use a range of isolates of the PAA pathogen in such resistance screening tests. All the best-performing genotypes identified in these trials were subsequently propagated and conserved for later field resistance trials and the development of seed-production orchards.

These findings have important implications for restoration plantings and for PAA resistance breeding programmes, as there is potential for selection in natural populations.

P 60

Populus nigra L. breeding strategies in the Czech Republic

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Populus nigra (PN) is an autochthonous European woody species that is endangered in the Czech Republic. Besides a fragmented population of PN that could led to inbreeding depression, the genetic purity of native populations of PN is threatened by the spread of hybrid poplars that are commercially grown in short rotation coppice (SRC) cultures. In the regions where it is not legal to plant allochthonous species, PN is irreplaceable and therefore a PN breeding programme was initiated in the 1980s for the selection of plus trees found in wild populations in the Czech Republic. The main breeding goals are 1) high yield potential; 2) suitability for SRC cultures; 3) suitability for growing on marginal agricultural land; and 4) resistance to the leaf rust pathogen *Melampsora larici-populina*.

The breeding strategy used is full-sib crossing. The obtained progeny are then tested in field trials. Subsequently, promising individuals are vegetatively propagated and planted in replicated plots on a range of sites with different growing conditions. Some of the selected individuals are then used in the next generation of breeding.

Within the PN breeding programme other potentially relevant properties are also studied, such as inbreeding depression within small PN populations or the correlation between rust susceptibility and biomass yields. The effect of crossings made between relatives on the offspring's vitality and fitness are based on field observations, but microsatellite markers have also been studied and inbreeding depression confirmed. In addition, some monoecious PN trees were found among the progeny of certain crosses, and their reproductive capability has been investigated. The feasibility of selecting for PN resistance to rust was demonstrated, along with a negative correlation between rust susceptibility and PN biomass production.

Selected clones are being used in commercial plantations mainly in protected areas. Some of them are now protected by plant breeder's rights.

P 61

Applying SE-technology for mass-propagation of high-quality Norway spruce in Finland

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Irregular flowering of seed orchards and problems caused by pests have caused periodic shortages in availability of genetically improved Norway spruce (*Picea abies* (L.) Karst.) seed over the past decade in Finland. Vegetative propagation based on somatic embryogenesis (SE) is expected to become a remedy to the supply problems and an effective way to produce high-quality clonal material for forest reforestation. The SE laboratory of the Punkaharju unit, the Finnish Forest Research Institute, is currently making rapid progress in producing SE clones of Norway spruce for genetic field testing.

Seed embryos used as a starting material in SE originate from controlled crosses between superior plus trees. The embryos are derived from immature seeds and used as explants for producing the SE clones. Suitability of the SE clones for mass-propagation is evaluated in multiple-stage tests carried out in the laboratory. The SE clones showing the highest embryo producing capacity and viability after cryopreservation will be selected for clonal field testing.

The first field trials with SE clones will be planted in 2014. Two-year-old emblings are used for the trials. The trees will be evaluated, e.g., for growth, phenology, lammas growth and resistance to various pathogens. Each set of clones will be tested in at least four sites with ten emblings per clone and site. Following the completion of field testing, the selected clones will be taken from the cryopreservation and delivered to commercial plant producers for large-scale multiplication.

In the future, emblings may be utilised in reforestation either as mixtures of tested clones or as untested full-sib families. However, dissemination of the new material requires implementation of the SE-technology in commercial plant production. Transferring the know-how and technology from research to practice has already begun in a pilot with Norway spruce special forms selected for ornamental use.

P 62

Development of varieties of Japanese cedar, Cryptomeria japonica, superior to resistance against damage from snow pressure in Japan

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Japanese cedar, *Cryptomeria japonica*, is an important endemic conifer tree species to produce timbers utilized as erect wooden columns in traditional Japanese houses, and its plantation is very common in Japan. We have also planted Japanese cedar trees in some regions having heavy snow, where serious damage from snow pressure is sometimes observed in the lower portion of their stems. In this context, three prefectures, i.e. Akita, Yamagata and Niigata, in the western part of the Tohoku breeding region in Japan and Tohoku Regional Breeding Office have been carrying out a breeding program for improving resistance to damage from snow pressure on Japanese cedar. As a result, we have selected 37 trees superior to resistance against damage from snow pressure, including two registered varieties, i.e. Dewa-no-Yuki #1 and Dewa-no-Yuki #2. This presentation will summarize the breeding program to improve snow pressure resistant varieties, which have not been developed in any other country. In addition, we will talk on a methodology for selection of trees superior to resistance against damages from snow pressure, upgrading the generation of snow pressure resistant varieties, e.g. development of the 2nd generation, and study on mechanism for resistance against damage from snow pressure in Japanese cedar trees.

P 63

Genetic variability in a population of Cariniana estrellensis (Raddi) Kuntze in southeastern Brazil

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The survival of natural populations depends upon its genetic variability that is related to the conditions of evolution and adaptation to different environments. The lack of genetic variability implies the impossibility of progress with the selection. *Cariniana estrellensis* is a forest species of great economic ecological importance is one of the largest and most long-lived trees native Brazil, occurs in low population density and is on the list of endangered species, has importance in medicine, landscaping, cellulose fiber and construction. The aim of this study was to evaluate the genetic variation of the diameter at breast height (DBH) and survival (SOB) in progenies *Cariniana estrellensis* in a Active Germplasm Bank (AGB), located in the municipality of Rosana in the state of São Paulo, Brazil. The AGB was installed in November 2000 and the experimental design was a randomized block design with 30 treatments (progenies), three replications, eight plants per plot spaced at 1.5m x 3.0m. Measurements were performed in the progenies at 13 years of age. Estimates of variance components by REML procedure/BLUP (Restricted maximum likelihood/best linear unbiased prediction) were obtained. It was found that families *Cariniana estrellensis* showed low survival on site (28.75%), which caused lower estimates of accuracy for DBH (27.51%) and high variation within plots (21.83%). However, the progeny show a further variation coefficient of genetic 14.07%. Thus, the progeny *Cariniana estrellensis* test its use has committed to a breeding program, but has potential for a program of ex situ genetic conservation such as the AGB.

P 64

The distribution, variability and breeding programme of Robinia pseudoacacia (L.) in Poland

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Black locust (*Robinia pseudoacacia* L.) was the first forest tree species introduced from North America to Europe at the beginning of the 17th century. The species was introduced by Dezydery Chłapowski into Poland 200 years ago as a valuable tree planting in mid-field woodlots in agricultural landscape of Wielkopolska region. In Poland *Robinia pseudoacacia* has become widespread in the south-west of country. According to Forest Inventory (data Bureau for Forest Management and Geodesy) on December 31, 2012 black locust is present in over 88 500 stands, covering a total of 273 000 ha on land managed by Polish State Forests. Total share of black locust as a dominant species in the area of Polish State Forest is 0,15%. However total forests with occurrence of black locust cover about 3,35% of the State Forest in Poland. Among over 420 forest districts in Poland, the total absence of black locust distinguishes only 11 of them. Distribution of black locust stands older than 50 years seems to be related to potential area planted with vines. The Belchatow Forest District in the central part of Poland stands out where for over 30 years black locust has been successfully used for land reclamation around the coalmine and power station. Polish black locust breeding programme aims mostly at obtaining straight growth form of trees. So far, there are 2 selected seed stands, 34 plus trees, 2 seed orchards. *Robinia pseudoacia* apart from precious wood (durable and rot-resistant wood is used as fire and pulp wood, for fences, construction and garden furniture) plays an important role in honey production. In Poland this species is the second best tree (linden is the best) regarding its melliferous value.

P 65

Selection of Basic Materials in Catalonia: A small country with a wide programme

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The selection on forest reproductive material (FRM) is scarce in many of the species used in reforestation/afforestation in Europe; particularly in those aimed to high quality timber and fruit production. In some Mediterranean regions like Catalonia, which have a good potential to develop productive and sustainable forest plantations, the lack of this selected FRM is an important bottleneck for developing public and private plantations. In this framework, a recent agreement between a Catalonian public tree nursery, Forestal Catalana S.A., and the research institute, IRTA, has permitted to start a programme to develop Basic Materials on some forest species. Each species and its growing context, environmental, economic or social, suppose a new and particular challenge. A wide ecological gradient characterizes this region and it is an advantage for breeding in a wide genetic basis; mainly because of some species are in the limit of their natural range and biotic and non-biotic pressures are highly intense.

The species included in the programme for timber purposes are common walnut (*J. regia* L.), hybrid walnut (*J. xintermedia*), narrow-leafed and common ash (*Fraxinus angustifolia* Vahl. and *F. excelsior L.*), wild cherry (*Prunus avium* L.), European hackberry (*Celtis australis* L.), mountain pine (*Pinus uncinata* Ram.), hazelnut (*Corylus avellana* L.) and sweet chestnut (*Castanea sativa* Mill). This last together with stone pine (*Pinus pinea* L.) are included also in the programme aimed to fruit production. Genetic material of ‘plus’ trees picked up from wild populations have allowed to install clonal banks, provenance and progeny tests. Selection on the base of the field trial data, which are located in different environments, leads to chose which are the most appropriated Basic Material for each species depending on its characteristics and productive aims. *Ex-situ*conservation and research on population genetics are underlying objectives related with this multispecies breeding programme.

P 66

cpSSR based breeding zones for Scots pine in Lithuania

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Owing to variable genomic function, microsatellites may be helpful not only in defining population genetic structure but also contribute information for delineation of breeding zones. Populations may have divergent evolutionary history having an effect on the present day genomic structure. Present-day adaptedness reflected by the functional markers is only one side of the long-term adaptation, when the DNA sequences in the neutral part of the genome may also have an effect. Our aim, therefore, was to evaluate how well genetic structuring produced by cpSSR markers represents the climatic zones and results of transfer effects in the field trials. We tested 6 cpSSR loci with 20 populations, 20 trees per population. The Bayesian clustering subdivided Lithuania into western (maritime climate) and eastern parts (continental), with the border following the midland lowland which splits the country into these two parts. cpSSR also showed differentiation of a population adapted poor sites and purely sandy *Cladiniosum* forest type in the south of the country. We speculate that this structure may reflect post-glacial migration history.

P 67

Utilization of Silver Fir Genetic Conservation Units in a Series of Measures Aimed at the Species Gene Resources Preservation and Reproduction in the Forest Management of the Czech Republic

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Objective: *This project, undertaken in the period 2009–2013, was focusing on the research of genetically conditioned variability of silver fir, in particular to testing progenies of valuable autochthonous stands of this species registered in the Czech Republic in genetic conservation units.*

Methods: Comprehensive assessment and the processing of results in the evaluation of silver fir populations and their progenies from genetic conservation units, which have been tested in research plots established up to now. These include measurement and evaluation of silver fir in genetic conservation units, including evaluation of ground vegetation. With the application of recent molecular biology methods (analyses of isozymes), another phase of these research activities was realized.

Results: On the base of evaluation of quantitative and qualitative characteristics in some of the Czech provenances, it was possible to identify healthy, well growing and productive variants. Good growth of some provenances proved that, when material of suitable origin is used, it is possible to breed qualitative stands of silver fir on the sites with conditions similar to those of the experimental sites.

Conclusions: Results of this project provided new findings on genetically conditioned variability of tested silver fir populations in the CzechRepublic, but it has also contributed to another successful series of measures aimed at the preservation and conservation of genetic resources of this species.

P 68

FitForClim – Procurement of highly productive and site-adapted forest reproductive material for future forests under climate change

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Timber is an important raw material, which has the benefit of sequestering carbon. However, in the face of changing climatic conditions it becomes challenging to sustain the supply with high quality timber. It is therefore important to foster the genetic adaptability of forests to those changes.

Thus, a Germany wide joint project has started recently and will be presented here. It aims at setting the foundation for a sustainable supply of markets with high-quality forest reproductive material (FRM). FRM needs to show a sufficient genetic diversity, in order to be productive in stable and adaptable stands, even under novel conditions due to a changing climate and under diverse site conditions. Apart from increased productivity (carbon sequestration) the project also aims to enhance wood-quality. This is a precondition for a multi utilisation of timber, which means that the material is used once or several times before it is used for energy generation.

In order to achieve this goal the project implements inter-institutional analyses of data from long-term tree breeding trials. Valuable information for the revision of provenance recommendations and for the establishment of breeding zones are expected from such analyses. Furthermore the selection of plus trees and their subsequent propagation for the establishment of seed orchards can be based on such information. Synergies and the remaining capacities in the field of forest tree breeding will be used efficiently due to the intensive networking of the partners in the joint project.

The intensity of breeding efforts will vary among the 6 target tree species of the project, spruce, pine, larch, Douglas fir, oak and sycamore. Possible trials could range from stand progeny trials (e.g. with oak) to controlled cross-breeding (e.g. with larch). After 15 years of breeding effort, the average gain in wood volume is expected to be around 10-30 %.

P 69

Growth potential of Scots pine (Pinus sylvestris) for reforestation in the lake states region of the USA

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Red pine (*Pinus resinosa*) is native to the Lakes States region of the USA, covers nearly 800,000 hectares, and is the most planted tree species in the region, but it possesses low genetic diversity raising concerns about its adaptability to a rapidly changing climate. Scots pine (*Pinus sylvestris*) is not native to the region, but it possesses some of the highest genetic variation in tree species, has wood characteristics similar to red pine, and may be a viable alternative to red pine in a changed climate. Currently, Scots pine is used very little for timber in the region due to unpredictability in growth and form likely resulting from mismatched seed source and/or silviculture. The objective of this study is to determine the viability of Scots pine as an alternative to red pine by comparing several long-term growth plots (99 years). A Riga variety Scots pine seed source was planted in 1915 at two sites at the Cloquet Forestry Center in northern Minnesota, USA at a spacing of approximately 1.5 meters. Naturally regenerated red pine was left on site for comparison. Four research plots, each approximately 0.04 acre in size, were established to monitor growth. The stands were thinned at years 1950, 1960, and 1970, and the plots were measured at roughly 20-year intervals. Results from the most recent measurement in 2012 revealed that Scots pine exceeded red pine by 5-8% for tree height, 14-25% for basal area, and 19-25% for volume. The exceptional growth and form of the Riga variety are likely due to ideal site factors, seed source, and silviculture suggesting a good fit regionally. When site factors and silviculture are properly matched to seed source, Scots pine can meet or exceed tree growth and form expectations for native species such as red pine.

P 70

Morphometric characteristics of buds of different European beech cultivars

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Objective: Along with significance as a forest species, European beech is renowned by ornamental cultivars, which are rare in Serbia. This paper presents results of analysis of morphometric characteristics of buds collected from nine ornamental beech trees (*Fagussylvatica* ‘Purpurea’,*Fagussylvatica* ‘Tricolor’ and*Fagussylvatica*’Purpurea Tricolor’) and Moesian beech. The aim of this research was to study the variability of the bud characteristics of selected ornamental beech cultivars and compare it with Moesian beech.
Methods: Ten trees were selected in Belgrade (Serbia) and one hundred buds were taken from each tree. The processing of data obtained by measurement of length and width on the sample of 1000 buds and one derived parameter (the length/width ratio), pointed out the differences in all characteristics. The data were processed by program “Statistica 6.0”, descriptive statistics and One-way ANOVA were performed. Statistical significance between selected trees and the homogeneity of the groups were tested by Fisher’s LSD test. Cluster analysis was used for grouping the similar trees.
Results: The results of analysis showed that the obtained differences between the analyzed characteristics are statistically significant. Minimum and maximum bud length values were in the range 7.09-30.30 mm and width between 1.10-4.74 mm. There are differences between cultivars, as well as in comparison with Moesian beech.
Conclusions: The recording of ornamental beech cultivars in Serbia and studing their variability can be the starting point for further breeding of this species in decorative traits. It is necessary to continue with similar investigations on other beech cultivars.

P 71

Characterization of water-use efficiency in Spanish common walnut populations

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Walnut timber plantations in Europe have increased in the last years. However, the planting stock frequently belongs to a few hybrid progenies, being the potential of autochthonous walnut (*Juglans regia* L.) underexploited. As a first step to quantify this potential and with a conservation background, a provenance trial was installed in 2005 in north-eastern Spain aimed at characterizing the performance of 44 representative Spanish wild populations. One of the multiple evaluation approaches consisted in determining the existing variability in intrinsic water-use efficiency (WUEi), a relevant trait under Mediterranean drought-prone conditions, and its relationship with growth. To this end, carbon isotope composition ($\delta^{13}C$) was determined in leaf tissues of nine trees per population during two consecutive years. In the second year, stomatal conductance and oxygen isotope composition ($\delta^{18}O$) were also analysed to discriminate between potential causes underlying variation in WUEi (either stomatal regulation or photosynthetic capacity). Annual diameter (DBH) and height (H) were recorded every year and climatic records at origin consisted in 50-year averages.

Six populations were clearly separated from the rest in terms of their WUEi. Growth (total height, H) was negatively correlated with WUEi (2nd year of $\delta^{13}C$ *vs.* H r=-0.30; p=0.05). In turn, WUEi was unrelated with altitude but negatively related to temperature at origin (two year average of $\delta^{13}C$ *vs.* Min. Temp. r=-0.33, p=0.05). Therefore, less water-use efficient populations, originating from warm sites, showed the most growing potential. These results have to analyse taking into account the trial conditions (under full irrigation in a warm environment) and also considering the effect of populations from high altitude and warm sites (Southeast of Spain) and the ones from lower altitudes, warm and rainy sites (Northwest of Spain). This information is discussed in order to an optimal deployment of genetic material to contrasting climatic conditions.

P 72

Morphological variation in the collection germoplasm of Genipa america L.

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Genipa americana L. is a native fruit tree of Brazil. This species has significant social and cultural importance in regions where it occurs naturally. Information on the morphological characteristics of this species is insufficient to support genetic conservation strategies that have previously been adopted. The proposal was to characterize *G. america* germplasm from morphological characters in juvenile age. The active germplasm bank of *G. americana*from Embrapa was composed of eighteen progenies/provenances from Northeast, Brazil and ten repetitions. This germplasm were evaluated at twelve and eighteen months after planting. The measurements were performed using the following characteristics: plant height, top diameter, stem diameter, leaf length, and leaf width. Data analysis of multivariate methods that were employed, were based on the average Euclidean distance and cluster analysis of Tocher's method. The phenotypic distances between pairs of progenies ranged from 0,08 to 3,35. The pattern of phenotypic structure of 18 progenies (provenances) of *G. americana* resulted in the formation of three groups one constituted by the progenies of municipalities of Sergipe State and another by two progenies from Sergipe and Bahia States and a third by one municipality of Sergipe State. The most divergent progenies are promising to increase genetic variability of active germplasm bank.

P 73

Relationship between parental genetic distance and offspring's heterosis of growth in Liriodendron: Implication for parent pair selection in cross breeding

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n plant cross-breeding, an important concern for breeders is to select the right parents that may produce hybrids with strong heterosis. Genetic distance (GD) is one informative parameter for parent-pair selection. *Liriodendron*, a genus from the Magnoliaceae family, is commonly used as a valuable timber and in landscape ornamentation.Interspecies hybrids in *Liriodendron* show significant heterosis and high levels of trait variations among combinations and among individuals within families. Therefore, the most effective strategy in *Liriodendron* improvement is making a large number of crosses and selecting elite combinations with strong heterosis. In the past two decades, dozens of interspecies crosses in *Liriodendron* have been made and their hybrids broadly planted for both ornamental and industrial uses in southern China. Conversely, the underlying mechanism of heterosis in *Liriodendron*remains poorly understood. Here, we reported the relationship between parental genetic distance and offspring's heterosis of growth in *Liriodendron*. The genetic distances among 13 parental trees were assessed across 30 microsatellites loci. Offspring heterosis was estimated based on six-year-old growth from a progeny testing plantation. A positive but non-significant correlation was found between parental genetic distance and offspring heterosis. Moreover, offspring's heterosis of growth reached a peak value when parental genetic distance was around 1.0. Our finding may be helpful for parent-pair selection in future cross-breeding of *Liriodendron*.

P 74

Proportion of vegetative reproduction in Mexican Populus tremuloides Michx. populations on the Sierra Madre Occidental

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Trembling aspen (*Populus tremuloides*Michx.) is the most widely distributed tree species in North America. It grows from Alaska across the Northwest Territories to Quebec and Newfoundland and south to Virginia, Missouri, Nebraska, and Central Mexico (Sierra Madre Occidental and Oriental). It colonizes all Canadian provinces and most of the states of the United States, but also some states in Mexico. In many parts of its distribution, this aspen is a unique and essential keystone species and is listed as a dominant species in over 100 habitat, plant community, and vegetation typings. *P. tremuloides* is one of the most important timber trees in Canada and the United States. In some forest regions, therefore, breeding programs have been developed for improving trembling aspen and aspen hybrids for fiber and wood production.

Several authors reported that *P. tremuloides* in the United States and Canada rarely reproduces sexually, but commonly regenerates through vegetative reproduction, or asexual root suckering. Disturbances, such as fire, often trigger root suckering that permits rapid regeneration and can occasionally lead to sexual regeneration. But there is a lack of reproduction information of Mexican trembling aspen.

Therefore, we hypothesized that Mexican *P. tremuloides* populations on the Sierra Madre Occidental also exhibit a high proportion of vegetative reproduction, which was tested using AFLP marker. Needles were sampled from about 76 randomly chosen trees coming from 50 x 50 m plots in seven populations. The results showed that all individuals were genetically different. The genetic diversity was very high in the populations. Thus, vegetative reproduction of trembling aspen probably plays only a secondary role on the Sierra Madre Occidental.

P 75

In vitro conservation of Castanea spp. hybrid clones using artificial seeds and slow growth storage

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Chestnut fruit production in Portugal declined due to ink disease (*Phytophthora cinnamomi*). Hybrids from one parental female and two resistant parental males obtained from controlled crosses between *Castanea sativa*x *Castanea crenata* (SC) and *C. sativa* x *C. mollissima* (SM) were micropropagated. From the 33 clones established *in vitro*, 25 were multiplied and plantlets were acclimatized to test pathogenic fungi resistance. *In vitro* clonal conservation is crucial.

Slow growth storage (4ºC, MS half strength, 0.09M sucrose, 0.45μM BA) was compared to synseeds. Nodal segments (5-7mm; without leaves) were isolated and prepared to perform the antioxidant treatment (sterile distilled water /1H). Then nodal segments were mixed in the same culture medium (lacking Ca component) added of Na-alginate (2.75% *vs* 3%). For encapsulation the nodal segments were released into $CaCl_2$ $2H_2O$ (3 concentrations were tested 50; 75; 100mM for 30 min.), then washed 3 times in sterile distilled water and after transferred to Petri dishes. The reactivation period (25/20ºC, in dark, 1st week; plus 16/8h, 2nd week) was accomplished one month (4ºC) later. The synseeds were exposed to the sterile air flow, during different periods (30 min. maximum) in a laminar-flow hood, before transfer to a new fresh medium. The survival and germination rates were recorded. After 4 weeks, shoots were cultivated and the multiplication rate was evaluated. The best results of survival rate were obtained when synseeds were tested. In this case, no significant differences were found in the germination and survival rates. The average time of germination of synseeds ranged from 7 to 21 days. The multiplication rate achieved to a maximum of 8 times from one synseed. Further studies using higher sucrose concentrations should be tested for slow growth storage, as well as bigger conservation periods to reduce labor and costs, hence assuring a most effective long-term conservation.

P 76

Micropropagation of selected clones of black locust (Robinia pseudoacacia L.)

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Future directions for forestry research include production the small-sized timber in short production cycles as an attractive, renewable energy resources. In this context, interest of black locust (*Robinia pseudoacacia* L.) forces to develop an effective method of micropropagation, allowing the production of vegetative cuttings on a commercial scale. The clonal material of black locust is required for setting up seed orchards and for wood production. Vegetative propagation of forest trees, allows more efficient than generative, keep desirable traits for breeding. In the case where the multiplication is not effective, or even impossible, plant tissue culture of forest tree species may provide a superior alternative to traditional cloning techniques for obtaining high multiplication of elite genotypes. In this study, we described the method of micropropagation by organogenesis selected clones of black locust. The current year 2-5 cm long shoots from early spring 2014, were collected as primary explants for clones regeneration. The explants were taken from 8 clones. Attention was also focused on possible factors responsible for successful propagation of adult donor trees. The sterilized plant material was transferred on the MS basal medium, supplemented with: 0,6 mg·l-1 BAP and 0.1 mg·l-1 NAA, and 30 g·l-1 of sucrose.Multiplied shoots were subcultured to root to a medium containing different concentrations of auxins. These rooted plantlets were transferred to pot in a greenhouse. The majority of the regenerated plants had adapted to in vivo conditions. The presented protocol provides relatively efficient rate of the multiplication and would be useful for forest tree breeding purposes.

P 77

Genetic divergence in select clones of Casuarina equisetifolia

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India is the largest planter of *Casuarina* in the world with about 0.5 million ha under this genus. Systematic tree improvement programmes are underway at the Institute of Forest Genetics and Tree Breeding since 1996 and IFGTB is the focal point of Casuarina research in India with the largest germplasm collection in the country. Genetic divergence studies were conducted in 87 select clones of *C.equisetifolia* at age 3 for tree height, DBH and volume. Application of Mahalanobis' D^2 statistics and Tocher's clustering method resolved the 90 treatments (87 clones and 3 seedling origin materials) into 10 clusters. The cluster strength varied from two in clusters III to VII and 37 in cluster I. Cluster IX, II and X included 18, 12 and 11 clones respectively. The largest intra-cluster distance was registered in cluster X (2.22) followed by cluster II (2.10). Cluster III recorded the smallest intra-cluster distance (0.12) followed by cluster IV (0.14). Inter-cluster distance revealed that maximum divergence existed between clusters IX and X (2.31). Cluster VIII showed maximum mean value for tree height (11.39) followed by cluster IX and VII. Maximum mean value for DBH (7.13) was registered by cluster III followed by cluster VII and IX. Cluster VII exhibited the maximum value for volume (55668.64) followed by cluster III and IX. Volume contributed the maximum towards genetic divergence (56.08%) followed by DBH (24.22%) and tree height (19.70%). Based on the results, five male clones namely, TNVM 2, CE 2003/4, CE 2003/3, CE 219 and CE 347 and ten female clones TNPP 2, TN 111, CE 2002/1, CE 220, CE 268, CE 243, CE 9, CE 303, CE 281 and CE 2003/5 could be selected for further breeding programmes. Five monoecious clones were also found to be productive and divergent. They could be used as pollen parents.

P 78

Evaluation of the internal morphology of Terminalia argentea Mart. et Zucc. (Combretaceae) seeds by the X-ray test

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Forest species, especially those from brazilian Cerrado, are characterized by the occurrence of predation, empty fruits and germ formation deficiency. The objective of this work was to evaluate the internal morphology of*Terminalia argentea* Mart. et Zucc seeds. By the x-ray test and to verify its relation with germination. It was taken 200 radiographs of each lot and the tests were taken with the seeds set at 28,0 cm from the source of x-rays, using the digital equipment Faxitron X-ray model MX-20 DC 12. Next, the seeds were classified according to its internal morphology as seen in the radiographs. It was established three categories of seeds: full, empty and malformed. For each seeds category germination tests were performed. Statistical design in factorial, with three lots and three categories. From the results obtained in this work, the use of x-ray test with *Terminalia argentea* seeds is promising in quality of seeds lots detection, assisting in empty and malformed seeds separation, invalidating its use immediately or for storage. For the 3 analyzed lots, the germination percentage of the Full categories diverged statistically from the others. The Lot 2 obtained a higher percentage of normal seedlings (61.74%), followed by Lots 1 and 3. The X ray test, in 26kV by 1,2 seconds intensity was efficiency in*Terminalia argenta* internal morpholy evaluation. The *Terminalia argentea* seeds germinal percentage can be compromised by fruits formed without embryonic tissue or malformed embryos.

P 79

Evaluation of the internal morphology of Platypodium elegans Vog. (Fabaceae) seeds by the x-ray test

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Forest species, especially those from brazilian Cerrado, are characterized by the occurrence of predation, empty fruits and germ formation deficiency. The objective of this work was to evaluate the internal morphology of *Terminalia argentea* Mart. et Zucc seeds. By the x-ray test and to verify its relation with germination. It was taken 200 radiographs of each lot and the tests were taken with the seeds set at 28,0 cm from the source of x-rays, using the digital equipment Faxitron X-ray model MX-20 DC 12. Next, the seeds were classified according to its internal morphology as seen in the radiographs. It was established three categories of seeds: full, empty and malformed. For each seeds category germination tests were performed. Statistical design in factorial, with three lots and three categories. From the results obtained in this work, the use of x-ray test with *Terminalia argentea* seeds is promising in quality of seeds lots detection, assisting in empty and malformed seeds separation, invalidating its use immediately or for storage. For the 3 analyzed lots, the germination percentage of the Full categories diverged statistically from the others. The Lot 2 obtained a higher percentage of normal seedlings (61.74%), followed by Lots 1 and 3. The X ray test, in 26kV by 1,2 seconds intensity was efficiency in *Terminalia argenta* internal morpholy evaluation. The *Terminalia argentea* seeds germinal percentage can be compromised by fruits formed without embryonic tissue or malformed embryos.

P 79

Effect of different doses of gamma radiations on the germination and seedling growth attributes of Albizia chinensis (obsbeck) Merr

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The impact of different doses of gamma irradiation on the germination and seedling growth of *Albizia chinenis* is described in this article. The air dried seeds of *Albizia chinenis* were irradiated with acute gamma rays at 10KR, 20KR, 40KR and 80KR dose levels using ^{60}Co source. The doses were given in two different ways i.e., Continuous treatment (C) and Fractionated treatment (F).The effect of gamma radiation on the germination and seedling growth were compared with the control plants. The germination percentage, germination energy index and germination value was enhanced by lower doses of gamma radiations i.e. 10KR (C & F) and 20KR(C & F) and the higher doses i.e. 40KR(C & F) and 80KR(C & F) causes reduction in these parameters. The dose levels of 10KR (C & F) & 20KR(C & F) enhanced the radical and hypocotyls length and 40KR(C & F) & 80KR (C & F) reduced them. The shoot growth increased in all the dose levels were as the number of the primary roots was decreased by treatment of gamma rays at all the dose levels but the number of secondary roots and tertiary roots were increased at all dose levels. Hence, lower doses of gamma radiation treatment may be used to increase the germination, growth & vigor and also the shoot and root growth parameters in *Albizia chinenis.*

TITLE INDEX

Estimation of fertility variation by strobili and cone productions in Taurus cedar (Cedrus libani A. Rich.) populations	Bilir, Nebi	
Estimation of genetic parameters for conservation and genetic improvement in progeny from Dipteryx alata Vogel in agroforestry system.	Beatriz de Souza, Francine	
Estimation of genetic parameters in Pinus tecunumanii	Beatriz de Souza, Francine	
Estimation of growth performance of Cryptomeria japonica clones in various environments based on the physiological process model	Hiraoka, Yuichiro	
Estimation of number and distribution of QTL effects in forest tree traits	Hall, David	
European Larch breeding programme in Poland	Kowalczyk, Jan	
Evaluation of characteristics of the cultivars with resistance to snow-damage of Japanese cedar	Takata, Katsuhiko	
Evaluation of Near Infrared (NIR) Spectroscopy to Predict Post-Harvest Needle Retention in Fraser Fir	Frampton, John	
Evaluation of the internal morphology of Platypodium elegans Vog. (Fabaceae) seeds by the x-ray test	Oliveira, Michelle Silva	
Evaluation of the internal morphology of Terminalia argentea Mart. et Zucc. (Combretaceae) seeds by the X-ray test	Paradelo Gomes, Kever Bruno	
Evolutionary and functional analyses of LysM-contained genes in Populus revealed two potential chitin receptors	Xu, Li-an	
Fertility variation and its effect on the effective number of parents at two seed stands of tropical species	Kang, Kyu-Suk	
Finnish Elite Seed Orchard Programme: A Progress Report	Antola, Jukka	
FitForClim – Procurement of highly productive and site-adapted forest reproductive material for future forests under climate change	Meißner, Meik	
Flowering variation as to years in Scots pine young clonal seed orchard	Ayan, Sezgin	
Forecast of Phenotypic Parameters for Growth Traits in a Clonal Seed Orchard of Scotch Pine (Pinus sylvestris L.) in the Erzurum Region in Turkey	Özel, Halil Barıº	
Future and present testing of the progeny of basic forest material in Poland	Magnuszewski, Michal	
Genetic analysis of survival and growth traits in Pinus radiata D. Don seedlings submitted to water restriction	Espinoza, Sergio	
Genetic architecture of local adaptation in widely-distributed, long-lived eastern white pine (Pinus strobus)	Rajora, Om	
Genetic differences in Scots pine resistance to needle cast (Lophodermium seditiosum Minter, Staley & Millar): possible impact of climate changes	Jansons, Aris	
Genetic differentiation and Phenotypic plasticity of Maritime pine in D13C: adaptive signifiance	Alia, Ricardo	
Genetic divergence among progenies of Pinus elliotii Engelm. var. elliottii in a subtropical region from Brazil	Kawahata Pagliarini, Maximiliano	
Genetic divergence in Pinus caribaea var. hondurensis progeny in Brazil	Torres-Dini, Diego	
Genetic divergence in select clones of Casuarina equisetifolia	Warrier, Kannan C.S.	
Genetic diversity and differentiation of Quercus petraea and Q. robur seed collection stands based on chloroplast microsatellites	Chmielewski, Michal	
Genetic evaluation – the breeders' perspective	McRae, Thomas	
Genetic gains in the selection in progenies of Copaifera langsdorffii (FABACEAE)	Manoel, Ricardo	
Genetic imprints and environment effects in a common garden of 500 Picea abies families from 160 sites throughout the Alps	Mosca, Elena	
Genetic improvement and breeding of Pinus massoniana in China	Ji, Kongshu	
Genetic improvement of Acacia senegal for gum arabic production: a question of ploidy level	Diallo, Adja	
Genetic parameter estimation for growth and form traits in Eucalyptus regnans progenies tested on two sites in New Zealand using spatial analysis	Suontama, Mari	

Genetic preservation ex situ of Myracrodruon urundeuva population in a germplasm bank	Lemos Souza, Danilla Cristina	
Genetic structure and diversity in two Norway spruce seed orchards of different size	Sønstebø, Jørn Henrik	
Genetic variability in a population of Cariniana estrellensis (Raddi) Kuntze in southeastern Brazil	Kubota, Thaisa	
Genetic variation for growth characters in progenies of Hymenaea stigonocarpa under Myracrodruon urundeuva in Selvíria, Brazil	Kubota, Thaisa	
Genetic variation for quantitative traits in progenies of Myracrodruon urundeuva Fr. Allem.	Lemos Souza, Danilla Cristina	
Genetic variation in dieback resistance in Fraxinus excelsior confirmed by progeny inoculation assay	Lobo, Albin	
Genetic variation in Dipteryx alata progenies in Brazil	Menezes Freitas, Miguel Luiz	
Genetic variation in Pinus caribaea var. hondurensis progenies for resin production	de Aguiar, Ananda Virginia	
Genetics of European ash versus the emerging invasive pathogen Hymenoscyphus pseudoalbidus	Kjaer, Erik Dahl	
Genome-wide analysis of heat shock protein 70 genes (Hsp70) in poplar	Nurten Yer, Esra	
Genome-wide SNP discovery and sequence variability in Fraser fir	Matallana, Lilian	
Genomic Selection for Embryogenic Clones Deployment on the Arauco's Radiata pine Program in Chile	Zapata-Valenzuela, Jaime	
Genomic selection of Eucalyptus breeding by SNP analysis in northern Brazil	Fukuda, Yujiroh	
Growth potential of Scots pine (Pinus sylvestris) for reforestation in the lake states region of the USA	Pike, Carrie	
How representative are plots in provenance trials? – An example from beech (Fagus sylvatica L.) genotyped with SSRs	Liesebach, Heike	
Hydric stress tolerance of Arbutus unedo L. selected trees	Gomes, Filomena	
Characterization of gene-based SSR markers polymorphisms of the Chinese fir and transferability in comparing with six conifers and model plant	Shi, Jisen	
Characterization of water-use efficiency in Spanish common walnut populations	Vilanova, Antoni	
Chloroplast genome assembly from NGS data, with applications to forest trees	Thompson, Stacey Lee	
In vitro conservation of Castanea spp. hybrid clones using artificial seeds and slow growth storage	Gomes, Filomena	
Inheritance of ring width, density, microfibril angle, and modulus of elasticity in wood of Norway spruce at two locations in south of Sweden	Chen, Zhiqiang	
Joint use of phenotypic, pedigree and genomic information in genetic evaluation: An example in Eucalyptus grandis	Cappa, Eduardo Pablo	
Management of the gene resources of broad-leaved species in Sweden	Stener, Lars-Göran	
Mapping Phytophthora Root Rot Resistance in Fir Species through Genotyping by Sequencing	Kohlway IV, William	
Massively multivariate genetic value prediction in tree breeding – experience from a variety of species.	Dutkowski, Gregory	
Matching Radiata Pine Genotypes to Current and Future Production Environments	Ivkovich, Milos	
Mating System in open-pollinated progenies of a natural population of Genipa americana L., by analysis of microsatellite Loci	Manoel, Ricardo	
Micropropagation of selected clones of black locust (Robinia pseudoacacia L.)	Szyp-Borowska, Iwona	
Molecular signatures of climate adaptation and range expansions in Mediterranean conifers	Gonzalez-Martinez, Santiago C.	
Morphological variation in the collection germoplasm of Genipa america L.	Freitas de Vasconcelos Mělo, Marília	
Morphometric characteristics of buds of different European beech cultivars	Nonic, Marina	

New genomic tools to enhance discovery of functional diversity in natural populations of Populus nigra L.	Faivre Rampant, Patricia	
New models for the definition of Scots pine deployment areas in Sweden and Finland.	Beuker, Egbert	
New transfer models to predict spruces plantation productivity in Québec (Canada) under the influence of climate change	Rainville, André	
Opportunities for genomic information in forestry	Tier, Bruce	
OPSEL – A tool for optimizing expected gain from clonal seed orchards while constraining relatedness and diversity among parents.	Perrson, Torgny	
Optimization of plant quantity for one family in Picea abies L. progeny tests	Bondarenko, Alexander	
Origins of the present day Portuguese Scots pine populations assessed by molecular markers	Gaspar, Maria João	
Orthology of Sinnapyl Alcohol Dehydrogenase gene (Sad) in Cedrus deodara (Deodar)	Goel, Neha	
Performance of seven tree breeding strategies under inbreeding depression	Wu, Harry	
Phenotypic and genetic correlations among tree volume, wood specific gravity and foliar traits in white spruce, Picea glauca (Moench) Voss and implications for selection	Pike, Carolyn	
Phenotyping Castanea hybrids from controlled crosses for resistance to Phytophthora cinnamomi	Costa, Rita	
Populus nigra L. breeding strategies in the Czech Republic	Novotná, Kateřina	
Progeny testing noble fir in Oregon and Washington for traits important to Christmas tree producers	Landgren, Chal	
Proportion of vegetative reproduction in Mexican Populus tremuloides Michx. populations on the Sierra Madre Occidental	Wehenkel, Christian	
Protocol: fast and high-performance Eucalyptus DNA extraction in 96-well plate method.	Nikichuk, Natalia	
Quantitative Genetic Survey of Scots Pine	Hong, Zhou	
Reduced background pollination using high tunnels	Wennström, Ulfstand	
Relationship between microfibril angle of S2 layer and mechanical properties in 10 open-pollinated families of Picea jezoensis	Tanabe, Jun	
Relationship between parental genetic distance and offspring's heterosis of growth in Liriodendron: Implication for parent pair selection in cross breeding	Li, Huogen	
Results from a joined Polish–French network of progeny trials with European larch	Kowalczyk, Jan	
Results of Scots pine provenance trial research in Siberia	Kuzmin, Sergey	
Review and Outlook on hybridization breeding of the Genus Liriodendron in China	Wang, Zhang-rong	
Review on advanced-generation genetic improvement of Chinese fir in China	Zheng, Renhua	
Scots pine adaptation depending on the reaction norm of geographical populations on the conditions of the West Transbaikalie	Novikova, Tatiana	
Seed orchards and supporting breeding	Lindgren, Dag	
Selecting Douglas fir provenances for future climates using universal response functions on basis of extensive provenance test series in Austria and Bavaria	Schueler, Silvio	
Selection of Basic Materials in Catalonia: A small country with a wide programme	Vilanova, Antoni	
Selection of European White Elm plus-trees based on half-sib variability in nursery progeny test	Šijaèiæ-Nikoliæ, Mirjana	
Shoot growth responded differently to imposed drought among half-sib families of Norway spruce	Chmura, Daniel J.	
Source to cold resistance in Eucalyptus breeding progrmams	Torres-Dini, Diego	
Studies of adaptive features of Siberian pine and Korean pine from provenance trial in the Asian part of Russia	Kuznetsova, Galina	

Temperature driven changes in transcriptomes and smallRNAomes during formation of an epigenetic memory in somatic embryos of Norway spruce	Yakovlev, Igor	
Testing progeny of beech plus trees in terms of height stability (preliminary study from northern Poland)	Klisz, Marcin	
The distribution, variability and breeding programme of Robinia pseudoacacia (L.) in Poland	Wojda, Tomasz	
The phenotypic effects of selective breeding on climatic adaptation in lodgepole pine and interior spruce	MacLachlan, Ian	
The potential of breeding for Phytophthora alni subsp. alni resistance in black alder	Novotná, Kateřina	
The rotation-aged performance of Chinese fir provenance trial in the northern Fujian	Bian, Liming	
The utility of GBLUP in forest genetic testing: examples from different genealogical complexities	Klápště, Jarosla	
The verification of the correctness of clone grafts planting in the seed orchard of Scots pine (Pinus sylvestris L.) in North-eastern Poland.	Przybylski, Pawel	
Today's Successes and Future Challenges for the NC State University Cooperative Tree Improvement Program	McKeand, Steve	
Towards a European Douglas-fir breeding population for long term breeding.	Michaud, Daniel	
Towards clever breeding: use of hyperspectral imaging for rapid assessment of field trials in genetic diversity	Danusevicius, Darius	
Tree Breeding in B.C. in 2014 – new tools, variable targets, and uncertain times	Yanchuk, Alvin	
Tree height estimation in progeny trials using UAS photogrammetry	Steffenrem, Arne	
Tree improvement of birch and alder for Ireland based on progeny testing	O‘ Connor, Ellen	
Tree improvement through breeding seed orchard; strategy and current situation in Nepal	Pokharel, Youba Raj	
Use of DNA markers in forest tree breeding programs	Szyp-Borowska, Iwona	
Use of prediction error co-variances for determining connectedness among test series	Kerr, Richard	
Utilization of Silver Fir Genetic Conservation Units in a Series of Measures Aimed at the Species Gene Resources Preservation and Reproduction in the Forest Management of the Czech Republic	Frýdl, Josef	
Validation of the Cryptomeria japonica seed and seedling transfer system by progeny tests analysis in conjunction with environmental factors	Miura, Masahiro	
Variability of biometric seed traits of Polish black locust (Robinia pseudoacacia L.) provenances	Jastrzêbowski, Szymon	
Variation of the timing of bud burst phenology in an old-growth forest stand composed of Fagus sylvatica and Quercus robur	Ulaszewski, Bartosz	

SPEAKER INDEX

Presenting author	Title	Page
Alia, Ricardo	Genetic differentiation and Phenotypic plasticity of Maritime pine in D13C: adaptive signifiance	
Almqvist, Curt	Effect of pruning and stand density on cone and pollen production in Pinus sylvestris seed orchards	
Antola, Jukka	Finnish Elite Seed Orchard Programme: A Progress Report	
Avila, Israel	Degree of hybridization in Pinus engelmanni in eight locations in the State of Durango Mexico	
Ayan, Sezgin	Flowering variation as to years in Scots pine young clonal seed orchard	
Beatriz de Souza, Francine	Estimation of genetic parameters for conservation and genetic improvement in progeny from Dipteryx alata Vogel in agroforestry system.	
Beatriz de Souza, Francine	Estimation of genetic parameters in Pinus tecunumanii	
Beuker, Egbert	New models for the definition of Scots pine deployment areas in Sweden and Finland.	
Bian, Liming	The rotation-aged performance of Chinese fir provenance trial in the northern Fujian	
Bilir, Nebi	Estimation of fertility variation by strobili and cone productions in Taurus cedar (Cedrus libani A. Rich.) populations	
Bondarenko, Alexander	Optimization of plant quantity for one family in Picea abies L. progeny tests	
Cappa, Eduardo Pablo	Joint use of phenotypic, pedigree and genomic information in genetic evaluation: An example in Eucalyptus grandis	
Cavers, Stephen	Collecting tree phenotypes in the field – TreeType: a new, open-source project from EVOLTREE	
Costa, Rita	Phenotyping Castanea hybrids from controlled crosses for resistance to Phytophthora cinnamomi	
Danusevicius, Darius	Towards clever breeding: use of hyperspectral imaging for rapid assessment of field trials in genetic diversity	
de Aguiar, Ananda Virginia	Genetic variation in Pinus caribaea var. hondurensis progenies for resin production	
Diallo, Adja	Genetic improvement of Acacia senegal for gum arabic production: a question of ploidy level	
Dutkowski, Gregory	Massively multivariate genetic value prediction in tree breeding – experience from a variety of species.	
El-Kassaby, Yoursy A.	Climate change and agile tree breeding	
Espinoza, Sergio	Genetic analysis of survival and growth traits in Pinus radiata D. Don seedlings submitted to water restriction	
Faivre Rampant, Patricia	New genomic tools to enhance discovery of functional diversity in natural populations of Populus nigra L.	
Frampton, John	Evaluation of Near Infrared (NIR) Spectroscopy to Predict Post-Harvest Needle Retention in Fraser Fir	
Freitas de Vasconcelos Mělo, Marília	Estimates of genetic parameters in progeny of G. americana test	
Freitas de Vasconcelos Mělo, Marília	Morphological variation in the collection germoplasm of Genipa america L.	
Frýdl, Josef	Utilization of Silver Fir Genetic Conservation Units in a Series of Measures Aimed at the Species Gene Resources Preservation and Reproduction in the Forest Management of the Czech Republic	
Fukuda, Yujiroh	Genomic selection of Eucalyptus breeding by SNP analysis in northern Brazil	
Gaspar, Maria João	Origins of the present day Portuguese Scots pine populations assessed by molecular markers	
Goel, Neha	Orthology of Sinnapyl Alcohol Dehydrogenase gene (Sad) in Cedrus deodara (Deodar)	
Gomes, Filomena	Assessment of Arbutus unedo L. clonal plants in a field clonal trial	
Gomes, Filomena	Hydric stress tolerance of Arbutus unedo L. selected trees	
Gomes, Filomena	In vitro conservation of Castanea spp. hybrid clones using artificial seeds and slow growth storage	

Gonzalez-Martinez, Santiago C.	Molecular signatures of climate adaptation and range expansions in Mediterranean conifers	
Haapanen, Matti	Applying SE-technology for mass-propagation of high-quality Norway spruce in Finland	
Hall, David	Estimation of number and distribution of QTL effects in forest tree traits	
Hiraoka, Yuichiro	Estimation of growth performance of Cryptomeria japonica clones in various environments based on the physiological process model	
Hong, Zhou	Quantitative Genetic Survey of Scots Pine	
Chen, Zhiqiang	Inheritance of ring width, density, microfibril angle, and modulus of elasticity in wood of Norway spruce at two locations in south of Sweden	
Chmielewski, Michal	Genetic diversity and differentiation of Quercus petraea and Q. robur seed collection stands based on chloroplast microsatellites	
Chmura, Daniel J.	Shoot growth responded differently to imposed drought among half-sib families of Norway spruce	
Ivkovich, Milos	Matching Radiata Pine Genotypes to Current and Future Production Environments	
Jansons, Aris	Genetic differences in Scots pine resistance to needle cast (Lophodermium seditiosum Minter, Staley & Millar): possible impact of climate changes	
Jastrzêbowski, Szymon	Variability of biometric seed traits of Polish black locust (Robinia pseudoacacia L.) provenances	
Ji, Kongshu	Cloning and analyzing of CAD and CCoAOMT genes from Pinus massoniana	
Ji, Kongshu	Genetic improvement and breeding of Pinus massoniana in China	
Kang, Kyu-Suk	Fertility variation and its effect on the effective number of parents at two seed stands of tropical species	
Kawahata Pagliarini, Maximiliano	Genetic divergence among progenies of Pinus elliotii Engelm. var. elliottii in a subtropical region from Brazil	
Kerr, Richard	Use of prediction error co-variances for determining connectedness among test series	
Kjaer, Eric Dahl	Genetics of European ash versus the emerging invasive pathogen Hymenoscyphus pseudoalbidus	
Klápště, Jaroslav	The utility of GBLUP in forest genetic testing: examples from different genealogical complexities	
Klisz, Marcin	Testing progeny of beech plus trees in terms of height stability (preliminary study from northern Poland)	
Kohlway IV, William	Mapping Phytophthora Root Rot Resistance in Fir Species through Genotyping by Sequencing	
Korecky, Jiří	Concept of intra-population analysis of genetic structure within populations of wild cherry (Prunus avium L.) in influenced urban area and selected superior trees originated from natural forest stands	
Kowalczyk, Jan	European Larch breeding programme in Poland	
Kowalczyk, Jan	Results from a joined Polish–French network of progeny trials with European larch	
Kubota, Thaisa	Genetic variability in a population of Cariniana estrellensis (Raddi) Kuntze in southeastern Brazil	
Kubota, Thaisa	Genetic variation for growth characters in progenies of Hymenaea stigonocarpa under Myracrodruon urundeuva in Selvíria, Brazil	
Kuzmin, Sergey	Results of Scots pine provenance trial research in Siberia	
Kuznetsova, Galina	Studies of adaptive features of Siberian pine and Korean pine from provenance trial in the Asian part of Russia	
Landgren, Chal	Progeny testing noble fir in Oregon and Washington for traits important to Christmas tree producers	
Lascoux, Martin	Clinal variation and the genetic basis of adaptive traits in trees	
Lemos Souza, Danilla Cristina	Genetic preservation ex situ of Myracrodruon urundeuva population in a germplasm bank	
Lemos Souza, Danilla Cristina	Genetic variation for quantitative traits in progenies of Myracrodruon urundeuva Fr. Allem.	

Li, Huogen	Relationship between parental genetic distance and offspring's heterosis of growth in Liriodendron: Implication for parent pair selection in cross breeding	
Liesebach, Heike	How representative are plots in provenance trials? – An example from beech (Fagus sylvatica L.) genotyped with SSRs	
Lindgren, Dag	Seed orchards and supporting breeding	
Lobo, Albin	Genetic variation in dieback resistance in Fraxinus excelsior confirmed by progeny inoculation assay	
Lstibůrek, Milan	Acceleration of genetic progress in tree improvement programs with modest financial support	
Luzia Dourado, Cecília	Estimates of genetic parameters for wood production in natural populations of rubber from the Brazilian Amazon	
MacLachlan, Ian	The phenotypic effects of selective breeding on climatic adaptation in lodgepole pine and interior spruce	
Magnuszewski, Michal	Future and present testing of the progeny of basic forest material in Poland	
Manoel, Ricardo	Genetic gains in the selection in progenies of Copaifera langsdorffii (FABACEAE)	
Manoel, Ricardo	Mating System in open-pollinated progenies of a natural population of Genipa americana L., by analysis of microsatellite Loci	
Matallana, Lilian	Genome-wide SNP discovery and sequence variability in Fraser fir	
McKeand, Steve	Today's Successes and Future Challenges for the NC State University Cooperative Tree Improvement Program	
McRae, Thomas	Genetic evaluation – the breeders' perspective	
Meißner, Meik	FitForClim – Procurement of highly productive and site-adapted forest reproductive material for future forests under climate change	
Menezes Freitas, Miguel Luiz	Genetic variation in Dipteryx alata progenies in Brazil	
Michaud, Daniel	Towards a European Douglas-fir breeding population for long term breeding.	
Miura, Masahiro	Validation of the Cryptomeria japonica seed and seedling transfer system by progeny tests analysis in conjunction with environmental factors	
Mosca, Elena	Adaptation of forest trees to climate change in the Alps: a study on ecologically different families of Pinus cembra and Picea abies	
Mosca, Elena	Genetic imprints and environment effects in a common garden of 500 Picea abies families from 160 sites throughout the Alps	
Nikichuk, Natalia	Protocol: fast and high-performance Eucalyptus DNA extraction in 96-well plate method.	
Nonic, Martina	Morphometric characteristics of buds of different European beech cultivars	
Novikova, Tatiana	Scots pine adaptation depending on the reaction norm of geographical populations on the conditions of the West Transbaikalie	
Novotná, Kateřina	Populus nigra L. breeding strategies in the Czech Republic	
Novotná, Kateřina	The potential of breeding for Phytophthora alni subsp. alni resistance in black alder	
Nurten Yer, Esra	Genome-wide analysis of heat shock protein 70 genes (Hsp70) in poplar	
O' Connor, Ellen	Tree improvement of birch and alder for Ireland based on progeny testing	
Oliveira, Michelle Silva	Evaluation of the internal morphology of Platypodium elegans Vog. (Fabaceae) seeds by the x-ray test	
Oribe, Yui chiro	Development of varieties of Japanese cedar, Cryptomeria japonica, superior to resistance against damage from snow pressure in Japan	
Özel, Halil Barıº	Forecast of Phenotypic Parameters for Growth Traits in a Clonal Seed Orchard of Scotch Pine (Pinus sylvestris L.) in the Erzurum Region in Turkey	
Paffetti, Donatella	Crossing Between Breeding popular cultivations and wild relatives in Mediterranean Environment	
Paradelo Gomes, Kever Bruno	Evaluation of the internal morphology of Terminalia argentea Mart. et Zucc. (Combretaceae) seeds by the X-ray test	

Persson, Torgny	OPSEL – A tool for optimizing expected gain from clonal seed orchards while constraining relatedness and diversity among parents.	
Pike, Carolyn	Early-season phenology impacts growth patterns in improved and wild sources of white spruce, Picea glauca (Moench) Voss	
Pike, Carolyn	Phenotypic and genetic correlations among tree volume, wood specific gravity and foliar traits in white spruce, Picea glauca (Moench) Voss and implications for selection	
Pike, Carrie	Growth potential of Scots pine (Pinus sylvestris) for reforestation in the lake states region of the USA	
Pokharel, Youba Raj	Tree improvement through breeding seed orchard; strategy and current situation in Nepal	
Przybylski, Pawel	The verification of the correctness of clone grafts planting in the seed orchard of Scots pine (Pinus sylvestris L.) in North-eastern Poland.	
Rainville, André	New transfer models to predict spruces plantation productivity in Québec (Canada) under the influence of climate change	
Rajora, Om	Genetic architecture of local adaptation in widely-distributed, long-lived eastern white pine (Pinus strobus)	
Ribeiro, Maria Margarida	Application of biophysical factors and molecular markers to explain spatial genetic structure in strawberry tree using GIS tools	
Rożkowski, Roman	Biomass productivity and distribution in half-sib progeny of pedunculate oak	
Ruotsalainen, Seppo	Altitudinal adaptation of Scots pine (Pinus sylvestris L.) in northern Finland	
Shi, Jisen	Characterization of gene-based SSR markers polymorphisms of the Chinese fir and transferability in comparing with six conifers and model plant	
Schueler, Silvio	Selecting Douglas fir provenances for future climates using universal response functions on basis of extensive provenance test series in Austria and Bavaria	
Smulders, M.J.M. (René)	Community genetics of poplar as a keystone species for insect and fungal species	
Sønstebø, Jørn Henrik	Double digested RAD tags in Norway spruce, a test of different restriction enzyme combinations	
Sønstebø, Jørn Henrik	Genetic structure and diversity in two Norway spruce seed orchards of different size	
Steffenrem, Arne	Tree height estimation in progeny trials using UAS photogrammetry	
Stejskal, Jan	Application of the Minimum Inbreeding (MI) seed orchard design in the Czech Republic	
Stener, Lars-Göran	Management of the gene resources of broad-leaved species in Sweden	
Suontama, Mari	Genetic parameter estimation for growth and form traits in Eucalyptus regnans progenies tested on two sites in New Zealand using spatial analysis	
Szyp-Borowska, Iwona	Micropropagation of selected clones of black locust (Robinia pseudoacacia L.)	
Szyp-Borowska, Iwona	Use of DNA markers in forest tree breeding programs	
Šijaèiæ-Nikoliæ, Mirjana	Selection of European White Elm plus-trees based on half-sib variability in nursery progeny test	
Takata, Katsuhiko	Evaluation of characteristics of the cultivars with resistance to snow-damage of Japanese cedar	
Tan, Biyue	Accuracy of genomic selection methods in Eucalyptus: a tentative exploration	
Tanabe, Jun	Relationship between microfibril angle of S2 layer and mechanical properties in 10 open-pollinated families of Picea jezoensis	
Thompson, Stacey Lee	Assessment of Genetic Diversity in Wild and Managed Stands of Norway Spruce	
Thompson, Stacey Lee	Chloroplast genome assembly from NGS data, with applications to forest trees	
Tier, Bruce	Opportunities for genomic information in forestry	
Torres-Dini, Diego	Genetic divergence in Pinus caribaea var. hondurensis progeny in Brazil	
Torres-Dini, Diego	Source to cold resistance in Eucalyptus breeding progrmams	

Ulaszewski, Bartosz	Variation of the timing of bud burst phenology in an old-growth forest stand composed of Fagus sylvatica and Quercus robur	
van den Berg, Gert	A comparison between two hybrid breeding strategies employed in the the production of Eucalyptus grandis x Eucalyptus urophylla in South Africa	
Vilanova, Antoni	Characterization of water-use efficiency in Spanish common walnut populations	
Vilanova, Antoni	Selection of Basic Materials in Catalonia: A small country with a wide programme	
Wachowiak, Witold	Divergence and selection on evolutionary and ecological timescales in closely related pine species from the Pinus mugo complex and P. sylvestris	
Wang, Zhang-rong	Review and Outlook on hybridization breeding of the Genus Liriodendron in China	
Warrier, Kannan C.S.	Genetic divergence in select clones of Casuarina equisetifolia	
Wehenkel, Christian	Proportion of vegetative reproduction in Mexican Populus tremuloides Michx. populations on the Sierra Madre Occidental	
Wennström, Ulfstand	Reduced background pollination using high tunnels	
Win, Thwe Thwe	Establishment of seed and planting zones of teak (Tectona grandis Linn) in Myanmar based on Bayesian genetic structuring with nuclear microsatellites	
Wojda, Tomasz	The distribution, variability and breeding programme of Robinia pseudoacacia (L.) in Poland	
Wu, Harry	Performance of seven tree breeding strategies under inbreeding depression	
Xu, Li-an	Evolutionary and functional analyses of LysM-contained genes in Populus revealed two potential chitin receptors	
Yakovlev, Igor	Temperature driven changes in transcriptomes and smallRNAomes during formation of an epigenetic memory in somatic embryos of Norway spruce	
Yanchuk, Alvin	Tree Breeding in B.C. in 2014 – new tools, variable targets, and uncertain times	
Zapata-Valenzuela, Jaime	Genomic Selection for Embryogenic Clones Deployment on the Arauco's Radiata pine Program in Chile	
Zheng, Renhua	Review on advanced-generation genetic improvement of Chinese fir in China	
Singh, SS	Effect of different doses of gamma radiations on the germination and seedling growth attributes of Albizia chinensis (obsbeck) Merr.	

NOTES

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