Phenotypic plasticity and genetic differentiation for morphological and functional leaf traits in nine metapopulations of black poplar (Populus nigra L.)

Justine Guet, Francesco Fabbrini, Maurizio Sabatti, Catherine Bastien, Franck F. Brignolas

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Stand of 10-year old hybrid poplar clone 53-242, a *Populus trichocarpa* (T) female crossed with a *Populus deltoides* (D) male; this hybrid clone was bred by Reini Stettler and Paul Heilman. The trees were planted in 1997 on 3x4 m spacing (approximately 825 trees per hectare) at the Huskcroft Farm near Creston, in southeastern British Columbia. The alluvial silty soils of the Kootenay River are deep with significant organic matter content. With a water table that never dropped below 1-1.5 m, this stand was never irrigated. Competing vegetation was controlled with tilling for four to five years. The trees were last measured at the end of the 14th growing season in 2010.

Photo by: Michael Carlson in the fall of 2006

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Informational Session

OPENING KEYNOTE - *Loren Rieseberg*

Plant domestication: Insights from evolutionary genetic studies of sunflower domestication and improvement

Department of Botany, University of British Columbia, Vancouver,
British Columbia V6T 1Z4, Canada

In his introduction to the Origin of Species, Darwin comments that 'a careful study of domesticated animals and of cultivated plants would offer the best chance of making out this obscure problem (the evolutionary process).' This prediction has been borne out, especially with the increased interest in recent years in the molecular genetic basis of phenotypic evolution. Investigations of the genetics of domestication traits typically use classic genetic approaches or scans of genomes at the population level to reveal gene variants that underlie acquisition of such traits.

Here I will illustrate the utility of both approaches when applied to the domestication of sunflower and discuss how results from sunflower compare to what we know about the genetics of domestication and improvement in other domesticated plants. I also will present a genome-wide analysis of the genomic ancestry of cultivated sunflower germplasm and show how different wild species have contributed importantly to the success of elite lines. I will end by discussing the prospects for rapid improvement of weakly domesticated crops and the potential advantages of re-domesticating some of our most widely cultivated crops.

CLOSING KEYNOTE - *Brian J. Stanton*

Plant domestication in a changing world: Poplar markets, plantations, and science

Biological Research Group, GreenWood Resources , Inc.,
Portland, Oregon 97201, USA

Poplar was among the earliest trees domesticated for wood-using industries in North America. The history of the way its markets and plantations advanced and the manner in which the science of genetics was practiced in domesticating plantation varieties for the industry is a fascinating story. Equally captivating are projections of where poplar markets and production strategies may be headed. But no matter where the future lies, the longstanding challenge of operating a plant domestication program with continuity through the cyclical downturns of the poplar-using industries will surely be encountered once more.

Three of the organizing principles of IPS-VI are: (1) Domestication is an essential activity and the sciences of genetics, genomics, and biotechnology must be effectively combined to ensure its success, (2) Future hybridization programs are linked to today’s efforts to conserve poplar genetic resources; and (3) Environmental certification is an indispensable component of an enduring poplar agricultural production system. The closing keynote will address these exigencies in proposing a domestication model that is: (1) Applied in nature and tailored to industrial requirements, (2) Responsive to societal priorities and environmental strictures, (3) Reliant on stable funding through private-public partnerships, and (4) International in species conservation. These features will be illustrated using the emergent renewable energy industry as a backdrop. The belief is that the IUFRO 2.08.04 working group through its IPS movement can be an effective leader in promoting sustainable domestication approaches as the poplar industry takes the next step in its exciting evolution.

SESSION I - GENETICS, BREEDING AND CONSERVATION OF POPLAR & WILLOW

Oral Presentations

S1 – O1

Key words:
hybrid fitness, gene flow, urban-forest interface, poplar plantations, forest certification

Impacts of human activities: the complex relationship between exotic and native poplar species

Nathalie Isabel[1], *Julie Godbout*[1], *Amanda Roe*[1]

[1]Natural Resources Canada, Canadian Forest Service, Laurentian Forestry Centre, Québec (Sainte-Foy), Canada G1V 4C7

Intentional or accidental introduction of exotic species into the wild raises concerns about their direct and indirect ecological impacts on native populations. For instance, interspecific hybridization and introgression of exotic species with native species might have long-term impacts on the genetic diversity of native populations leading to maladaptation of the species. However, interspecific hybridization is a natural evolutionary phenomenon allowing new genetic combinations to rapidly accumulate spurring the emergence of novel traits, a mechanism that has been long exploited in artificial settings.

North American poplars provide an excellent model system for studying interspecific hybridization and introgression. Native poplars form distinct natural hybrid zones and many exotic poplar varieties exist in close proximity to these native hybrid populations. This mosaic of pure poplars, native hybrids, and exotic hybrids provides opportunities for exotic genetic material to escape into native gene pools. We present results on interspecific gene flow and hybrid fitness in both natural and artificial hybrid zones in order to identify factors modulating the hybridization process and develop a generalized model of exotic gene introgression. Since it is impossible to eliminate exotics from the landscape it is essential that we consider a conciliatory approach in order to managing the exotic gene flow by minimizing the risks and accepting potential benefits of this novel genetic material.

S1 – O2

Key words:
aspen, triploid, Intermountain West, clonality, seedling establishment

New perspectives on aspen (*Populus tremuloides*) in the western US: phylogeography, regeneration ecology, and triploidy

<u>Karen E. Mock</u>[1], Paul G. Wolf[2], John D. Shaw[3], James N. Long[1]

[1]Utah State University Ecology Center and Department of Wildland Resources, 5230 Old Main Hill, Logan, Utah 84322 [2]Utah State University Ecology Center and Department of Biology, 5305 Old Main Hill, Logan, Utah, 84322 [3]US Forest Service, Rocky Mountain Research Station, 507 25th St., Ogden, Utah, USA 84401

New genetic information on aspen in the western US is causing managers and silviculturists to revisit strategies for sustaining aspen in these landscapes. First, aspen in the southwest portion of the species range (i.e. in the western US) are genetically distinct from aspen in the northern portion of its range, likely due to the dynamics of post-glacial recolonization. Second, southwestern aspen populations are more isolated and more genetically depauperate than northern aspen, likely due to the natural fragmentation of western landscapes. Third, a variety of indicators suggest that seed reproduction in southwestern aspen is not negligible and may represent an important source of adaptive potential. Finally, very high rates of triploidy have been described in southwestern aspen populations, and most of the large clones appear to be triploid. Triploids are expected to have low fertility and distinct physiology compared to diploids, but the particular advantages and disadvantages of triploidy in aspen have yet to be described. We will discuss the possible evolutionary, ecological, and management implications of these findings.

Funding support: The authors thank the United States Department of Agriculture (USDA) Forest Inventory and Analysis Program, NASA Biodiversity Program, USDA National Research Initiative, USDA Natural Resource Conservation Service, Utah Agricultural Experiment Station, Utah State University (USU) Cedar Mountain Initiative, and USU ADVANCE program, and for financial support.

S1 – O3

Key words: poplar breeding, yield improvement, variance component analysis

Two decades of poplar breeding and field testing in Minnesota, USA: results and implications to future genetic improvement

<u>Bill Berguson</u>, Bernie McMahon and Don Riemenschneider

University of Minnesota-Duluth, Natural Resources Research Institute, Duluth, Minnesota, USA 55811

Poplar breeding and field testing has been underway at the University of Minnesota-Duluth, Natural Resources Research Institute since 1996. The goal of this work is to increase genetic diversity and improve yield and disease resistance among a variety of poplar species. Breeding includes intraspecific crosses of Populus deltoides as well as interspecific crosses among P. deltoides, P. nigra, P. maximowiczii and P. trichocarpa. As part of this effort, genotypes produced in our breeding program were established and evaluated in a series of large field tests over a five to ten year period. These trials include hundreds of genotypes contained within an experimental framework designed specifically to estimate additive and non-additive components of genetic effects among multiple families and clones-within-families. The results of these analyses on multiple sites will be presented which provide insight on the optimal design of poplar breeding programs and the magnitude and rate of yield improvement to be expected in similar programs worldwide. Results of pure-species clones and commercial standards relative to new genotypes produced in the Minnesota breeding program will be presented. In addition to relative performance data, a discussion will be included which compares expected growth and yield of current commercial poplar clones to that of new genotypes.

Funding support: US Department of Energy, Sun Grant Regional Feedstock Development Partnership

S1 – O4

Key words:
clonal-selection,
wood-density,
mechanical-properties,
growth,
statistical-genetics

Genetic variation in wood traits and covariation with growth in a clonal population of selected poplar hybrids in Chile

Francisco Zamudio

Poplar Technology Center. Universidad de Talca, Chile

Between 1999 and 2002, the Poplar Technology Center (PTC) introduced in Chile more than 2000 hybrids of thirteen different crosses among five species, and generated by the former Poplar Molecular Genetics Cooperative (University of Washington, USA). At the end of 2005, more than 100 hybrids were selected among the best performers in nursery trials to begin a specific selection program of poplar hybrids able to produce high wood quality products. Several candidacy trials were established in 2006. The field experimental design was a randomized block design, with two cuttings per hybrid in each one of ten blocks. At the end of 2008, one cutting per hybrid and block was fell from two trials and wood density and mechanical properties were measured. Statistical and genetic analyses were performed to assess the genetic variation and relationship of growth and wood properties within crosses and trials. Crosses included in the analyses are: *Populus deltoides x P. nigra*, *P. trichocarpa x P. deltoides*, (*P. trichocarpa x P. deltoides) x P. deltoides*, and (*P. trichocarpa x P. deltoides) x* (*P. trichocarpa x P. deltoides)*. Preliminary results involve the study of juvenile wood. Our research is confronting data correlated at multiple levels and we cannot use the traditional approach for measuring statistical differences among clones or interactions in the field experimental design. Therefore, the use of the Linear Mixed Model theory as a robust methodology to work with repeated measures (over time, space, or both) obtained from our clonal trials is discussed. Here, a summary of main results is presented. Significant genetic differences among crosses were observed in: a) the genetic control of wood density, MoE, and MoR; b) the genetic correlation between growth and wood properties; and c) the effects of genotype-by-micro-environmental interaction affecting juvenile wood properties.

Funding support: FONDEF-CONICYT grants program, Universidad de Talca, Chile.

S1 – O5

Key words: breeding, phenomic selection, genomic selection, Near Infrared Spectroscopy, black poplar

The concept of phenomic selection: using Near InfraRed Spectroscopy (NIRS) to predict quantitative phenotypes.

Vincent Segura[1], Jean-Paul Charpentier[1,2], Kévin Ader[1,2], Redouane El Malki[1], Justine Guet[1,3], Véronique Jorge[1], Patrick Poursat[4], Jean-Charles Bastien[1] and Catherine Bastien[1]

[1]INRA, UR0588 Amélioration Génétique et Physiologie Forestières, Orléans, France [2]INRA, Plateau technique Génobois, Orléans, France [3]INRA, USC 1328 Laboratoire de Biologie des Ligneux et des Grandes Cultures, Orléans, France [4] INRA, UE0995 Génétique et Biomasse Forestières, Orléans, France

Evaluating as precisely as possible the genetic variability of quantitative traits is crucial in the context of breeding. It requires accurate, fast and cost-effective evaluation tools. We hypothesized that near infrared spectroscopy (NIRS), which is traditionally used to indirectly evaluate the physical and chemical properties of biological samples, could capture genetic variation and thus be used to predict the heritable variation of quantitative traits. To test this hypothesis, we collected NIRS spectra on wood samples harvested in a cloned association population of *Populus nigra*. After verifying that NIRS could capture a significant amount of genetic variation, we used this information to predict various phenotypes of interest related to growth, branching, phenology and rust resistance. The prediction accuracies estimated within a cross-validation scheme varied greatly depending on the phenotype, reaching a maximum of 0.74 for the circumference of the stem whose wood was collected for NIRS analyses. More surprisingly, accuracies around 0.7 were also found for traits related to bud flush despite a low correlation between bud flush and stem circumference (|rho] ~ 0.25-0.3). In addition, the accuracies of NIRS-based predictions were in the range of those obtained in the same dataset through genomic prediction with *ca.* 8,000 SNPs, demonstrating the usefulness of NIRS for predicting quantitative phenotypes. Moreover, phenomic and genomic selection accuracies were correlated together and to both the broad- and narrow-sense heritabilities of the corresponding traits, validating our initial hypothesis that NIRS is able to capture a significant amount of genetic information which can in turn indirectly be used to predict the heritable variation of quantitative traits.

S1 – O6

Key words: *Populus trichocarpa*, growth, water use efficiency, wood chemical composition, metabolome

Analysis of variation in growth, water use efficiency, chemical properties and metabolome of wood, in *Populus trichocarpa* provenances

Fernando Guerra[1]*, Oliver Fiehn*[3]*, James Richards*[4]*, Brian Stanton*[5]*, Robert Sykes*[6]*, Mark Davis*[6] *and David Neale*[1,2]

[1]Department of Plant Sciences, University of California at Davis, Davis, CA 95616, USA; [2]Bioenergy Research Center, University of California at Davis, Davis, CA 95616, USA; [3]Department of Molecular and Cellular Biology & Genome Center, University of California, Davis, CA 95616; [4]Department of Land, Air and Water Resources, University of California, Davis, CA 95616; [5]Genetic Resources Conservation Program, Greenwood Resources, Portland, OR 97201, USA; [6]National Renewable Energy Laboratory, Golden, CO,USA.

Populus trichocarpa is an important candidate species for the development of biofuel plantations. As a first step toward developing marker breeding technologies for cellulosic ethanol production, we have estimated genetic variation in a suite of quantitative traits. In this study we analyzed 460 *P. trichocarpa* clones, representing 101 provenances collected from Oregon and Washington, for a series of traits, including growth, water use efficiency (WUE), and chemical properties and metabolome of wood. Clones were established in a field trial located in Davis, California in 2009, and sampled in 2011. WUE was estimated by carbon isotope discrimination, whereas the characterization of the wood chemical composition and metabolome was performed by pyrolysis MBMS and gas chromatography-TOF-MS, respectively. A significant clonal effect was observed for traits related to growth (height, diameter), WUE ($\delta^{13}C$, $\delta^{15}N$, C and N contents in leaves) and wood chemical composition (five and six carbon sugars, lignin and the syringyl:guaiacyl ratio). In general, moderate correlations and heritabilities were detected for most traits. In addition, analysis of wood metabolome allowed identifying and quantifying a set of 632 metabolites, representing pathways related to the sugar and amino acid metabolism, and the citrate cycle, among others. Significant clonal variation was also observed for these traits. The obtained results contribute to a comprehensive phenotypic characterization of *P. trichocarpa*, useful for further applications, including genomic-based breeding.

Funding support: Advanced Hardwood Biofuels Northwest Project, supported by Agriculture and Food Research Initiative Competitive Grant no. 2011-68005-30407 from the USDA National Institute of Food and Agriculture.

S1 – O7

Key words:
SRC,
bioenergy,
selection, wood
properties

Wood quality parameters to assess potential of poplar and willow clones for short rotation coppice bioenergy plantations.

Joris Van Acker[1], Jan Van den Bulcke[1], Nele Defoirdt[1], Marijke Steenackers[2], Boudewijn Michiels[2]

[1]Laboratory of Wood Technology (Woodlab), Ghent University (UGent), Coupure Links 653, 9000 Ghent, Belgium
[2]Research Institute for Nature and Forest (INBO), Kliniekstraat 25, 1070 Brussels, Belgium

Since several decades there has been a close cooperation between the team involved in selection and breeding of poplars and willows in Geraardsbergen, Belgium and the laboratory of wood technology (Woodlab) at the Ghent University (UGent). Recent emphasis on using poplar and willow clones more for bioenergy has shifted key wood properties used for selection. Also the methodology has evolved and now parameters like green moisture content, macrochemical distribution, bark thickness all are becoming more relevant when considering short rotation coppice rather than timber production plantation trees. Starting from cross section discs a range of parameters and properties is assessed both on an overall integrated and local spotsized basis. Extra tools used to obtain data that not only relate to average values but also gives insight in spatial distribution are a flatbed scanner, a NIRS camera, a coupled DSC-TGA equipment and a standard bomb calorimeter. Based on some case study data the relevance of each of the wood quality parameters and tools used are discussed.

S1 – O8

Key words:
poplar hybridisation,
cross breeding,
gene conservation,
Populus nigra

Hybridisation of poplar holds much potential if conservation of genetic resources is integrated in improvement work

Georg von Wuehlisch

Thuenen Institute for Forest Genetics, Sieker Landstr. 2, 22927
Grosshansdorf, Germany

The immense success of poplar cultivation is due to heterosis effects of the hybridisation of differing species and sub-species, which naturally would not come into contact. However, the choice of germplasm to be exchanged among breeders has mostly not been systematic due to insufficient knowledge about variation patterns because of lacking field trials. Many of the exceptional cultivars are the fortunate result of the availability of exotic and flowering poplars for cross breeding, e. g. in botanical gardens, e. g. NM6. Examples of such successful crosses are given which may show the way for possible future breeding programmes and markers may give information about the parentage of such cultivars. Altogether there is uncertainty in the predictability of the success of improvement programmes and they may even prove inefficient in relation to their costs. A more systematic approach and utilization of available knowledge for the choice of germplasm adapted to specific regions of a species may raise the breeding success substantially. Gene conservation programmes are crucial to ensure survival of necessary germplasm for future breeding. At the same time gene conservation programmes can serve to provide knowledge to support the choice of appropriate crossing partners. As an example, elements of the ongoing gene conservation programme for endangered natural *Populus nigra* is presented. Tree improvement work should integrate the conservation of genetic resources of target species already at an early stage.

SESSION I - GENETICS, BREEDING AND CONSERVATION OF POPLAR & WILLOW

Poster Presentations

S1 - P1

Key words:
poplar,
tetraploid,
drought
tolerance

Breeding of tetraploid aspen for dry land sites

Anne Hennig, Silvia Köhler, Jörg Kleinschmit, Alwin Janssen

Northwest German Forest Research Institute (NW-FVA), Department Forest Genetic Resources, Prof. Oelkers-Str. 6, D-34346 Hann. Muenden, Germany

There is still a high demand for breeding new poplar varieties which are well-adapted to short rotation coppice (SRC). As crossings between some species are difficult, we used the technique of somatic hybridization to combine favorable properties of different poplar species. Besides the propagation by cuttings also drought tolerance plays a key role in SRC. Plants are threatened by dry periods after planting and as SRC are considered to be planted on marginal sites with low water and mineral supply, clones with adequate biomass production under dry conditions are required. Therefore, we examined drought tolerance of tetraploid plant material of protoplast fusion experiments between hybrid aspen (*Populus tremula* x *P. tremuloides*) and black poplar (*P. nigra*) in comparison to their corresponding diploid clone in a greenhouse experiment. Physiological parameters as stomatal conductance, relative water content, the ability to recover after drying, height and leaf area index provides a first insight into the potential of biomass production and drought tolerance of tetraploid poplar. According to current results tetraploid plant material shows higher drought tolerance than their diploid clone. Thus, they might be interesting candidates for dry land sites and for further breeding as diploid pollen or diploid ovum donor.

Funding support: Federal Ministry of Food and Agriculture (BMEL, FKZ: 22005010)

S1 – P2

Key words:
poplar, SRC,
breeding,
FastWOOD

Evaluation, breeding and genetic characterization of black and balsam poplars

Meike Borschel, Christina Fey- Wagner, Steffen Fehrenz, Alwin Janssen

Northwest German Forest Research Institute (NW-FVA), Department Forest Genetic Resources, Prof. Oelkers-Str. 6, D-34346 Hann. Muenden, Germany

The cultivation of fast-growing poplars has ecological and economical advantages for production of energy wood. Short rotation coppices (SRC) are regarded as one of the most promising methods for the production of utilizable wood and energy biomass. Due to the increase in importance of biomass produced in SRC and the expansion of opportunities for wood-energy use, the need for improved poplar clones rises as well as the demand for a broad assortment of specially adapted varieties. One aim of the joint research project “FastWOOD” is breeding of suitable black and balsam poplars for biomass production in SRC. Inter- and intraspecific crosses are carried out to create improved clones. The progeny is selected in terms of leaf rust tolerance and high biomass production. An analysis for identification of selected clones is made with microsatellite markers. These investigations lead to the recommendations of SRC-operators and land users as well as to improvement economic cultivation of fast growing tree species for biomass production in SRC. An overview over the breeding results achieved in the joint research project “FastWOOD” is given.

Funding support: Federal Ministry of Food and Agriculture (BMEL, FKZ: 22002511)

S1 – P3

Key words:
poplars, SNPs,
clv1, tb1, phyB2

Identification of SNP’s associated with phenotypic characters for poplar breeding

Karl Gebhardt, Marion Hoffmann, Alwin Janssen

Northwest German Forest Research Institute (NW-FVA), Department of Forest Genetic Resources, Prof. Oelkers-Str. 6, D-34346 Hann. Muenden, Germany

Since the genome of a black cottonwood was sequenced in 2004 and became available in public domains numerous attempts have been made in order to assign elements of function to the genes of poplar species. Here the result of a candidate gene approch is reported which could be helpful in breeding of poplars. Evaluations of the growth characters of a set of poplar cultivars including members of the sections Aigeiros and Tacamahaca as well as intra- and intersectional hybrids were made in experimental fields situated nearby the breeding station in Hann.Muenden (Germany) and documented by Fröhlich & Grosscurth (1973) for the first time. The SNP character of each cultivar was determined by resequencing of five candidate genes [cinnamyl alcohol dehydrogenase-like (cad-like, gibberellic acid 20-oxidase (GA20ox), C-repeat binding factor 1 (cbf1), teosinte branched-like1 (tb1), phytochrome B2 (phyB2) and clavata1 (clv1)]. The data from Sanger sequencing (both directions) of the PCR products allowed the creation of a consensus sequence using the software CodonCodeAligner 3.7.1. Heterozygous alleles could be simply recognized as single nucleotide overlays. Using the internet-based program SNPStats (Sole et al., 2006) and the R-Package: SNPassoc [R, version 2.14.1., (R development core team, 2011)] significant associations of SNPs with phenotypic characters like height and diameter growth, crown shape and density of branches, became obvious. Since the statistic model used compares genotypes heterozygous and homozygous for the variant allele to the genotypes homozygous for the most frequent allele different modes of inheritance are considered for each SNP. The SNP markers described will allow the genotyping of future breeding populations. With the use of genotyping platforms nowadays available the genotyping for breeding purposes will become more attractive and cost efficient.

Funding support: German Federal Minister of Food and Agriculture (BMEL, FKZ: 22013709 PT-FNR)

S1 – P4

Key words:
poplar,
breeding, SRC,
projects

Breeding of black and balsam poplars at the Northwest German Forest Research Institute – Status report

Alwin Janssen, Christina Fey-Wagner, Karl Gebhardt, Anne Hennig und Martin Hofmann

Northwest German Forest Research Institute, Department of Forest Genetic Resources, Prof.-Oelkers-Str. 6, D-34346 Hann. Muenden, Germany

Poplar breeding is carried out at the Northwest German Forest Research Institute (NW-FVA) in Hann. Muenden since 1955. The overall game is to breed new Poplar clones for use in forests and in short rotation coppices (SRC). At this time 4 projects are executed:

Breeding of fast-growing tree species for the production of renewable resources in short rotation coppices (FastWOOD): Focus of the subproject managed by NW-FVA is on evaluation, breeding, genetic characterization, and clone tests on yield and resistance with black and balsam poplars as well as willows. First results from the trial series established 2010 and 2011 show that newly bred clones could double yield of recently used clones.

Clone-site-relations of Poplars and Willows on agricultural land in short rotation coppices (ProLoc): The Joint Research Project is making the so far unique attempt to thoroughly assess the site-yield-interaction on a supraregional scale for environmental conditions typically found in Germany. At 43 trial sites different management regimes are tested and evaluated in order to implement a yield prediction model.

Breeding of novel Poplars as energy crops for Germany (ZÜEND):

This project focuses on combining poplar species, which are difficult to hybridize under natural conditions, with SRC relevant traits. Therefore, we use the technique of somatic hybridization.

Single Nucleotide Polymorphism (SNP) diagnosis of relevant breeding characters of Salicaceae: Using SNP markers, Populus genotypes can be characterized and optimized with respect to their growth, local adaptation and the properties of the cell wall of the timber by taking advantage of natural variation in the genes that encode these features (SMART-Breeding).

Funding support: German Agency of Renewable Resources (FNR) by order of the Federal Ministry for Food and Agriculture (BMEL)

S1 – P5

Key words:
poplar breeding,
Populus nigra,
parent
population
improvement

A *Populus* breeding and clone development program in Minnesota: Status update on a *P. nigra* collection and parent population improvement efforts

<u>Bernie McMahon</u>, Bill Berguson, Dan Buchman, Don Riemenschneider

University of Minnesota - Duluth, Natural Resources Research Institute,
Duluth, Minnesota, USA 55811

Since 1996, an applied *Populus* breeding program has been operating in Minnesota at UMD's Natural Resources Research Institute. In an effort to produce a broadly adapted and genetically diverse base breeding population, existing and new plant material collections were utilized or assembled. Building upon an existing *Populus deltoides* collection at the University of Minnesota, additional species (*P. nigra; P. trichocarpa and P. maximowiczii*) were sought for inter-specific hybrid combinations. Breeding efforts evolved and focused on generating more first-generation (F1) crossing combinations among eastern cottonwood and black poplar parent selections. The focused direction on DxN breeding was based on results from large-scale nursery and replicated field trials established over the past twenty years.

In 2005, a large exchange of open-pollinated seed of *P. nigra* was coordinated with researchers working within the EUFORGEN *Populus nigra* Network in Europe. Seed source progeny trials were established initially in two Minnesota nurseries. Observations and data for relative adaptability, initial growth and incidence of disease were gathered. Replicated clone trials and a long-term breeding archive were established in 2009. Parent population improvement efforts utilizing *P. nigra* in Minnesota will be outlined and presented. Simultaneous parent population improvement efforts among *P. deltoides* and *P. nigra* collections in Minnesota will provide breeding materials for future intra- and inter-specific improvement efforts moving forward for existing wood fiber demands and emerging biomass feedstock markets.

Funding support: US Department of Energy, Sun Grant Regional Feedstock Development Partnership

S1 – P6

Key words: poplar breeding, hybridization, clonal selection, *P. deltoides*

Breeding of *Populus deltoides* in Argentina: selection in populations derived controlled crossing

Silvia Cortizo[1,2], *María Silvana Monteverde*[3,4]

[1]E.A.S. Delta del Paraná, National Institute of Agricultural Technology (INTA) [2]Faculty of Agronomy, University of Buenos Aires, [3]Domestication and improvement of native and introduced species for high value applications Program. (PROMEF). BIRF 7520-AR. MAGyP.
[4]Faculty of Agricultural Sciences, University of Concepción del Uruguay

In Argentina, there are 40,500 ha of poplar plantations and 23,500 ha under agroforestry systems, mainly distributed in Delta del Paraná, Mendoza and Río Negro valley. From an economical point of view they provide an average of about 354,000 tons/year of round wood. Afforestation programs need a continuous production of high quality reproductive material. As Argentina does not have any native poplar population, commercial plantations began in the late nineteenth century using clones introduced mainly from the U.S.A. and Italy. In the sixties, the National Institute of Agricultural Technology began a poplar breeding program with the objective of selecting superior genotypes based on the overall quality of the tree to improve wood industrial performance. Seeds belonging to *P. deltoides* trees, that had shown good behavior in areas ecologically similar to Delta del Paraná within the natural range of this specie, were also introduced. This strategy allowed the selection of genotypes adapted to the needs of growers and industries such as 'Carabelas INTA' and others in the diffusion process such as "Ñacurutú INTA". Recently, a program of controlled crosses using well-adapted, good performance and healthy genotypes was initiated. Between 2006 and 2013, 11,245 new genotypes were obtained and installed in progeny banks in the Paraná Delta Experimental Station. So far, 280 individuals were selected focusing on rooting ability, *Melampsora medusae* and *Septoria musiva* tolerance, growth rate, stem form and cloned. In order to get an accurate estimation of genetic values, the evaluation of performances was a combination of nursery and clonal tests planted at high density. Thirty four experimental clones have exceeded the thresholds considered in our protocol and were established in the network of comparative trials planted in 2013 in five different sites of Delta area. The results of these studies will be presented, as well as an overview of the present status of the breeding program.

Funding support: INTA, MAGyP, Carabelas Group.

S1 – P7

Molecular markers benefit reliable poplar breeding

Key words:
poplar species,
SNP marker,
genealogy

Hilke Schröder, Matthias Fladung

Thünen Institute of Forest Genetics, 22927 Grosshansdorf, Germany

Hybrids between various *Populus* species belonging to the same or different sections are commonly used in short rotation plantations for biomass production. Many years of breeding work including repetitive crosses and back-crosses has caused, however, that for many commercially available hybrid clones the background information of the *Populus* species originally used is not available anymore. Hence, a high number of interspecies-hybrids has artificially been produced in huge breeding programs during the last 100 years, besides naturally occurring hybrids possibly also used for breeding purposes. Therefore, determination of the single poplar species used either as maternal or paternal crossing partner for the production of "multi-species-hybrids" is often difficult, and represents a great challenge for the use of molecular markers for species identification and to unravel hybrid genealogy.

Therefore, a part of the project "FastWOOD" devotes on the development of molecular markers for unambiguous identification and genetic characterization of commonly used poplar species and hybrids (*Populus trichocarpa, P. alba, P. tremula, P. tremuloides, P. nigra, P. deltoides, P. maximowiczii)*. For this purpose, we developed species-specific chloroplast markers for the differentiation of the above mentioned at least seven poplar species to determine maternal contribution. We found much more SNPs within intergenic spacers than in the coding regions often used for barcoding approaches. To identify the parental contribution of the hybrids, we developed SNP and InDel markers using the nuclear genome. Especially within the genes gibberellin 20 oxidase, TB1, and KNOX, we found a lot of species-specific SNPs. A combination of cp and nuclear SNP markers gives us the possibility (i) to identify the direction of a crossing and (ii) to follow the genealogy of poplar hybrids.

Funding support: Federal Ministry of Food, Agriculture and Consumer protection via the Agency for Renewable Resources (FNR)

S1 – P8

Key words:
sex determination, *Populus*, aspen, next generation sequencing

Next generation sequencing of phenotypic bulks for the development of a genomic marker for sex in aspen (*P. tremula* L. and *P. tremuloides* Michx.)

Birgit Kersten, Birte Pakull*, Matthias Fladung*

Thünen Institute of Forest Genetics, Sieker Landstrasse 2, 22927 Großhansdorf, Germany *equal contribution

Most species of the genus *Populus* are dioecious, and sex determination is thought to be under genetic control. However, different *Populus* species seem to vary in the position of the sex-linked region on chromosome 19 and the apparent heterogametic sex. In contrast to *P. trichocarpa*, where sex determination was reported to be located in a peritelomeric position with female heterogamety (WZ-system), the sex determining region in aspen (*P. tremuloides*) was shown to be located on a central position with male heterogamety (XY-system). The boundaries of the sex-linked region in *P. tremuloides* Turesson 141 were recently defined by genetic mapping. However, as a generally applicable diagnostic marker for aspen sex is not available so far, the sex of an aspen individual can only be determined when the respective tree starts to flower; usually at the age of 6 to 10 years.

Aiming at the development of a diagnostic genomic marker for sex in aspen, we applied Next Generation Sequencing of phenotypic bulks. Four genomic DNA pools were generated: male *P. tremula,* male *P. tremuloides,* female *P. tremula* and female *P. tremuloides* pools. Single and paired-end reads of 100 bp were generated with Illumina HiSeq 2000 by GATC (Konstanz, Germany). The sequence region flanked by the borders of the sex-linked region in *P. tremuloides* was extracted from the genomic sequence of *P. trichocarpa* (v3.0) at Phytozome and used as a reference for subsequent mappings of the reads of each pool using CLC Genomics workbench (CLC bio, Aarhus, Denmark). The bioinformatic analysis aiming to identify potential candidate genes for sex determination and genomic markers to differentiate between both sexes early in plant development is under way.

S1 – P9

Key words:
somatic hybridization, chromosome-specific SNP marker, somatic fusion hybrids

Development of chromosome-specific SNP-markers in different poplar genotypes to detect single chromosomes in aneuploid *Populus* fusion lines

Matthias Fladung, Caren Heitmann, Katrin Groppe

Thünen-Institute of Forest Genetics, 22927 Grosshansdorf, Germany
Email:

The development of SNP-markers in general in *Populus* and, in particular chromosome-specific SNP-markers, is hindered by the possible presence of paralogous genes and intergenic regions of similar evolutionary age due to several genome-wide duplications occurred during evolution of this genus, with a most recent one about 8 to 13 Million years ago. This recent duplication affected nearly 92% of the *Populus* genome including about 8000 pairs of genes and intergenic regions mostly duplicated as blocks and formed chimeric chromosomes.

The application of chromosome-specific SNP-marker is manifold. Several poplar species within a section but also belonging to different ones are cross-compatible, hence interspecies- and intersectional hybrids occur naturally but also artificially produced. Having a range of species- and chromosome-specific SNP-markers in hand, hybrids can be analyzed in respect to their hybridization genealogy, and possibly, also crossing-over events can be detected physically.

Further, there is a high demand for breeding of poplar varieties that are adapted to short rotation coppice (SRC) cultivation. The technology of somatic hybridization of protoplasts (Phytowelt GreenTechnologies GmbH, Germany) is used to increase the genetic diversity through new combinations of poplar lines from the various *Populus* sections. Somatic hybridization allows the combination of chromosomes from difficult or not cross-compatible *Populus* species. To analyze the obtained tetraploid somatic fusion hybrids, clone-specific microsatellite- or SNP-markers are needed.

Besides tetraploid fusion hybrids, also the occurrence of fusion hybrids identified as aneuploids or with aberrant chromosome sets is well known. To analyze the aneuploid somatic hybrids lines, chromosome-specific SNP-markers have to be developed to assign each single chromosome to one of the two fusion partners. Further, chromosome-specific SNP-markers can also be applied to detect possible chromosome breakages.

Funding support: Federal Ministry of Food, Agriculture and Consumer Protection (BMELV) on behalf of the Agency for Renewable Resources (FNR)

S1 – P10

Key words:
early flowering,
heat treatment,
gene stacking

Strategies for improvement of flower development in transgenic early flowering poplar (*Populus tremula* L.)

Hans Hoenicka, Denise Lehnhardt, Doris Ebbinghaus, Matthias Fladung

Thünen-Institute of Forest Genetics, Sieker Landstr. 2, D-22927
Grosshansdorf, Germany

Tree species produce flowers only after many years of non-reproductive development. This fact has been a severe impediment for forest tree breeding. Induction of early flowering using genetic transformation with different gene constructs, e.g. HSP::*AtFT*, has been successful in poplars. However, “early flowering” poplar models still show drawbacks.

First crossings with transgenic early flowering poplar were successfully carried out in our lab with pollen from HSP::*AtFT* poplar in 2013. Modifications on the heat treatment, used for flowering induction, improved significantly performance in HSP::*AtFT* poplar, and fertile flowers were obtained. This approach allowed shortening the reproductive phase from more than ten to less than three years. However, the HSP::*AtFT*-based early flowering system for poplar still requires improvement. The number of fertile flowers is still low and anthers show a disturbed dehiscence.

We carried out gene stacking approaches, with the aim of reaching improved flower fertility. Gene stacking with relevant “flowering genes” was performed with HSP::*AtFT* poplar. Gene constructs 35S::*SOC1*, HSP::*AtLFY,* HSP::*AtAP1,* HSP::*PtAG1* and HSP::*PTD* were used for genetic transformation. Transgenic lines showed some changes in flower development. Gene stacking with 35S::*AtSOC1* promoted faster flower development under *in vitro* conditions. HSP::*AtLFY* promoted sex change. However, no significant improvement on flower fertility was observed through gene stacking in early flowering poplar so far.

Funding support: DFG (German Research foundation), BLE (Federal Office for Agriculture and Food)

S1 – P11

Key words:
mitochondrion,
somatic
hybridization,
SNP marker

Development of mitochondrial SNP-markers in different *Populus* species

Mina Voss, Birgit Kersten, Matthias Fladung

Thünen-Institute of Forest Genetics, D-22927 Grosshansdorf, Germany

Through somatic hybridization of protoplasts (Phytowelt GreenTechnologies GmbH, Germany), nuclei and cytoplasm (mitochondria and chloroplasts) of two not cross-compatible *Populus* species can be combined in one protoplast. So far, the analysis of hundreds of tetraploid fusion lines by using chloroplast SNP markers revealed that through the regeneration process, the chloroplasts of only one species remain along with the related nuclei. To further deepen our understanding on the combination of the cytoplasms of two different *Populus* species, the destiny of the mitochondria during the regeneration process will be analyzed. Thus, the goal was to develop species-specific SNP-markers for the poplar mitochondrial genome.

As mitochondrial DNA is known to evolve slowly in sequence, we decided to develop SNP markers based on mitochondrial gene sequences that include or border introns and/or intergenic spacers. First, mitochondrial sequences were selected by mapping all *Populus trichocarpa* scaffolds (Phytozome version 1.0) to mitochondrial genome sequences of other species available at NCBI. Next, *P. trichocarpa* transcript sequences, as well as ESTs were mapped to the selected mitochondrial genomic scaffold sequences. Transcript mappings with gaps point to transcripts expressed by intron-including genes. Introns in the RPS3 gene and in other genes as well as intergenic spacer on scaffolds 1007, 438, 846 and 2812 were selected. For intron amplification, PCR-primers were placed in the exon regions flanking the intron of interest. Using the designed primer pairs, the introns of interest were amplified in 14 different *Populus* species and sequenced. The initial screening of the introns resulted in the identification of a SNP in the RPS3 intron that allows the differentiation of *Populus tremula* from eight other species. The analysis of other sequences is in progress.

Funding support: Federal Ministry of Food, Agriculture and Consumer Protection (BMELV) on behalf of the Agency for Renewable Resources (FNR)

S1 – P12

Key words:
biomass,
candidate gene,
transformation

Development and use of novel gene technologies to increase biomass yield in the woody perennial *Populus* spec.

Tobias Brügmann, Matthias Fladung

Thünen Institute of Forest Genetics, D-22927 Grosshansdorf, Germany

Trees with increased biomass are of high economic interest serving as feed material for the direct retrieval of energy or as a source of biopolymers in the pulping industry. The main objective is the generation of qualitatively optimized poplar trees (*Populus sp*.) with increased wood yield, modified lignin content or optimized plant architecture. With *Arabidopsis* mutants, it was shown that the flowering genes *SOC1* (*SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1*) and *FUL* (*FRUITFULL*) have an impact on wood formation. Beside *SOC1* and *FUL*, five other genes expressed in developing xylem and catkins or developing xylem and roots but with so far widely unknown function were chosen.

Two different clones were used for *Agrobacterium*-mediated transformation: the fast growing *P.* × *canescens* INRA 717-1B4 and hybrid aspen Esch5. Until February 2014, in total 37 transformations were accomplished yielding in more than 100 different independent transgenic lines for both overexpression and down-regulation. Verification of resistance gene(s) and gene(s) of interest in the transgene lines is accomplished. Furthermore, Southern blots were executed for detection of copy numbers. Single copy lines will be used for subsequent expression analyses. Selected two, three and four copy lines were observed for possible quantitative effects.

Since genetic analyses are accomplished, currently, the transgenic poplar plants are being analysed for both their transgene expression and wood property characteristics. Beside *in vitro* cultivation, the *in vitro* plants are being transferred to soil and cultivated under greenhouse conditions for phenotype analyses.

A notable phenotype is shown by two double transgene lines with overexpression of both *SOC1* and *FUL*. These plants lost their tree-like growth and have denticulate leaves, fragile roots as well as weak wood formation.

Funding support: Federal Ministry of Education and Research (BMBF) on behalf of the Project Management Jülich (PTJ)

S1 – P13

Key words:
poplar, genetic mapping, RAPiD-seq, QTL analysis

A dense genetic linkage map of poplar based on “genotyping by sequencing” for QTL analysis of pest resistance.

Giorgia Carletti[1], *Giampiero Valè.*[2], *Gianni Allegro*[1], *Lorenzo Vietto*[1], *Luigi Cattivelli*[3] *Giuseppe Nervo*[1]

[1]Consiglio per la Ricerca e Sperimentazione in Agricoltura, Research Unit for Intensive Wood Production (CRA-PLF), Strada Frassineto 35, I-15033 Casale Monferrato (AL) [2]Consiglio per la Ricerca e Sperimentazione in Agricoltura, Rice Research Unit (CRA-RIS), S.S. 11 to Torino, Km 2,5, 13100 Vercelli [3]Consiglio per la Ricerca e Sperimentazione in Agricoltura, Genomics Research Center (CRA – GPG), Via S. Protaso 302, I-29017 Fiorenzuola d'Arda (PC), Italy

Poplar is a tree of economic importance in the temperate regions of the world where represents the major source of domestic round-wood and also have significant potential for paper and biomass production. Interspecific *P. x canadensis* (*P.deltoides x P.nigra*) clones at present represent the bulk of European intensive poplar cultivation and new poplar clones characterized by fast growth, high resistance to diseases and pests, high wood quality for several purposes have been recently selected and proposed to the market.

Nevertheless as poplar genome has been sequenced and the introduction of new desirable traits by classical breeding is a time-consuming process because of the long sexual generation cycles and the long time required to evaluate adult traits, a genome mapping project has been implemented to identify the genetic bases on important agronomic traits.

In this work, the development of a genetic linkage map based on an F1 *P. x canadensis* hybrid population derived from an inter-specific mating of *Populus nigra* and *Populus deltoides* accessions is described. A population of 131 F1 plants have been initially genotyped with 113 Single Sequence Repeat (SSR) markers and further subjected to genotyping by sequencing using the innovative Randomly Amplified Polymorphic DNA Sequencing (RAPiD-seq) protocols (http://www.rapid-genomics.com/). The combination of both genotyping technologies has led to a highly dense molecular map with 64 SSR and 5672 SNP markers organized in 19 linkage groups. The genetic linkage maps of *Populus nigra, Populus deltoides and P. x canadensis* represent the base of a QTL mapping program for resistance to *Phloeomyzus passerinii*, since the two parents differ for their resistance response to the Woolly poplar aphid.

S1 – P14

Key words:
Salix erioclada,
bioenergy,
genetic map,
growth, QTL
mapping

A genetic map of *Salix erioclada* constructed for QTL mapping bioenergy traits

Hu Jianjun, Jia Huixia, Lu Mengzhu

Research Institute of Forestry, Chinese Academy of Forestry, Wan Shou Shan, Beijing 100091, P. R. China.

Short rotation coppice willow with fast growth and high biomass has been applied to bioenergy and biofuel production. *Salix erioclada*， a shrub willow, is one of very important bioenergy species. To understand the genetic basis of energy characteristics, a genetic linkage map of *Salix erioclada* using AFLP and SSR markers was constructed and the QTLs for bioenergy traits such as growth, biomass and calorific value etc. were mapped. 520 F_1 individuals were obtained from a cross between *S. erioclada* (2n=38) 'S25' and 'S287' and 280 of which were used for constructing a genetic linkage map based on 609 amplified fragment length polymorphism (AFLP) and 8 simple sequence repeat (SSR) markers. The 19 major linkage groups spanned 2481.75 cM with an average interval of 4.07 cM between markers, about 99% coverage of genome length. Five bioenergy traits (stem height, stem ground diameter, stem number, biomass and calorific value) were subjected to QTL analysis and 38 QTLs associated with bioenergy traits were detected and most of them were specific for each trait. Our results provide basic information for breeding programs on willow biomass yield.

Funding support: The National 863 Program of China (No. 2011AA100201)

S1 – P15

Key words:
Salix, genome, genetics, QTL, transcriptomics

Progressing gene discovery in *Salix*: genetic and genomic approaches to the dissection of key productivity and quality traits

Steve Hanley, Rachel Rossiter, Femke De Jong, Jennifer Cunniff, Tim Barraclough, William Macalpine and Angela Karp

Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK

Moving from the identification of QTL to the underlying genomic determinants of observed phenotypic variation remains a major challenge in crop improvement programmes. Although not a prerequisite for selection and breeding *per se*, knowledge of the genes and mechanisms underlying QTL is extremely valuable in that it can offer new insights into fundamental biological processes as well as providing novel targets for use breeding programmes and genes of relevance to industrial biotechnology.

By applying an integrated genetics and genomics approach, we have made progress in the identification of some key genes underlying QTL for phenology, growth and architecture, drought response and more recently, saccharification potential and secondary metabolism. These traits have been studied in our collection of large and diverse willow mapping families, for which genetic maps and QTL are available. Although genome sequences are now being generated for all parental lines as part of our willow genome sequencing programme, segregating haplotypes for some important and finely mapped loci have already been sequenced using BACs and gene content and polymorphism content determined. To help identify causal polymorphisms, high-resolution genetic mapping using association mapping or additional crosses in which plants recombinant in QTL regions have been specifically selected for using markers, have then been applied. Similarly, transcriptomic analysis of QTL regions (RNA-Seq) has also proved informative.

Another approach to identify key genes and useful allelic variation has been functional screening of multiple willow alleles of selected candidate genes in Arabidopsis. Although not applicable for all traits of interest, we successfully developed this approach to identify *SxMAX4* as a key gene in the coppicing response, with a single base pair substitution responsible for the observed phenotypic variation.

S1 – P16

Key words:
willow, shrubs, bioenergy, genetics, association mapping

Analysis of phenotypic and genetic diversity of a *Salix purpurea* association mapping population

Fred E. Gouker[1]*, Ran Zhou*[2]*, Luke Evans*[2]*, Stephen P. DiFazio*[2]*, and Lawrence B. Smart*[1]

[1]Dept. of Horticulture, Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456 [2]Department of Biology, West Virginia University, Morgantown, WV 26506, USA

Shrub willow (*Salix* spp.) is a widely adapted and genetically diverse genus that improves soil characteristics, provides habitat for wildlife, and can be grown on marginal lands. These characteristics make willow particularly advantageous as a sustainable high-yielding biomass crop. There is great potential for genetic improvement for yield and other important traits, but little trait mapping or tools for marker-assisted selection (MAS) have been accomplished in *Salix*. We have utilized high-density genotyping-by-sequencing to develop SNP markers across the genome of *Salix purpurea*, a reference species that is key to breeding willow in North America, to help understand the genetic basis for key traits and identify QTL and markers that can be utilized for marker assisted selection. A *S. purpurea* association mapping population with 113 accessions has been established at three experimental sites in 2012 to identify candidate genes for high biomass yield and test for variation amongst traits relating to biomass yield and composition. This population was phenotyped extensively for several key growth and physiological traits, specific gravity, and composition. Broad-sense heritability estimates, variance components and BLUPs were calculated for each genotype to assess the relative performance in each location. SNP markers were used to assess population structure and for selection of candidate genes for re-sequencing to test for marker-trait variation and causal polymorphisms. This study addresses the underlying genetic basis of yield and biomass composition for the Northeast US and will allow for the development of tools for MAS to accelerate breeding and selection.

Funding support: USDA-NIFA grant from the Northeast Sun Grant Center and a USDA-NIFA Bioenergy CAP Center grant

S1 – P17

Key words:
willow, volume index, genotypic coefficient of variation, multilocation trial

Four years' growth performance of superior clones of tree willows (*Salix* species)

Jai Pal Sharma[1] and *Naresh Bahadur Singh[2]*

Department of Tree Improvement and Genetic Resources
Dr YS Parmar University of Horticulture and Forestry,
Nauni, Solan HP, India 173230

Willow (*Salix*) has multifarious uses and recognized under short rotation forestry crop throughout the world. Keeping in view its economic and social importance a research programme was started in the department to develop more productive clones. Over the years 200 clones/hybrids/ species were procured from 20 different countries of the world. These clones were screening in nursery and thereafter in departmental field. The selected 17 clones were planted under multilocation trial of willow at Devamanal, district Sirmour, H. P. India in the year 2010. The four years growth data were recorded in February 2014. Perusal of the data depicts the growth of clones varied significantly among the clones. Maximum plant height (10.42 m), diameter at breast height (8.58 cm) and volume index (0.095 cm^3) were recorded in the clone J799. Diameter at breast height recorded by clones NZ-1040 (7.46 cm), SI-64-017 (6.44 cm), Kashmiri willow (6.37 cm), 131/25 (5.17 cm) and SE-63-016 (4.65 cm) and volume index by NZ-1040 were at par with highest recorded clone at 5 percent of significance. Number of branches was also recorded highest (40) in the clone J 799 which was at par with the clones PN 722 (26.00), Kashmiri willow (23.67) and NZ-1140 (20.33). The analysis of genetic parameters was done, for all these characters. Genotypic (87.88 %), phenotypic (162.78%) and environmental (105.04%) coefficients of variation along with genetic gain (110.86%) were highest for volume index. Heritability in broad sense ranged from 0.37 (Plant height) to 0.63 (number of branches).

S1 – P18

Key words:
Salix, short rotation woody crops, yield, annual increment

Yield of 30 shrub willow cultivars over two rotations in a yield trial at Middlebury Vermont

Shun Shi[1]*, Mark Eisenbies*[1]*, Eric Fabio*[2]*, Mike Moser*[3]*,Timothy Volk*[1]

[1]Department of Forestry and Natural Resources Management, State University of New York College of Environmental Science and Forestry
[2]Department of Horticulture, Cornell University, Geneva, NY
[3]Middlebury College, Middlebury, VT, USA

Short rotation willow biomass has the potential to become a dedicated source of biomass. Identifying and selecting high yielding cultivars is an essential step in facilitating the commercialization of shrub willow biomass. A yield trial with 30 willow cultivars was established in 2007 at Middlebury, Vermont. The trial was coppiced after the first growing season and harvested three years after the coppice, and will be harvested for the second time in March 2014. Yield from the 1st rotation ranged from 7.4 Mg $ha^{-1}yr^{-1}$ to 14.6 Mg $ha^{-1}yr^{-1}$, indicating a wide range of variation across cultivars. The top 3 yield cultivars ('Canastota', 'Marcy', and 'Preble') had an average yield of 14.3 ± 0.2 Mg $ha^{-1}yr^{-1}$, which was 25.1% higher than the average yield (11.5 ± 0.5 Mg $ha^{-1}yr^{-1}$) of the four reference cultivars ('SX61', 'SX64', 'SX67', and 'SV1'). Stem diameters were collected at the end of each growing season in non-harvest years and were used to predict above ground biomass using allometric equations for 'SX64' and 'Fish Creek'. At the 3rd year of the first rotation, both cultivars had similar mean annual increment (11.1 Mg $ha^{-1}yr^{-1}$ and 11.2 Mg $ha^{-1}yr^{-1}$ for 'SX64' and 'Fish Creek', respectively). Current annual increment(CAI) and mean annual increment(MAI) curves of 'SX64' crossed at the end of the 3rd year, indicating the maximum yield production for 'SX64' was reached during the 3 year rotation. The CAI and MAI curves of 'Fish Creek' did not intersect, indicating there is potential to increase the yield of this cultivar by extending the time length of the 1st rotation. This paper will also report on changes in yield from first to second rotation among the different cultivars. Characterizing these changes is important for modelling yields of willow biomass crops over multiple rotations.

S1 – P19

Key words:
broad sense heritability, Type B genetic correlation, clonal poplars, breeding for dendroenergy plantations

Estimates of genetic parameters for poplar biomass production after two harvesting cycles in Chile

Carlos Gantz[1], *Mario Olmedo*[2]

[1]Managing Director, Biological Research Group, GreenWood Resources Inc., Portland, Oregon, USA. [2]Propagation and Field Test Manager, GreenWood Resources Chile S.A., Los Angeles, Chile.

GreenWood Resources Inc. (GWR) has been introducing proprietary hybrid poplar varieties in south central Chile since 2002. The first 36 clones were introduced after one and a half year of required quarantine and several trials were established through a collaborative work with the University of Talca. In 2006, GreenWood Chile S.A. was created and developed its own nursery, quarantine facilities and testing areas at the Santa Julia Demonstration Center near Los Angeles (37°30'S). Since then, several trials were established using the same set of clones evaluated at different plot spacing (i. e. 4 × 4 m and 3 × 0.6 m) and different trial designs. Three clonal trials were established between 2008 and 2010 at a 3.0 x 0.6 m spacing (equivalent density of 5,550 trees per ha), to mimic a dedicated biomass plantation. A randomized complete block design was used in all the trials with 16 tree square plots and four replications. Trials were measured every year and data were used to estimate genetic parameters and to predict genotypic values to select the best clones for deployment to the GreenWood's biomass production plantations. Estimated broad sense heritabilities were moderate for all traits, indicating that good genetic gains can be obtained through clonal selection. Low Type B genetic correlations among trials suggest a high genotype by environment interaction. One trial was harvested at age two and allowed to coppice. Height and diameter measurements were taken after coppicing and age-age genetic correlations for biomass were estimated to determine the best selection age. A strong genetic correlation was determined between age two (before coppicing) and age three (after coppicing), suggesting that selection during the first growing cycle could be used to select for clonal performance during the coppicing phase of a biomass trial.

S1 – P20

Key words: hybrid poplars, species poplar, wood plantations, linear, massive plantings, growth, productivity

Growth and productivity of poplar species cultures in the South-East of Kazakhstan

Dani N. Sarsekova

S.Seifullin Kazakh Agrotechnical University, Astana, 010011, Republic of Kazakhstan

Small forested territory of Kazakhstan and an acute shortage of wood requires foresters to find ways to improve the productivity of forests and their sustainable using. This deficit in the wood can be to some extent offset by the growing plantation crop of fast-growing tree species, including the species and best hybrid poplars form. Increased interest in the poplar in the world is due to its biological characteristics and economic value: quick growth and ability to produce technically suitable timber at the cutting cycle in 20 years or less; use in most industries, based on the wood using; the ability to grow on land, not always suitable for agricultural using; widespread using possibility in protective, greenery and recreational plantings; the ability of most species and hybrids of poplars for vegetative reproduction.

Poplar culture in the country, especially in the south and south-east, are engaged quite extensively. Basically, it's landing in settlements, along roads, in protective forests. However, they cannot practically serve as a source of business and wood of commerce. In this regard, there is a need to establish in the country poplar plantation cultures growing on the wood. In this regard, there is a need to adjust in the country growing plantation poplar culture for wood. Research of growth study and productivity of culture species poplars found in South-Eastern Kazakhstan in greenery and massive planting the most common types of poplars are Deltoid, Algerian and Bolle; main factors that ensure high productivity of poplar plantations are soil fertility, good aeration and adequate moisture; the greatest current increase of the poplar comes aged 5-10 years in height and 4-9 years in diameter. Culminating, increments in height and diameter begin to decline gradually and 14-16 years abruptly decrease; the high productivity of poplar plantations noted when placing 2.5-3 x 1.5 -2m from 2000-3000 trees per 1 hectare. The tangle creation of 5000-7000 tree trunks per 1 hectare leads to rapid growth of trees in height and quite early growth reduction in diameter.

Funding support: grant of MES of the RK “Best teacher of 2014 year”.

S1 – P21

Key words:
poplar, hybrid, mini-cuttings, disease, progeny

Growth and disease resistance in hybrid poplar genetics trials in Minnesota and Michigan

Daniel Keathley[1], Paul Bloese[2], Bill Berguson[3], Bernie McMahon[3], and Raymond Miller[4]

[1]Department of Horticulture, Michigan State University, East Lansing, Michigan, USA [2]Department of Forestry, Michigan State University, East Lansing, Michigan, USA [3]Natural Resource Research Institute, University of Minnesota Duluth, Duluth, Minnesota, USA [4] Michigan State University Forest Biomass Innovation Center, Escanaba, Michigan, USA

This paper reports the results of five-year growth data from hybrid poplar tests growing on nine different sites in Minnesota and northern Michigan. Planting material for these tests was 4" mini-cuttings from the progeny of controlled crosses that were rooted in the greenhouse and field planted in two multiple-site tests during the 2008 and 2009 growing seasons. Six ramets per clone were established at each site, planted in single tree plots. Variation in family means for dbh was significant across the sites, with the family means ranging from 68% to 118% of the 2008 test mean, indicating strong potential for selection for improved growth rates. This was also shown by the high single-site family h^2 values, which ranged from 0.40 to 0.64. Interestingly, for dbh the paternal effects were stronger than maternal effects in both tests. Spearman correlations of clone means for disease resistance data, scored as leaf retention in the 2009 plantations and canker development in the 2008 plantations, showed strong correlations for disease resistance/susceptibility of individual genotypes across all sites, also indicating the potential for selection of superior lines with general resistance for planting throughout the region.

Funding Support: Natural Resource Research Institute, DOE Sun Grant Regional Feedstock Partnership, MSU AgBioResearch.

S1 – P22

Key words: biomass, short rotation coppice, optimal growth

Comparison of clones and rotation lengths in commercial plantations of hybrid poplar in central Spain

Maria José Hernandez-Garasa[1], Carlos Alvarez[2], Manuel Ibarra[2], Isabel Cañellas[1], Hortensia Sixto[1]

[1]Centro de Investigación Forestal (CIFOR) del Instituto Nacional de Investigación de Tecnología Agraria y Alimentaria, INIA. Ctra. de la Coruña km 7,5, 28040- Madrid, Spain [2]ENCE, Energía y Celulosa, P^{o} Castellana, 35 , 28046- Madrid, Spain

The current interest in the production of renewable energy from lignocellulosic biomass has led to the establishment of commercial plantations, mainly of poplar and eucalyptus, in the area of influence of these production plants. The optimization of production by selecting the most productive genotypes and determining the best moment to harvest is key to improving its economic viability. The objective of this study is to compare the productive performance of a series of hybrid *Populus* genotypes in the area of influence of an electricity production plant of 16.5 MW, taking into consideration not only the genotype but also the optimal rotation length to obtain maximum production.

The plantations, which were established with a density of 5,555 trees/ha, include the following clones: \`AF2´, \`AF8´, \`MC´, \`I-214´,\`Dorskamp´ and \`Oudenberg´ (*P.x canadensis* Moench.), \`Beaupre´ and \`Raspalje´ (*P. x generosa* Henry) and \`Monviso´ ((*P. x generosa)* x *P.nigra*). The rotation lengths, based on which the optimal growth for each of the clones is determined, were of two, three and four years. That is, the period corresponds to the first crop rotation in all cases. The plantations were carried out under Mediterranean climatic conditions (Lat.39.14, Lon.-4.15), characterized by a marked summer drought period being irrigation essential. Weed control was performed using pre-emergent herbicide (oxyfluorfen 4 l ha^{-1}) in the establishment year along with timely plowing in those plots where weed infestation was important. Aerial fresh biomass (stem and branches) was evaluated in 25 trees per 4 replicates per genotype and age. A subsample of the biomass obtained from each one of the studied plants was oven dried to constant weight in order to estimate dry biomass. The most productive hybrids (Mg ha^{-1} year^{-1},dry matter) for the study area were \`I-214´, and \`Raspalje´ for a rotation of four years and \`AF2´ for a rotation of three years. For \`Monviso´ and \`AF2´ in which two, three and four years rotation were compared, the maximum growth rate was obtained in the third year. Funding support: RTA2011-00006-00-00

S1 – P23

Key words:
Populus nigra, phenology, genetic variation, population differentiation, G×E interaction

Genetic characterization of phenology in a *Populus nigra* European association population grown in different environments

Francesco Fabbrini[1], Justine Guet[2,3], Catherine Bastien[2], Franck Brignolas[3], Antoine Harfouche[1], Giuseppe Scarascia-Mugnozza[1], Maurizio Sabatti[1]

[1]Department for innovation in biological, agro-food and forest systems (DIBAF), University of Tuscia, via S. Camillo de Lellis snc, 01100 Viterbo, Italy.[2]INRA, UR0588, National Institute for Agricultural Research, Orléans 2 F-45075, France.[3]UFR, Faculté des Sciences, UPRES EA 1207 'Laboratoire de Biologie des Ligneux et des Grandes Cultures' (LBLGC), Université d'Orléans, rue de Chartres, BP 6759, F–45067 Orléans Cedex 2, France

Adaptation of the annual cycles of growth initiation and cessation to the environment is critical for survival of woody plants. Thus, understanding the regulation of the seasonal cycles on perennial species is crucial for our ability to confidently predict how trees respond to local climate. This research will extend our knowledge on phenological responses of black poplar (*Populus nigra*), a European native species with substantial economic impact in worldwide active breeding programs. For this purpose, bud flush (BF) and bud set (BS) were taken as the cornerstones of the seasonal growth cycle in an integrative approach by dissecting the phenotypic level into several components. The genetic variability and the phenotypic plasticity of BF and BS were assessed in 13 natural metapopulations spanning 12° of latitude and grown in two common garden experiments in France and Italy. Genetic differences in the rates and degrees of adaptive responses to phenology between northern and southern populations were detected within and between sites. We found that broad-sense heritability for BF was generally higher ($0.81 \leq H^2 \leq 0.85$) than for BS ($0.35 \leq H^2 \leq 0.54$), suggesting that abundant genetic variation exists for phenological response to changes in the length of the growing season. Q_{ST} values, which estimate the proportion of the total trait variation among populations were generally higher for BS ($0.35 \leq Q_{ST} \leq 0.47$) than for BF ($0.26 \leq Q_{ST} \leq 0.33$). As indicated by the considerable role of G×E interaction in the observed phenotypic variance, the studied metapopulations respond differently to the two sites. Furthermore, G×E interaction for BS was explained by rank and scale changes between locations, while it was mainly attributed to the differences in the scale of genotypic responses across sites for BF. These results provide new insights into the study of phenology in *P. nigra* germplasm and will speed up breeding to improve growth and adaptation to future climatic conditions.

Funding support: the study was carried out with financial support from the NOVELTREE project (FP7 – 211868).

S1 – P24

Key words:
Populus nigra,
bud flush,
temperature,
G×Y interaction,
QTLs

Influence of temperature on bud flush phenology in *Populus nigra*: A comparison between two growing seasons

Riccardo Ludovisi[1], Muriel Gaudet[1], Francesco Fabbrini[1], Catherine Bastien[2], Antoine Harfouche[1], Giuseppe Scarascia Mugnozza[1], Maurizio Sabatti[1]

[1]Departement for Innovation in Biological, Agro-food and Forest systems (DIBAF), University of Tuscia, via S. Camillo de Lellis snc, 01100 Viterbo, Italy. [2]INRA, UR 0588 'Amélioration, Génétique et Physiologie Forestières' (AGPF), centre de recherche Val de Loire, CS 40001 Ardon, F–45075 Orléans Cedex 2, France.

Plant phenology represents a serious ecological and evolutionary trade-off between survival and growth. Besides, it is responsive to global warming, especially spring bud flush that is predominantly controlled by temperature. The aim of this study was to improve the knowledge on the bud flush dynamics focusing on the influence of temperature on this process. A protocol was developed for black poplar (*Populus nigra*) to dissect the bud burst spring phenology in different stages, from the swelling of the buds to complete leaf formation, passing through three intermediate phases. A *P. nigra* full-sib family originating from a controlled cross between parental genotypes from contrasting climate was studied at the same location and in two different growing seasons. Cumulative average temperature above 10°C (CAT_{10}) and cumulative minimum temperature below 5°C (CMT_5) were used to describe phenotypic and genetic variance of the bud flush stages. Both growing seasons showed similar CAT_{10} values for the initial stage of bud flush, despite seven days of difference observed between the years. Southern parent was more sensible to temperature variation than the northern parent. Greater fluctuations in temperatures increased the extent of phenotypic variance among genotypes during the process than stable temperatures. Moderate to low values of heritability ($H^2<0.50$) were observed for the different stages in the two years. The relative importance of year, genotype and genotype × year (G×Y) interaction effects on total phenotypic variation of bud flush stages was estimated. No significant G×Y interaction was observed. A large number of QTLs (28 and 22 on paternal and maternal maps, respectively) was found, corresponding to 9 genomic regions for each parent. QTLs were characterized by small or modest effect (average PVE=7.1%), without common genomic regions between parental maps. These results highlight the complex nature of the traits involved during bud flush and leaf elongation. Funding support: the study was carried out with financial support from the NOVELTREE (FP7 - 211868) and WATBIO projects (FP7 - 311929)

SESSION II – GENOMICS OF POPLAR & WILLOW

Oral Presentations

S2 – O1

Key words: functional genomics, wood chemistry, development, leaf and tree architecture

Activation tagging in poplar for gene function discovery

Arianna Latini[1], Denis Lachance[1], Marie-Josée Morency[1], Gervais Pelletier[1], Don Stewart[1], Lee Gunter[2], Gerald A. Tuskan[2], Jin-Gui Chen[2], Sharon Regan[3] and Armand Seguin[1]

[1]Laurentian Forestry Centre, Natural Resources Canada, Québec, QC G1V 4C7, Canada [2]Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA [3]Department of Biology, Queen's University, Kingston, ON K7L 3N6, Canada

Activation tagging is an approach by which a T-DNA fragment containing an engineered tetramer of the CaMV35S enhancer randomly integrates into the plant genome to activate the transcription of flanking genes. A total of 1164 activation tagged lines of hybrid Populus (P. tremula x P. alba) were evaluated at the Laurentian Forestry Centre research station starting in August 2006. The trees were grown for four years to monitor appearance of specific phenotypes. Close to 100 mutants were identified and a large proportion showed dwarfism. However, many trees were lost during the second growth season, leaving 14 mutants for further analyses. Another group of about 20 mutants showed early or late bud break, winter marcescent foliage and change in tree or leaf architecture phenotypes. In December 2010, trees were cut and 727 stem sections were collected for wood chemistry analysis. A total of 18 lines were selected (from 400 analysed) based on important variation of cell wall chemistry (e.g. lignin percentage, S/G ratio).

Identification of T-DNA insertions in the poplar genomes was achieved and genes located within the 15 kb region flanking the T-DNA insertion site were selected for further investigations. For each mutant we gathered information on gene annotation and expression profiling (database mining and our own quantitative RT-PCR analysis) on the selected candidate genes. Our analysis already revealed several potentially interesting proteins related to wood chemistry (e.g. glucanase, hydrolase, F-box protein and HRGP) and bud development (e.g. bHLH transcription factors and protein kinases) for instance. Association of other candidate genes, (e.g. stress related genes; wound-inducible, dehydrin) with specific mutant phenotypes such as tree architecture and dwarfism could however be more challenging. We are pursuing further characterization of those genes and related proteins to improve our understanding of their roles in key physiological processes.

Funding support: This work is supported by the Genomics R & D Initiative of Canada and the Arborea project (www.arborea.ca funded by Genome Canada and Genome Quebec). Support from the United States Department of Energy (DOE) BioEnergy Science Center project is also acknowledged. The BioEnergy Science Center is a Research Center supported by the Office of Biological and Environmental Research in the DOE Office of Science.

S2 – O2

Using metabolomics for trait selection in willow QTL studies

Key words: metabolomics, willow, QTLs, mapping populations, NMR, traits

Jane L. Ward, *Claudia Harflett, Delia I. Corol, Steven Hanley, Ian Shield, Angela Karp, Michael H. Beale*

Rothamsted Research, West Common, Harpenden, Herts. AL5 2JQ. UK.

Research into woody bioenergy crops is looking towards metabolomic profiling as an aid to crop improvement for a number of industrial applications. As well as increases in biomass yield, calorific value, and pest resistance we are researching such crops as non-petrochemical sources of feedstock for the synthesis of fuels, industrial and high value chemicals.

We have established a high throughput metabolomics screening system for the bioenergy crop willow, for which Rothamsted Research maintains the UK National Collection and has also developed large-scale genetic mapping populations, grown in different agronomic environments. Tree/shrub species present a new set of problems to the plant metabolomics community. In particular the interplay of metabolism across very different tissue types, the large plant size and seasonal developmental differences in plant structure, make sampling choices especially important when trying to map specific traits.

We will report on willow genotype metabolite screening by combined NMR and direct infusion ESI-MS, a project that has required completely new solutions in sample preparation. Baseline experiments such as temporal lifecycle studies have informed on the variation of carbon flow within the plant in relation to primary and secondary biosynthetic pathways. Comparison of different genotypes has highlighted major differences in metabolism. We will demonstrate how metabotyping amongst multiple willow mapping populations has revealed a selection of compositional traits that can be targeted for candidate genes to be used in crop improvement programmes towards added value woody biomass.

S2 – O3

Metabolic phenotype of disrupted cellulose synthesis and assembly in *Populus deltoides*

Key words: *Populus deltoides*, cellulose synthesis, KORRIGAN, UDP-glucose pyrophosphorylase

Timothy J. Tschaplinski, Raja S. Payyavula, Nancy L. Engle, Madhavi Z. Martin, Gerald A. Tuskan and Udaya C. Kalluri

BioEnergy Science Center and Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN

Cellulose synthesis and assembly genes have been prime targets for manipulation to understand the mechanisms underlying the production of the most abundant polymer on earth, and more recently, to potentially lower the recalcitrance of plant cell walls to deconstruction as a means of releasing cell wall sugars, ultimately, for conversion to biofuels. RNAi mutants of two of such genes in Populus deltoides (Pd), including KORRIGAN (KOR1 and KOR2), plasma-membrane-bound EGase from subclass γ required for appropriate cellulose synthesis and assembly, and overexpression lines of UDP-glucose pyrophosphorylase2 (UGPase2), which produces the substrate for cellulose synthesis, have been generated. Leaf, stem, xylem and phloem samples were collected for RNAi transgenic lines (two to four) of Pd(KOR1), Pd(KOR2), and overexpression lines of Pd(UGPase2), and four to six lines of empty–vector control P. deltoides with three to four replicate plants per line. All plants were subjected to broad spectrum metabolite profiling by gas chromatography-mass spectrometry using electron impact ionization of their trimethylsilyl derivatives. Despite these genes regulating markedly different reactions, the metabolomic analyses indicated that a common metabolic phenotype was evident that reflected the disruption of cell wall formation. Although these genes are primarily involved in cellulose synthesis and assembly, there were pronounced effects on aromatic metabolism with direct effects on lignin biosynthesis and effects extending to higher-order salicylates, other benzoic acid conjugates, and flavanols. The significance of the results in the context of the integration of broadly distant metabolic pathways and their role in controlling cell wall recalcitrance will be

discussed.

S2 – O4

Key words: poplar, genomics, karyotype, ploidy, breeding

A *Populus* gene-dosage resource for functional genomics and breeding

Andrew Groover[1,2], *Luca Comai*[2,3], *Isabelle Henry*[3], *Matthew Zinkgraf*[1]

[1]US Forest Service, Pacific Southwest Research Station, Davis CA USA [2]Department of Plant Biology, University of California Davis
[3]Genome Center, University of California Davis, USA

Gene dosage relationships are complex in Populus, as illustrated by naturally occurring ploidy variants (e.g. triploid aspen) and interactions between parental genomes in interspecific hybrids. We describe a new functional genomics resource that can be used to investigate the role of gene dosage in heterosis and the regulation of traits of interest such as biomass production and wood formation. High frequency deletion and additions of chromosomal segments were induced through irradiation of P. nigra pollen, followed by controlled cross to P. deltoides. Dosage variation was identified in individual F1 progeny using Illumina sequencing to precisely define deletions and additions of chromosomal segments. In an initial survey of 74 F1 individuals, 55% of individuals carried at least one deletion or addition. We will present preliminary examination of dosage variation and phenotypes in a larger population of over 600 individuals, and describe ongoing efforts to make this resource available to the Populus research community. Importantly, the approaches we have developed are supportive of creating novel genotypes and phenotypes at a rapid rate, and could be an important tool for future poplar breeding.

Funding support: This research was supported by the Office of Science (BER), U. S. Department of Energy.

S2 – O5

Key words:
DNA methylation, microRNA, gene expression, *Populus*, flower development

Comprehensive analysis of the methylome, microRNAome and transcriptome reveals the effects of DNA methylation in flower development of andromonoecious poplar

Yuepeng Song and Deqiang Zhang

National Engineering Laboratory for Tree Breeding, College of Biological Sciences and Technology, Beijing Forestry University, No. 35, Qinghua East Road, Beijing 100083, P. R. China

Cytosine methylation functions as an important regulator of transposon silencing, genomic imprinting, and gene expression. Many studies have focused on the relationship between methylation and expression of protein coding genes, but the regulatory relationship between microRNAs (miRNAs) and methylation remains unclear. The methylome, microRNAome and transcriptome data were obtained by genome scale bisulfite sequencing of single-base solution, microRNA sequencing and Affymetrix microarray, respectively. Bisulfite sequencing of candidate sequences in gynomonoecious and normal poplar, respectively, was used to validate the methylation patterns of differently methylated regions.

We identified 113 methylated miRNA genes (28%) and showed that the miRNA and protein coding genes have different methylation patterns. In coding genes, the cytosine methylation level was lower at the borders of the genes and slightly higher in the gene body regions than in the 5′ and 3′ flanking regions. By contrast, in miRNA genes, the methylation levels of cytosine were significantly lower in the gene body regions. Hierarchical cluster analysis indicated significantly negative and strand specificity correlation of methylation and gene expression ($r=0.79$, $P<0.05$). Eight lmiRNAs direct methylation at their target genes resulting in transcriptional gene silencing were detected. Strand specificity methylation and gene expression analysis showed methylated on the minus strand has a stronger correlation with repression of gene expression than methylation on the plus strand, both in coding and microRNA genes. Among 11 flower development relate miRNAs, miRNA172b might play an important role in bisexual flower development in poplar, *via* modification of methylation. The results imply a regulatory role for methylation in mediating miRNA and their targets expression, demonstrating the potential of methylation, microRNAs and gene expression might have a complex regulatory network in plant development.

S2 – O6

Key words:
SNP-trait association, *Arabidopsis*, Black cottonwood, wood properties, lignin

Validation of SNP-trait associations identified in black cottonwood (*Populus trichocarpa*)

Steffi Fritsche[1], Ilga Porth[3], Athena McKown[2], Michael Friedmann[1], Jaroslav Klapste[2], Rob Guy[2], Shawn Mansfield[2], Yousry El-Kassaby[2], Jürgen Ehlting[4], Carl Douglas[1]

[1] Department of Botany, University of British Columbia, Vancouver, BC V6T 1Z4, Canada, [2] Department of Forest Sciences, University of British Columbia, Vancouver, BC V6T1Z4, Canada, [3] Department of Wood Science, University of British Columbia, Vancouver, BC V6T1Z4, Canada, [4] Centre for Forest Biology, University of Victoria, Victoria, BC, V8W 3N5, Canada

Populus trichocarpa is an important model tree species in forest tree research with high economic and ecologic relevance. Extensive genomic resources, e.g. the full genome sequence and tools for the identification of single nucleotide polymorphisms (SNP) are available. With the advent of SNP genotyping arrays and more recently, low-priced sequencing technologies, genome-wide SNP profiles from hundreds of individuals can be assayed. We and others are using this information in combination with extensive phenotype data from trees grown in common gardens for genome-wide association studies (GWAS) to study the correlation between genetic variants and quantitative trait differences based on linkage disequilibrium (LD). These studies identify a considerable number of SNPs that that are significantly associated with various traits. However, our GWAS have revealed many SNP-trait associations in genes of unknown function or genes not previously known to be related to the associated trait. Also, many SNPs appear not affect protein function or are located non-coding regions. Major questions thus include: How can we corroborate the allelic effects of these SNPs? Which strategies can be applied to identify causal SNPs (QTN) that directly impact trait variation? In our work we are analyzing selected genes and SNPs identified in two *Populus* GWAS that focused on wood property traits with respect to these questions. We will discuss our strategies and initial results for experimental validation of the SNP-trait associations including analysis of expression level variation and/or transgenic expression of the target alleles.

Funding support: Genome Canada, Genome BC

S2 – O7

Key words:
Populus,
demography,
genome
sequencing,
Quaternary

Quaternary demography of *Populus trichocarpa* throughout its range

Luke M. Evans[1], *Wellington Muchero*[2], *Gerald A. Tuskan*[2], *& Stephen DiFazio*[1]

[1]Biology Department, West Virginia University, Morgantown, WV, 26506
[2]BioSciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831

Temperate zone forest trees, ranging across broad geographic areas, have experienced drastic change throughout the Quaternary (ca. 2.5 million years). It is important to understand the demography of temperate tree populations, in terms of population size (N_e), gene flow and divergence, because those changes impact the extant distribution of genetic variants across the landscape and can provide insights into the effects of current and future warming on such populations. Here, we report estimates of population size, migration rates and divergence times among populations of *Populus trichocarpa* from throughout its range using 544 complete genome sequences and two fundamentally different approaches: an individual-genome based coalescent method and a forward simulation method using the multi-population allele frequency spectrum. Patterns of polymorphism throughout the species' range are consistent with demographic history models including strong ancestral bottlenecks and recent population divergence. There is also evidence of rapid growth in the center of the range and rapid decline of populations at the southern range margin after the last glacial maximum. Our analyses indicate that *Populus trichocarpa* has experienced diverse climatic conditions across its range at different points in time; consistent with the hypothesis that climate has influenced recent demographic changes. These results contribute to our understanding recent and future climate change, as well as the current and potential future distribution of genetic variation across the landscape. This knowledge will aid breeding and conservation efforts in this ecologically and commercially important genus.

Funding sources: Department of Energy Bioenergy Science Center

S2 – O8

Key words:
phenolic
glycosides,
salicortin,
salicin,
biorefining

The phenolic glycoside biosynthetic pathway in Salicaceae:– what do we know and what do we need to know for future exploitation?

Michael H. Beale, Jane L. Ward, Femke de Jong, Steven Hanley and Angela Karp

Rothamsted Research, Harpenden, Herts, AL5 2JQ, UK

From a biotechnological perspective, small aromatic compounds from bulk biomass are an attractive target to replace the highly valuable BTX aromatic stream that is currently produced in petrochemical refining. The relatively simple, and hugely successful, drug aspirin (acetylsalicylic acid) was developed by the Bayer company in 1897, from a lead provided by the study of salicyl glycosides in herbal preparations of *Salix alba* bark. It is however very surprising that, after over 100 years of natural product chemistry, our knowledge of the pathways involved in the biosynthesis of these metabolites is very limited. Against a background of the varied structures that occur across the Salicaceae, the presentation will take a critical look at the published biosynthetic investigations. Using new knowledge on the co-occurrence and stability of known compounds such as salicortin and salicilin, as well as the identification of additional analogues we have re-evaluated the likely biosynthetic routes Examination of genome information and screening of mapping populations add insight into genes that may be involved. As well as the *ortho*-substituted salicylate family of compounds, *para* substititued aromatics, such as triandrin and salidroside also occur in *Salix sp.* The presentation will thus explore the prospects of improving Salicaceae crops for biorefining applications as well as a source of genes for heterologous production of pharma products.

S2 – O9

Key words: comparative genomics, gene expression, trait mapping, *Salix*, transcriptomics

Genomic approaches to improve yield and biofuels conversion efficiency of shrub willow

Lawrence B. Smart[1], *Fred E. Gouker*[1], *Craig H. Carlson*[1], *Michelle J. Serapiglia*[1], *Christopher D. Town*[2], *Haibao Tang*[2], *Vivek Krishnakumar*[2], *Stephen P. DiFazio*[3], *Eli Rodgers-Melnick*[3], *Ran Zhou*[3], *Shengqiang Shu*[4], *David M. Goodstein*[4], *Kerrie W. Barry*[4], *Erika A. Lindquist*[4], *Jeremy Schmutz*[5], *and Gerald A. Tuskan*[4, 6]

[1]Dept. of Horticulture, Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456 [2]J. Craig Venter Institute, Rockville, MD 20850 [3]Department of Biology, West Virginia University, Morgantown, WV 26506 [4]DOE Joint Genome Institute, Walnut Creek, CA 94598 [5]HudsonAlpha Institute for Biotechnology, Huntsville, Alabama 35806 [6]Oak Ridge National Laboratory, Biosciences Sciences Division, Oak Ridge, TN 37831

Shrub willow (*Salix* spp. and hybrids) has numerous traits that make it well suited for the sustainable production of biomass for bioenergy on marginal land. *Salix* is also an interesting model for the study of polyploidy, dioecy, apical dominance, dormancy, bud phenology, nutrient transport, wood composition, biotic interactions and speciation in the Salicaceae. We have established a long-term willow breeding program to develop novel species hybrids with improved yield, pest and disease resistance, and biomass quality. We have documented significant variation in biomass composition among different genotypes and across diverse sites that results from genotype-by-environment interactions. To gain a greater understanding of the genetic basis for variations in the traits mentioned above, in heterosis for yield, and in biomass chemistry, and to develop tools to accelerate breeding, we have examined next-generation sequencing data generated through a DOE Joint Genome Institute initiative to produce an draft *de novo* assembly and annotation of the *S. purpurea* genome and transcriptome. The *Salix* genome is largely syntenic with that of *Populus*, but is significantly smaller and includes a few major chromosomal rearrangements. We have developed a high-density linkage map of *S. purpurea* using genotyping-by-sequencing that enabled the assembly of sequence scaffolds into chromosome-length pseudomolecules. We have also established and extensively phenotyped and genotyped an association panel of *S. purpurea* across three contrasting sites. One of our primary interests is to apply these tools in understanding the mechanisms of heterosis in triploid *Salix* species hybrids.

Funding support: US Dept. of Energy-Office of Bioscience, US Dept. of Agriculture-NIFA

S2 – O10

Key words:
XY, genome-wide association, recombination

An assay for gender determination in cottonwoods reveals the occurrence of unexpected rare recombinants

Armando Geraldes[1], Charles A. Hefer[1*], Arnaud Capron[1], Natalia Kolosova[1], Felix M. Nunez[1], Raju Y. Soolanayakanahally[2], Brian Stanton[3], Rich Shuren[3], Michael C. Friedman[1], Robert D. Guy[4], Shawn D. Mansfield[5], Carl J. Douglas[1] and Quentin C. B. Cronk[1]   [*]equal contribution*

[1]Department of Botany, University of British Columbia, Vancouver, Canada
[2]Agroforestry Division, Agriculture Agri-Food Canada, Indian Head, Saskatchewan, Canada [3]Greenwood Resources, Portland, OR, USA
[4]Department of Forest and Conservation Sciences, University of British Columbia, Vancouver, Canada [5]Department of Wood Science, University of British Columbia, Vancouver, Canada

Trees in the genus *Populus* are dioecious and gender in this genus is a genetic trait. Early identification of tree gender could prove hugely beneficial for breeding programs as well as physiological, ecological and evolutionary studies. Despite many years of research into the identification and development of genetic markers for gender prediction, to our knowledge, no genetic test is yet available. We used a genome-wide association approach to identify gender-specific polymorphisms in *Populus trichocarpa* and *Populus balsamifera*. We identified hundreds of single nucleotide polymorphisms associated with gender. The segregation pattern of these polymorphisms indicates that, in these species, males are the heterogametic gender (XY system). Unexpectedly, despite being in strong linkage disequilibrium, these gender-specific polymorphisms were mapped to several different genomic regions. Sequence data reveals that these gender-specific regions arose recently, after the divergence between cottonwoods and aspens. We used these data to develop two expedient and economic assays for gender prediction. Validation of these assays revealed that one assay is fully diagnostic for tree gender in cottonwoods (*P. trichocarpa*, *P. balsamifera*, *P. deltoides* and *P. nigra*), and, as expected, not in aspens (*P. tremuloides*). For the other assay, a very small percentage of cottonwood male accessions were incorrectly identified as females. Analyses of whole genome sequence data show that in these individuals a recombination event occurred in the male specific region of the genome. We used this assay to predict gender in large population samples of *P. trichocarpa* and *P. balsamifera*, as well as in the progeny of several controlled crosses to estimate the frequency of recombinants in the wild and the rate at which recombination occurs.

S2 – O11

Key words: *Salix*, transcriptome, paralogous genes, evolution, gene expression

Analyses of gene expression variation among paralogous genes in *Salix* spp.

Pascal Pucholt, Lei Liu Conze, Ann Christin Rönnberg-Wästljung and Sofia Berlin

Department of Plant Biology, Swedish University of Agricultural Sciences and Linnean Center for Plant Biology, Box 7080, 75007 Uppsala, Sweden

In this study we have used RNA seq data to assemble and analyse a *Salix* spp. transcriptome and used this transcriptome to study gene expression variation across paralogous genes in *Salix* spp. The main aims were to assemble and describe the *Salix* spp. transcriptome and use the RNA seq data to study the fate of genes after duplication events. Many paralogous genes in *Salix* spp. originate from the Salicoid event, or the genome duplication that is shared between species in the *Populus* and *Salix* genera. Also paralogous genes can result from gene duplications. We ask specific questions, like how many paralogous genes are there in the *Salix* spp. transcriptome and if more than one copy is present, how much have they diverged? We will estimate gene expression levels at the paralogous genes and in that way identify differentially expressed genes. This we will correlate with K_s values. We are also interested if there is a correlation between tissue and genotype specific expression and K_s. To answer these questions we have generated RNA seq data (774×10^6 raw reads) using the Illumina technology (18 libraries, paired-end reads, 100 bp read lengths) from leaves (five biological replicates) and roots (four biological replicates) from two sibling genotypes from the S_1 experimental cross (*S. viminalis* x *S. schwerinii* x *S. viminalis*). We have assembled the RNA seq data with Trinity using the whole dataset (all transcriptomes) where the sequence reads were clustered in isogroups, each assumed to represent one transcribed gene. The member of each isogroup proposed by Trinity that had the highest expression level was selected. This was done to eliminate the redundancy of the assembly. Transcripts likely originating from reading errors were filtered out based on expression level. By this strategy we obtained a set of high quality transcripts that are being used in further analyses.

Funding support: The Swedish Research Council, the Swedish Energy Agency (30599-3) and the NL-faculty at the Swedish University of Agricultural Sciences.

S2 – O12

Key words: willow, genomics, gene-space, de novo assembly, aTRAM, breeding, phytoremediation, biomass

Building genomic resources for Canadian willows: a *de novo* gene-space assembly for *Salix eriocephala* and comparison to the *Populus* gene-space

Daisie I. Huang[1], *Carl J. Douglas*[1], *Robert D. Guy*[2], *Shawn D. Mansfield*[3], *William R. Schroeder*[4], *Michael K. Deyholos*[5], *Quentin C. B. Cronk*[1], *Raju Y. Soolanayakanahally*[4], *Julie. M Allen*[6], *Kevin P. Johnson*[6]

[1]Department of Botany, University of British Columbia, Vancouver, British Columbia, V6T 1Z4 Canada [2]Department of Forest and Conservation Sceinces, University of British Columbia, Vancouver, British Columbia, V6T 1Z4 Canada [3]Department of Wood Sciences, University of British Columbia, Vancouver, British Columbia, V6T 1Z4 Canada [4]Agroforestry Development Center, Agriculture and Agri-Food Canada, Saskatchewan, S0G 2K0 Canada [5]University of Alberta, CW405 Biological Sciences, Edmonton, AB T6G 2E9, Canada [6]Illinois Natural History Survey, University of Illinois, Champaign, IL, 61820, USA

The genus *Salix* (willows) comprises some 450 species (with over 100 native to North America). Willows are of great ecological importance in the northern hemisphere inhabiting ecosystems from the Arctic to sub-tropical regions and from mountains to coastal wetlands. Here we report progress on a *Salix eriocephala* gene-space assembly generated via a novel strategy of gene-targeted *de novo* assembly implemented by the program aTRAM, assisted using the *Populus trichocarpa* reference genome. For convenience the gene-space collection will be made available in a Gbrowse browser environment. The gene-space assembly permits genome-level comparisons with poplar, and with other willows for which genome resources are also in progress (e.g. *S. sitchensis* Sanson ex Bong., *S. scouleriana* Barratt ex Hook., *S. exigua* Nutt. and *S. bebbiana* Sarg.). *Salix eriocephala* Michaux has numerous advantages as a focal species for genome work. It is widespread in North America east of the Rocky Mountains. It is generally abundant, easy to propagate, and has been used as a parent in breeding work. In recent years, fast growing short rotation shrub willows (including *S. eriocephala*) are emerging as an attractive source of biomass for bioenergy purposes across North America. In addition, willows are gaining attention in agroforestry systems for intercepting surface runoff of nutrients from crop fields, phytoremediation, reclamation of marginal lands/sites affected by excess salinity and improving biodiversity by providing wildlife habitat. We suggest that genome-enabled breeding of native North American species has the potential to produce clones with a range of useful traits and promising energy-economy-environment synergy.

Funding support: Genome Canada, Agriculture and Agri-Food Canada

SESSION II – GENOMICS OF POPLAR & WILLOW

Poster Presentations

S2 – P1

Key words: willow, poplar, genomics, phylogeny, trait analysis, novel crops, biomass, biodiesel

The expanding circle: lessons from close relatives of willows and poplars

Quentin C. B. Cronk[1], *Carl J. Douglas*[1], *Shawn D. Mansfield*[2], *Daisie I. Huang*[1]

[1]Department of Botany, University of British Columbia, Vancouver, British Columbia, V6T 1Z4 Canada [2]Department of Wood Sciences, University of British Columbia, Vancouver, British Columbia, V6T 1Z4 Canada

The family Salicaceae is a large group, distributed mainly in tropical and subtropical regions, and comprising 55 genera and over 1000 species. Two temperate genera, *Populus* and *Salix*, are among the most important, ecologically and economically, but other genera, such as *Azara*, are used in commercial horticulture. Recently, attempts have been made to develop another member of the Salicaceae, *Idesia polycarpa* Maxim. (the ligiri tree or “Chinese wonder tree”), as a biomass crop for the production of biodiesel or lubricant from its fruit oil. Here we present phylogenetic analyses that illustrate the relationships between poplar and willow and their closest relatives (including *Idesia*) using recently generated plastid and nuclear genomic resources. The morphological and growth traits of close relatives of poplar and willow will be examined in order to shed light of the origin of biologically and economically important characteristics in poplar and willow.

Funding support: Genome Canada, NSERC

S2 – P2

Key words:
poplar,
breeding, SRC,
projects

Poplar species and hybrids – molecular differentiation by microsatellites within and between all poplar-sections

Steffen Fehrenz, Serge Havel, Egbert Schönfelder , Alwin Janssen

Northwest German Forest Research Institute, Department of Forest Genetic Resources, Prof.-Oelkers-Str. 6, D-34346 Hann. Muenden, Germany

The basis for breeding of new varieties is the knowledge about the species we choose as parents, since this is the requirement to incross targeted characteristics. Classification of species and varieties as well as the placement of different poplar clones within the natural section has traditionally been based on morphological and reproductive features. However, convergent morphology on different species, hybrids and varieties hinders determination exceptionally. Based on 24 nuclear microsatellites amplified for 247 individuals of 28 different poplar species and 17 different hybrids we use a cluster algorithm to group this dataset gaining most information about its structure. Multiscale analyzes of different distance measures and description by principal coordinates offers insights into intra- and intersectional relationships as well as classification of hybrids and varieties. Under these methods we demonstrate that genetic markers as microsatellites are powerful and effective tools for determination different levels of relationship in the genus *Populus*.

Funding support: German Agency of Renewable Resources (FNR) by order of the Federal Ministry for Food and Agriculture (BMEL)

S2 – P3

Key words:
poplar, ssr,
molecular
systematic,
evolution,
phylogeny

New aspects in molecular phylogeny of the genus *Populus* (*Salicaceae*)

Steffen Fehrenz, Serge Havel, Egbert Schönfelder , Alwin Janssen

Northwest German Forest Research Institute, Department Forest Genetic Resources, Prof.-Oelkers-Str. 6, D-34346 Hann. Muenden, Germany

The genus Populus (*Salicaceae*) includes about 30 species worldwide. Based on morphological and reproductive characters the known poplar species are actually subdivided into 6 sections named *Aigeiros*, *Abaso*, *Tacamahaca, Populus*, *Leucoides,* and *Turanga*. Molecular analyzis on intra- and interspecific relationships in the genus give evidences of a phylogeny that clearly differ from the classical view. Approaches such as fragment and sequence analysis add new perspectives to resolve the genus-wide relationship for the poplars. But a final classification at the sectional and species level is still pending and controversial discussed. In the ongoing German breeding project “FastWOOD” we analyzed data from 24 nuclear microsatellites amplified for 28 poplar species of all sections. Based on the generalized stepwise mutation model we calculated microsatellite length-differences and generated a molecular dataset of systematic and phylogenetic relevance using a modification of the known distances based on allel size distributions. After reconstruction the phylogenetic tree we linked the data to a molecular clock calibrated by fossil records. The results lead to new aspects into poplar systematics and phylogeny.

Funding support: German Agency of Renewable Resources (FNR) by order of the Federal Ministry for Food and Agriculture (BMEL)

S2 – P4

Key words: marker assisted selection, association mapping, growth, phenology, candidate gene

Association mapping in Salix viminalis L. (Salicaceae) – identification of candidate genes associated with growth and phenology

Henrik R. Hallingbäck[1]*, Johan Fogelqvist*[1]*, Sviatlana Trybush*[2]*, Stephen J. Powers*[2]*, Martin Weih*[3]*, Nils-Erik Nordh*[3]*, Niclas Gyllenstrand*[1]*, Angela Karp*[2]*, Ulf Lagercrantz*[4]*, Steven J. Hanley*[2]*, Sofia Berlin Kolm*[1]*, Ann-Christin Rönnberg-Wästljung*[1]

[1]Department of Plant Biology, Swedish University of Agricultural Sciences and Linnean Center for Plant Biology, Box 7080, 75007 Uppsala, Sweden
[2]Rothamsted Research, Harpenden, Hertfordshire, UK [3]Department of Crop Production Ecology, Swedish University of Agricultural Sciences, Box 7043, 75007 Uppsala, Sweden [4]Department of Ecology and Genetics, Uppsala University, 752 36 Uppsala, Sweden

The importance of *Salix* spp. as a short rotation biomass crop has created a demand for faster genetic improvement and breeding for this species through deployment of molecular marker assisted selection (MAS). In this respect, association mapping constitutes a powerful tool because associations between molecular genetic and phenotypic variation observed in populations of largely unrelated individuals may be relevant and applicable in many different genetic backgrounds. This approach has so far been little used in *Salix* research, but here we present results of an association mapping effort using 315 *Salix* accessions. The accessions were sampled from a number of regions in northern Europe and were established in two field experiments situated in Pustnäs in Sweden (59º48' N, 17º39' E) and at Woburn in the UK (52º01' N, 0º35' W). They were genotyped using a panel of 1502 SNP markers developed from candidate genes for phenology and growth and from genes earlier observed to be differentially expressed in contrasting environments. Field measurements were also recorded for important and heritable traits including bud burst, leaf senescence, number of shoots developed per plant and shoot diameter growth. Association mapping between SNPs and the measured traits was performed taking into account the population structure estimated from an independent set of SSR markers. At a false discovery rate (FDR) below 0.2, 1 - 4 associations were detected for bud burst while 0 – 10 and 0 – 3 were detected for leaf senescence and growth traits respectively. A SNP located in the FLD_FLOWERING_LOCUS_D gene was detected at a FDR<0.05 for diameter growth at Pustnäs and for leaf senescence in Woburn and explained 4.6 - 6.6% of the phenotypic variance of clone means. Another SNP, ELF3b appeared to be associated with budburst both at Pustnäs and at Woburn, explaining up to 7.8% of the phenotypic variance. Our results indicate these SNPs to be promising targets for application in MAS. Funding support: EraNet grant from the Biotechnology and Biological Research Council of the UK (BB/G00580X/1) and from the Swedish Energy Agency (31468-1). SAMBA2 grant from the Swedish Energy Agency (30599-3) and the NL-faculty at the Swedish University of Agricultural Sciences.

S2 – P5

Key words: additive, dominance, epistasis, haplotypes-based association approach, *Populus tomentosa*

Integration of multiple genes-wide association studies reveals allelic variation underlying growth and wood properties in *Populus tomentosa*

Deqiang Zhang

National Engineering Laboratory for Tree Breeding, College of Biological Sciences and Technology, Beijing Forestry University, No. 35, Qinghua East Road, Beijing 100083, P. R. China

Given the polygenic nature of complex traits of interest in trees, previous association studies have typically focused on examination of single locus at single candidate gene level, which often lacks the power to uncover the relatively small effect sizes conferred by most genetic variants. Here we used multiple genes-based association approach to detect whether a group of related candidate genes in the biological pathway of wood formation are jointly associated with traits of interest. First, 11 candidate target genes were cloned from xylem tissues of stem in *Populus tomentosa*, with the full length ranging from 1693 to 6944 bp. Linkage disequilibrium (LD) test revealed that all genes harbor low LD ($r^2 > 0.1$, within ranges from 500 to 1800 bp, respectively). By combining multiple loci-based association study in an association population of 460 natural individuals, we identified 92 interactive loci affecting ten traits ($P < 0.0001$) under the additive model, with a total of heritability explained from 16.97% to 36.00%; in the dominance model, 121 significant associations were detected, and the total heritability explained for each trait, varies from 1.81% to 13.06%. We further examined the epistasis among unlinked loci by multifactor dimensionality reduction models, and result reveals that 58 SNP-SNP pairs ($P < 0.0001$) were significantly related to these nine traits except for microfiber angle, with the epistatic effects ranging from -7.03% to 0.85%. Most interestingly, a unique epistasis interaction of three nonsynomous loci was observed for diameter at breast height and stem volume. These data revealed that each quantitative trait was controlled by multiple loci from various genes with three main genetic effects, and additive effect appears to the most prominent. Further correlation analyses of tissue-specific transcription abundances among these genes provides a better understanding of the multiple-genes coordinate network in the shared pathway.

S2 – P6

Key words: association studies, chromosomal localization, glycosyl hydrolase family 9, phylogenetic analyses, *Populus tomentosa*

Populus endo-ß-1,4-glucanases gene family: genomic organization, phylogenetic analysis, expression profiles and association mapping

Qingzhang Du and Deqiang Zhang

National Engineering Laboratory for Tree Breeding, College of Biological Sciences and Technology, Beijing Forestry University, No. 35, Qinghua East Road, Beijing 100083, P. R. China

In higher plants, endo-1,4-β-glucanase (cellulase), belong to glycosyl hydrolase family 9 (GH9), have the capacity to hydrolyse the β-1,4 linkage of the cellulose chain, and have been shown to be more active on cell wall synthesis, remodeling, degradation. To better understand the regulatory mechanisms of GH9 family in cellulose biosynthesis and xylem development, an extensive characterization of GH9 family in even one tree species is essential. Here we characterized the 25 putative members of GH9 family with 3 subclasses (A, B, and C) in the model tree species, *Populus*. Phylogenetic analyses of 114 GH9 proteins from plant (dicot, monocot, and conifer) and bacteria species, demonstrated that all plant GH9s are monophyletic with respect to bacteria GH9s. Identification of chromosomal localization and duplications of *GH9s* in the *Populus* genome showed that 75% of *GH9s* were located in the paralogous blocks associated with the recent salicoid duplication event, suggesting that the poplar *GH9* genes mainly generated from a genome-wide reorganization and diploidization of the duplicated chromosomes. By examining tissues-specific expression profiles for all 25 *GH9* members, we revealed that *GH9* members exhibited distinct but partially overlapping expression patterns, while certain members have higher transcript abundance in mature or developing xylem. Furthermore, on the basis of knowledge of intraspecific variation and linkage disequilibrium of two *Populus tomentosa GH9As* (*PtoGH9A1* and *PtoGH9A2*) in a natural population of *P. tomentosa*, two non-synonymous SNPs in *PtoGH9A1*, remarkably associating with fiber width and holocellulose content, were identified by candidate gene-based association approach. The extensive characterization of poplar GH9 family provide insights into its function and evolution in woody species and also offer a genetic engineering or marker-aided selection breeding strategy.

S2 – P7

Key words:
CRISPR, 4CL,
Populus, lignin,
flavonoid

CRISPR/Cas genome editing in *Populus*: A case study with the 4CL family

Xiaohong Zhou[1,2], *Thomas Jacobs*[3], *Wayne Parrott*[3], *Chung-Jui Tsai*[1,4]

[1]School of Forestry and Natural Resources, University of Georgia
[2]College of Forest Resources and Environment, Nanjing Forestry University, China [3]Institute for Plant Breeding, Genetics & Genomics, University of Georgia [4]Department of Genetics, University of Georgia

CRISPR (clustered regularly interspaced short palindromic repeats), first discovered in immune systems of bacteria and archaea, has become a powerful tool for genome editing in eukaryotes. Co-expression of the CRISPR-associated endonuclease (Cas9) with a chimeric guide RNA (gRNA) targeting a gene of interest with a $GN_{20}GG$ motif results in cleavage of the target gene near NGG. Repair by the error-prone non-homologous end joining process often leads to small indels that can disrupt the reading frame, rendering the gene non-functional. The CRISPR/Cas system has been successfully applied to several plant systems, including *Arabidopsis*, rice, wheat, tobacco and soybean. Here, we demonstrate its application for targeted mutagenesis in *Populus* using the 4-coumarate:CoA ligase (4CL) gene family as a case study. 4CL is a key phenylpropanoid pathway enzyme and is encoded by six genes in the *Populus* genome. Three 4CL genes were selected for CRISPR-based mutagenesis. 4CL1 is known to be involved in lignin biosynthesis. 4CL5 is a paralogs of 4CL1, sharing 90% coding sequence identity with as-yet-unidentified function. 4CL2 is thought to be associated with biosynthesis of flavonoids and flavonoid-derivatives. PCR analysis of transgenic hairy roots and whole plants confirmed the presence of the Cas9 and gRNA transgenes. Sequencing of transformed events shows that the CRISPR system is able to induce indels at the targeted genes. We found evidence of reduced condensed tannin accumulation in hairy root lines derived from all three constructs, suggesting that all three 4CL genes may support the flavonoid pathway in roots. Data from stem wood lignin analysis will be presented.

S2 – P8

Key words: transcriptome, *de novo* assembly, RNA-seq, *Salix*, willow

Transcriptome sequencing and *de novo* analysis for *Salix babylonica* and *S. suchowensis* using 454 GS-FLX

Xudong He[1]*, Jiwei Zheng*[1,2]*, Kaiyue He*[2]*, Dezong Sui*[1]*, Jie Zhou*[1]*, Shizheng Shi*[1]*, Baosong Wang*[1]

[1]Department of Tree Genetics and Breeding, Jiangsu Academy of Forestry, Nanjing, Jiangsu Province, 211153 China [2]Department of Botany, Nanjing Forestry University, Nanjing, Jiangsu Province, 210037 China

The genus *Salix* (willow) serves as a great important wood source for industries, landscape planting and bioenergy. In this study, the transcriptomes of *S. babylonica* and *S. suchowensis* were sequenced using Roche 454 GS-FLX pyrosequencing technology. Totally, 280 074 and 267 030 reads with an average length of 432 bp and 398 bp for the leaves of *S. babylonica* and *S. suchowensis* were obtained each. The *de novo* assemblies yielded 4 701 contigs and 35 570 singletons for *S. babylonica*, 7 793 contigs and 47 290 singletons for *S. suchowensis* and 13 458 contigs 59 773 singletons for combined samples. Most unigenes of *S. babylonica* (80.4%) and *S. suchowensis* (77.1%) were annotated against non-redundant database. Comparison analyses showed that 58 and 15 genes significantly expressed in *S. babylonica* and *S. suchowensis*, respectively. PPI (Protein-Protein interaction) network showed a hub node of KOG3017 (POPTRDRAFT_550049). Transcription factor analysis indicated that WRKY played important roles in *S. babylonica* and *S. suchowensis*. Our work is the first deep transcriptomic analysis of *Salix*, which will be helpful for understanding the genetic architecture of *Salix* transcriptome and provides useful resources for functional genomic research in future.

Funding support: This work was financially supported by Jiangsu Provincial Natural Science Foundation (BK2011871).

S2 – P9

Key words: *Salix*, willow, expressed sequence tag (EST), simple sequence repeat (SSR), microsatellites

Development, characterization and cross-amplification of eight EST-derived microsatellites in *Salix*

Xudong He[1]*, Jiwei Zheng*[1]*, Michelle J. Serapiglia*[2]*, Lawrence B. Smart*[2]*, Shizheng Shi*[1]*, Baosong Wang*[1]

[1]Department of Tree Genetics and Breeding, Jiangsu Academy of Forestry, Nanjing, Jiangsu Province, 211153 China [2]Department of Horticulture, Cornell University, New York State Agricultural Experiment Station, 630 West North Street, Geneva, NY, 14456 USA

A set of eight simple sequence repeat (SSR) markers were developed from 707 *Salix* expressed sequence tags (ESTs) deposited in GenBank. Each of the EST-SSR amplicons was identical to the original EST, with sequence identity 60.90-96.03% and presence of the expected repeat motifs. Of the eight EST-SSR loci, five were polymorphic among 14 individuals of *S. eriocephala*, with the number of alleles per locus (N_a), observed heterozygosity (H_o), expected heterozygosity (H_e) and polymorphic information content (*PIC*) being 2-7 (mean 4.8), 0.29-0.85 (mean 0.65), 0.25-0.84 (mean 0.65) and 0.21-0.78 (mean 0.58), respectively. High rates of cross-species/genus amplification were also observed within fourteen different species. The primer sequences for the eight EST-SSRs have been deposited in the Probe database of GenBank (IDs Pr031820546–Pr031820553). The EST-SSRs developed herein would be a valuable addition of functional markers for genetics and breeding applications in a wide range of *Salix* species.

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S2 – P10

Key words:
EST-SSR,
Microsatellites,
Salix, *Populus*,
Eucalyptus

Characterization and comparison of EST-SSRs in *Salix*, *Populus* and *Eucalyptus*

Xudong He[1]*,Jiwei Zheng*[1,2]*,Dezong Sui*[1]*, Kaiyue He*[2]*, Shizheng Shi*[1]*, Baosong Wang*[1]

[1]Department of Tree Genetics and Breeding, Jiangsu Academy of Forestry, Nanjing, Jiangsu Province, 211153 [2]Department of Botany, Nanjing Forestry University, Nanjing, Jiangsu Province, 210037 China

Simple sequence repeats (SSRs) are molecular markers with great potential for plant genetic studies. With the development of high-throughput sequencing techniques, transcriptome sequencing projects provide valuable resources of expressed sequence tags (ESTs) for recovering SSRs. To investigate the variation of SSRs in different trees, three large EST databases of genus *Salix,* genus *Populus* and genus *Eucalyptus* were characterized for EST-SSRs. EST databases of *Populus* and *Eucalyptus* were downloaded from National Center for Biotechnology Information, and EST database of *Salix* was generated from 454 GS-FLX pyrosequencing in our previous work. Among the ESTs from *Salix*, *Populus* and *Eucalyptus*, 4.3%, 8.4%, and 12.2% contained SSRs, respectively. Trinucleotide repeats were the most abundant repeat class in *Salix* (47.7%)*, Populus* (38.8%) and *Eucalyptus* (44.5%), followed by dinucleotide repeats. The AG dinucleotide repeat motif was the most abundant motif detected in these trees. The length diversification of SSRs in di-, tri-, tetra-, penta- and hexanucleotide repeats in these trees was similar. There was a negative correlation between repeat motif length and the variation of repeat numbers in *Salix*, *Populus* and *Eucalyptus*. 89.9% and 86.7% primer pairs for *S. babylonica* and *S. suchowensis* were successfully used for PCR amplification. SSR loci of *Salix* in noncoding regions represented a bit higher rate than that in coding regions. Tandem repeat sequences (TRS) analysis represented the highest TRS ratio of *Populus* and the lowest ratio of *Salix*. We provide a deep insight into the characteristics of EST-SSRs of *Salix, Populus* and *Eucalyptus* in this study.

Funding support: This work was financially supported by Jiangsu Provincial Natural Science Foundation (BK2011871) and the National Natural Science Foundation of China (31300556).

S2 – P11

Key words: *Populus tremula×P. tremuloides*, cutting propagation, rooting mechanism, digital gene expression profile, Solexa sequencing

Transcriptome profile during the early development of the cuttings adventitious root of hybrid Aspen using Solexa sequencing

Qiuyu Wang[1], Shaopeng Yan[1], Daping Liu[2]

[1]School of Life Science, Northeast Forestry University, China [2]Harbin Institute of Technology, China

In this paper, hybrid aspen (*Populus tremula* × *P. tremuloides*) was used as material for plant propagation by stem cutting. Based on the cutting's morphological and anatomical changes, and physiological and biochemical characteristics during the rooting process, the cutting's transcriptome profile in different rooting stages was investigated by Solexa sequencing. The purpose is to screen rooting-related genes of the cutting's adventitious roots, as well as to comprehensively explore the molecular mechanism of rooting.

Anatomical changes of cuttings during different rooting stages shows that adventitious roots only come from root primordiums of the cortex, which originates from the phloem parenchyma cells. The callus formation on the cutting's base is not directly related to the occurrence of the adventitious roots. In other words, a callus present at the base of a cutting makes it difficult for roots to grow.

The samples in two rooting stages of the root primordia induction (the 6th day after cutting) and adventitious elongation (the 12th day after cutting) were collected for Solexa transcriptome sequencing. 21 508 differential expression genes were found and conducted the functional annotation referred to *Populus trichocarpa* (Torr. & Gray) genome database and the other public databases involved in poplar molecular resources. Most differentially expressed genes are closely related to the biological process of metabolism, signaling pathways, material transport, growth and development, energy metabolism, cell cycle by GO and Pathway analysis; some of which are being researched, including auxin transport components and transcriptional regulation factors, cell division cycle, ubiquitin-dependent protein, oxidoreducatase system, etc. Also, a number of the unreported genes are found, indicating that the rooting of cuttings is a complex biological process, which needs to be studied intensively.

Funding support: The major science and technology program of Heilongjiang province, China, Grant No. GA06B301

S2 – P12

Key words:
seed hairs,
trichomes,
fiber, poplar,
transcriptome

Comprehensive transcriptome characterization of seed hair development in *Populus tomentosa*

Meixia Ye, Zhong Chen, Lexiang Ji, Jia Wang, Weihua Liao, HuanDi Ma, Xinmin An

National Engineering Laboratory for Tree Breeding, Key Laboratory of Genetics and Breeding in Forest Trees and Ornamental Plants, The Tree and Ornamental Plant Breeding and Biotechnology Laboratory, Beijing Forestry University, Beijing 100083, P. R. China.

Poplar seed hair is an environmental annoyance in northern China due to its abundance and widespread airborne distribution. The morphogenesis and molecular mechanisms of its development are not well understood, and little attention has been focused on the dynamics of its development. To understand the mechanism of seed hair development, paraffin sections were used to examine the initiation and elongation of poplar seed hairs. RNA-seq technology was also employed to provide a comprehensive overview of transcriptional changes during seed hair development. The placenta at the base of ovary, was identified as the origin of seed hair development, which is in sharp contrast to cotton fibers that originate from epidermal cells of the seed coat. An enlarged cell nucleus in seed hair cells was also observed, which was supported by our gene ontology enrichment analysis. The significant enriched GO term of “endoreduplication” indicated that cycles of endoreduplication, bypassing normal mitosis, is the underlying mechanisms for the maintenance of the uni-cellular structure of seed hairs. By analyzing global changes in the transcriptome, many genes regulating cell cycle, cell elongation, cell well modification were identified. Additionally, in an analysis of differential expression, cellulose synthesis and cell wall biosynthesis-related biological processes were enriched, indicating that this component of fiber structure in poplar seed hairs is consistent with what is found in cotton fibers. Differentially expressed transcription factors exhibited a stage-specific up-regulation. A dramatic down-regulation was also revealed during the mid-to-late stage of poplar seed hair development, which may point to novel mechanisms regulating cell fate determination and cell elongation. This study revealed the initiation site of seed hairs and also provided a comprehensive overview of transcriptome dynamics during seed hair development. The dynamic changes in the transcriptome presented may serve as a valuable resource for developing a more complete understanding of this biological process.

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S2 – P13

Abiotic stress gene networks in poplar

Key words: abiotic stress, poplar genomics, gene networks, tree improvement

Palitha Dharmawardhana[1], *Vindhya Amarasinghe*[1], *Dylan Beorchia*[1], *Teague Green*[1], *Justin Elser*[1], *Sergei Filichkin*[1], *Eric Beers*[2], *Amy Brunner*[3], *Pankaj Jaiswal*[1]

[1]Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR, 97331 [2]Department of Horticulture, Virginia Tech, Blacksburg, VA 24061 [3]Department of Forest Resources and Environmental Conservation, Virginia Tech, Blacksburg, VA 24061, USA

Improving abiotic stress tolerance (i.e. tolerance to drought, salt and temperature) of poplar clones used in plantation forestry is of significant economic importance. We used a network based approach to better understand the complex physiological, cellular, and molecular processes that underlie abiotic stress response in poplar. To this end we have utilized, 1) a baseline poplar functional gene network constructed by orthology driven projection from the Arabidopsis probabilistic gene network, and, 2) gene co-expression networks constructed from RNAseq based abiotic stress response transcriptomes of poplar tissue types. The predicted functional gene network and co-expressed gene clusters were used to identify abiotic stress-responsive gene modules and enriched biological processes. We will discuss, 1) the sub-networks of genes and their hubs that are likely to play crucial roles during abiotic stress response, and, 2) alternative splicing pattern changes during abiotic stress response.

Funding Support: U.S. Department of Energy, award # DE-SC0008570

S2 – P14

Key words: willows, phytoremediation, soil contamination, gene expression, RNAseq

Identification of the genes induced in willow (*Salix purpurea* cv Fish Creek) in response to soil contamination

Frederic Pitre[1,2]*, Emmanuel Gonzalez*[2]*, Julie Marleau*[2]*, Vanessa Grenier*[2]*, Hafssa Kadri*[2]*, Werther Guidi Nissim*[2]*, Simon Joly*[1,2] *, Michel Labrecque*[1,2]

[1]IRBV, Montreal Botanical Garden, 4101 Sherbrooke East, Montreal, Québec, Canada. [2]IRBV, Universite de Montreal, 4101 Sherbrooke East, Montreal, Québec, Canada.

The GenoRem project develops innovative phytoremediation procedures for polluted soils, deploying the decontamination potential of willows associated with fungal and bacterial symbionts. A greenhouse trial was established with *Salix purpurea* ‘Fish Creek’, the model willow cultivar used in the project, in order to identify the transcripts differentially accumulated in response to soil contaminated with PAH, PCBs and petroleum hydrocarbons.

Information on plant physiology and organic soil pollutants was collected and a *de novo* transcriptome of leaves, stems and buds of *Salix purpurea* ‘Fish Creek’ growing in clean and contaminated soils was assembled and annotated in Trinity. The trees tolerated the pollution of the soils and did not show any reduction in the biomass produced. The transcriptome data was filtered for contaminant (i.e., non plant) transcripts, leaving transcripts of very high quality (N50 = 3385) that correspond to 47,815 clusters that indicate potential genes.

Gene expression analyses were performed on leaves, stems and buds between plants growing in contaminated and non-contaminated soil. Results show that several genes are differentially expressed (more than 5000 in the leaves only) and that several transcripts were found in more than one plant tissue. Twenty-four (24) sequences were differentially accumulated in all the tissues analyzed. One such gene was found in all aerial tissues encodes a cytochrome P450 gene family, which is involved in the oxidation of organic compounds.

The findings obtained in this project should provide basis for the development of molecular markers that can be used in the selection of superior willow genotypes for the phytoremediation of polluted soils.

Funding support: Genome Canada and Genome Quebec

S2 – P15

Key words: biofuels, biomass, salinity, *Populus alba*, transcript profiling, secondary cell wall, transcription factors

Characterization and transcript profiling of wood-related genes in poplar genotypes growing under salt stress conditions

Hortensia Sixto[1], Juan Jesús Molina-Rueda[2], Mario Sanchez[1], Alicia Garrido-Aranda[2], Isabel Cañellas[1], Fabien Mounet[3], Jacqueline Grima-Pettenati[3], Ricardo Alia[1,] Jean-Charles Leplé[4], Fernando Gallardo[2], Francisco R. Cantón[2]

[1]Centro de Investigación Forestal (CIFOR) del INIA. Ctra. de la Coruña Km 7,5, 28040, Madrid, Spain. [2]Dpto. Biología Molecular y Bioquímica. Universidad de Málaga, 29071-Málaga, Spain [3]LRSV, Laboratoire de Recherche en Sciences Végétales, Université Toulouse III, UPS, CNRS, BP 42617, Auzeville, 31326 Castanet Tolosan, France [4]INRA, UR0588 Amélioration Génétique et Physiologie Forestières (AGPF), F-45075 Orléans, France

The possibility of using lignocellulosic biomass produced from forest crops in order to obtain second generation biofuels is a key issue in the current context of climate change and the promotion of renewable energy sources. It is therefore important to make progress in the functional characterization of the candidate genes (CGs) associated with wood property traits relevant to this use as well as to determine the effect of different environmental factors (saline stress among others) on the expression of those genes. *Populus* spp. are among the forest species which rouse the most interest in the short term. Our objective is to carry out a dasometric, physiological and candidate gene expression characterization in hybrid genotypes of *Populus* which are suitable for use in short rotation growing under saline stress conditions.

The trials were conducted under controlled greenhouse conditions. *P. x canadensis* 'Oudenberg', *P. tremula x P. alba* `717-1B4 'and two autochthonous *P. alba* clones were tested from cuttings in individual pots. Two stress levels were applied: NaCl 68 and 137 mM, The parameters evaluated were, i) survival and expression of leaf necrosis symptoms, ii) relative height and diameter growth rate, iii) dry aerial and root biomass iiii) functional variables: net photosynthesis rate (A), stomatal conductance (gs) to infer intrinsecal water use efficiency (IWUE), and chlorophyll content. Significant interspecific differences were revealed with regard to salt stress responses, both in terms of damage symptomology and growth, production and functional variables. *P. alba* genotypes displayed the greatest tolerance. Transcript levels were analyzed in stem samples by Fluidigm technology for 80 selected genes related to secondary cell wall deposition (MYB and NAC transcription factors, hormone signaling, lignification, etc.). All genotypes showed changes in transcript profiles in response to salt, although the magnitude of the response was different among genotypes. About 77 % of the selected genes showed lower transcript level in plants under salt stress when compared to the salt-free treatment. The impact of salt stress on the expression of selected genes was lower in both *P. alba* genotypes, in agreement with the greatest tolerance. Funding support: MINECO (SP)-PIM2010PKB-0702; TreeforJoules (Plant KBBE ANR(FR)-10-KBBE-0007; BMBF (G)-0315914A; FCT(PT) AGR-GPL/0001/2010. Some of the candidate genes were selected in the frame of the project ANR(FR)-06-GPLA-0007, 2006-2009. RTA2011-00006-00-00.

S2 – P16

Key words:
sex differentiation
RNAseq
differential expression
positive selection

Gender-specific expression patterns and patterns of selection on gender-specific genes in *Populus balsamifera*

Li Wang[1], Zhi-qiang Wu[1], Peter Tiffin[2], and Matthew S. Olson[1]

[1]Department of Biological Sciences, Texas Tech University, Lubbock, Texas, 79409 USA [2]Department of Plant Biology, University of Minnesota, St. Paul, MN 55108, USA

Poplars are among the minority of Angiosperms that are dioecious, and one of only a handful on Angiosperms with a well annotated genome. Physiological and ecological differences between the sexes in poplars and willows have been identified in several previous studies, and we were interested in whether these differences could be detected as differential transcription. Moreover, genes with sex-biased expression exhibit elevated rates of purifying and positive selection in relatives of Arabidopsis. Using RNA-seq on flowers and leaves from 5 male and 5 female Populus balsamifera individuals, we explored three main questions: 1) to what extent do the transcriptomes differ among male flowers, female flowers, and leaves?, 2) are genes with sex-biased expression more commonly found in regions of the genome associated with sex determination?, and 3) are genes with sex-biased expression more likely to show signatures of positive selection than non-DE genes? We identified 144 genes expressed only in female flowers, 141 genes expressed only in male flowers, and only 7 genes that were differentially expressed in the leaves of males and females. These results suggest that ecological differences between males and females may not be strongly exhibited in the leaf transcriptome, or our sample sizes were too small to detect minor and important differences. Differentially expressed genes were not over-represented on chromosome 19, the chromosome predicted to carry the sex determination region, possibly resulting from dosage compensation. Overall, genes with male-specific expression were significantly more likely to exhibit dN:dS>1 than genes with female-specific expression, leaf-specific expression, and genes that were expressed in all three tissues. These results indicate a pattern of strong selection on genes associated with sex determination and flower development in Poplars.

S2 – P17

Key words:
RNA-Seq, xylem, leaf, sex linked, gene expression

Sex-influenced gene expression in *Populus trichocarpa* developing xylem and leaf tissues

Charles A. Hefer[1], *Nima Farzaneh*[1], *Armando Geraldes*[1], *Natalia Kolosova*[1], *Felix M. Nunez*[1], *Michael C. Friedman*[1], *Robert D. Guy*[2], *Juergen Ehlting*[3], *Carl J. Douglas*[1], *Shawn D. Mansfield*[4], *Quentin C. B. Cronk*[1]

[1]Department of Botany, University of British Columbia, Vancouver, Canada [2]Department of Forest and Conservation Sciences, University of British Columbia, Vancouver, Canada [3]Department of Biology and Centre for Forest Biology, University of Victoria, Victoria, Canada [4]Department of Wood Science, University of British Columbia, Vancouver, Canada

Recent advances in understanding *Populus* gender determination have identified gender-specific SNP polymorphisms, allowing the development of inexpensive and accurate genotyping assays for sex determination in *Populus trichocarpa*. Several reports have suggested that there are underlying differences in physiological, morphological and biochemical traits between genders. We have developed genetic tests for gender determination and generated large-scale gene expression datasets (whole genome transcriptome data) that together allow identification of sex-influenced gene expression patterns. We have sequenced the developing xylem and young leaf transcriptomes of 200 *Populus trichocarpa* trees, grown in a common garden (Illumina paired end sequencing). We assigned gender to these same trees using the newly developed gender determination assay We report here the gene expression patterns of genes identified within regions significantly associated with gender, and highlight differences and similarities between male and female gene expression in the total xylem and leaf transcriptome datasets. We report molecular pathways influenced by a difference in gene expression between males and females. The analysis of genes influenced by gender may aid in unraveling the molecular underpinnings of reported economically important trait differentiation between males and females in the genus *Populus*.

SESSION III

PHYSIOLOGY: INTEGRATING FORM & FUNCTION IN THE *SALICACEAE*

Oral Presentations

S3 – O1

Key words: drought resistance, phenotypic plasticity, water relations, xylem embolism

Drought-induced cavitation in poplar: synthesis and future opportunities

Régis Fichot[1], *Franck Brignolas*[1], *Reinhart Ceulemans*[2]

[1]University of Orléans, INRA, LBLGC, EA 1207, Rue de Chartres, F-45067, Orléans, France [2]University of Antwerp, Department of Biology, Research Group of Plant and Vegetation Ecology (PLECO), Universiteitsplein 1, B-2610, Wilrijk, Belgium

To assimilate carbon dioxide, trees unavoidably lose large amounts of water through opened stomata. Leaf transpiration thus creates tensions (negative pressures) in the hydraulic pipeline placing the xylem conduits under the constant threat of cavitation, i.e. a sudden phase change of water from liquid to vapor. Drought-induced cavitation events take place in the bordered pits at the interface between water-filled and air-filled conduits, when the xylem tension overcomes the capillary forces holding water in the pit membrane. This leads to embolized (non-functional) conducting elements and decreased hydraulic conductivity ultimately threatening plant survival. During the last 25 years, considerable knowledge has been gained with regard to the anatomical, physiological and ecological correlates of vulnerability to drought-induced cavitation. The number of species characterized has steadily expanded and recent findings suggest that vulnerability to drought-induced cavitation is a key trait in determining tree maximum recoverable water stress and therefore the adaptive potential of tree species.

Poplars are among the fastest growing temperate hardwood trees, a characteristic which comes at the expense of a high sensitivity to water limitation. Therefore, the response of poplar to drought has been extensively investigated, although most studies have focused on acclimation mechanisms within physiological boundaries. Comparatively, much less is known regarding functional limits. In the current context of global change, xylem vulnerability to cavitation may be especially relevant in the prospect of selecting resistant genotypes to severe drought and in evaluating the adaptive potential of natural populations. We propose here a synthesis of all literature published data on drought-induced cavitation in poplar; genetic variations among/within pure species or hybrids, acclimation in response to environmental factors (drought, nutrients, light…) and coordination with other aspects of tree water and carbon relations will be discussed. Practical implications, actual gaps in knowledge and future research opportunities will be finally presented.

S3 – O2

Key words:
water-use efficiency,
variability,
plasticity,
environment,
biomass production

Water-use efficiency in hybrid poplars: an overview of 15 years of research

Nicolas Marron[1,2,3], Romain Monclus[1,3], Ludovic Bonhomme[1,4,5], Régis Fichot[1], Sylvain Chamaillard[1], Fahad Rasheed[1,3,6], Julien Toillon[1,2,3], Catherine Bastien[7], Erwin Dreyer[2,3], Franck Brignolas[1]

[1]University of Orléans, INRA, LBLGC, EA 1207, Rue de Chartres, F-45067, Orléans, France [2]Université de Lorraine, UMR 1137 Écologie et Écophysiologie Forestières (EEF), F-54500 Vandœuvre-lès-Nancy, France [3]INRA, UMR 1137 Écologie et Écophysiologie Forestières (EEF), route d'Amance, F-54280 Champenoux, France [4]Université de Clermont-Ferrand, UMR 1095 Génétique, Diversité et Écophysiologie des Céréales (GDEC), F-63171 Aubière, France [5]INRA, UMR 1095 Génétique, Diversité et Écophysiologie des Céréales (GDEC), F-63039 Clermont-Ferrand, France [6]Institut des Sciences de la Forêt Tempérée (ISFORT), Université du Québec en Outaouais, Gatineau (Québec), Canada [7]INRA, UR 588 Amélioration, Génétique et Physiologie Forestières (AGPF), 45075 Orléans, France

Because of their impressive growth rates, interspecific hybrid poplars are among the most extensively cultivated trees in temperate latitudes. In line with their general riparian origin, poplar trees are generally highly water-demanding such that the productivity of poplar plantations is tightly dependent on adequate water availability. However, in the current context of rational management of water resources, optimizing the carbon-water trade-off of such plantations is particularly important. Water-use efficiency (WUE, *i.e.* the amount of plant biomass produced per unit water used) has therefore emerged as a new relevant criterion to be accounted for in poplar selection, in conjunction with other common criteria already used such as disease resistance, productivity and wood quality.

We propose here an overview of nearly 15 years of research on WUE in poplar hybrids (*Populus deltoides* × *P. nigra* and *P. deltoides* × *P. trichocarpa*, the two most commonly interspecific combinations used in Europe). These results were obtained under the framework of seven Ph.D. theses conducted along the 2000-2013 period. Four main questions will be addressed: *(1)* Is bulk leaf carbon isotope discrimination a good estimator of WUE assessed either at the leaf level (intrinsic WUE, *i.e.* net CO_2 assimilation rate / stomatal conductance to water vapour) or at the whole-plant level (transpiration efficiency, *i.e.* biomass produced / water loss)? *(2)* To what extent does WUE vary among and within hybrid crosses and are hybrid rankings for WUE stable with ageing? *(3)* How do environmental conditions (drought, planting density, soil fertility) affect WUE? and *(4)* Are WUE and biomass production related and is this relationship modulated by environmental factors?

The practical implications opened up by the results and the future research opportunities will then be presented.

S3 – O3

Key words: black poplar, leaf traits, phenotypic plasticity, genetic variation, meta-population differentiation

Phenotypic plasticity and genetic differentiation for morphological and functional leaf traits in nine metapopulations of black poplar (*Populus nigra* L.)

Justine Guet[1,2,3], *Francesco Fabbrini*[4], *Maurizio Sabatti*[4], *Catherine Bastien*[3], *Franck Brignolas*[1,2]

[1]UFR–Faculté des Sciences, UPRES EA 1207 'Laboratoire de Biologie des Ligneux et des Grandes Cultures' (LBLGC), Université d'Orléans, rue de Chartres, BP 6759, F–45067 Orléans Cedex 2, France [2]INRA, USC1328 'Arbres et Réponses aux Contraintes Hydriques et Environnementales' (ARCHE), rue de Chartres, BP 6759, F–45067 Orléans Cedex 2, France [3]INRA, UR 0588 'Amélioration, Génétique et Physiologie Forestières' (AGPF), centre de recherche Val de Loire, CS 40001 Ardon, F–45075 Orléans Cedex 2, France [4]Department for Innovation in Biological, Agro-food and Forest systems (DIBAF), University of Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy

Black poplar (*Populus nigra* L.) is a major tree species of riparian ecosystems in Europe that covers a wide range of pedoclimatic conditions and river dynamics, which may have promoted the development of adaptive strategies in natural populations. This species is of economic importance as a parental pool used in many breeding programs for the development of *P. deltoides* × *P. nigra* hybrids cultivated for biomass and wood production. The present study aimed at deciphering the components of phenotypic variation for a set of morphological, structural and functional leaf traits related to growth and water-use efficiency. Twenty-four to 62 *P. nigra* individuals sampled in nine European natural metapopulations were established in replicated clonal tests in two experimental sites contrasted for soil texture and fertility. The relative importance of site, genetic and Genotype × Site (G × S) interaction effects on total phenotypic variation of leaf traits was estimated. In the most favourable site for growth, individual area of mature and fully illuminated leaves increased by an average of 150 % while bulk leaf carbon isotope discrimination ($\Delta^{13}C$) decreased by an average of 2.5 ‰. In the two sites, substantial genetic variation was expressed within the nine *P. nigra* metapopulations for leaf traits. As indicated by G × S interaction variance comparable or even greater than genetic effects, the expression of genetic variation was significantly modulated by the site for all metapopulations. For individual leaf area, G × S interactions were explained by both changes in genotype ranking and increased genetic variation in the most favourable site while these interactions were mainly attributed to changes of genotype ranking across sites for $\Delta^{13}C$. Despite high intra-metapopulation genetic variation, the genetic differentiation among the nine metapopulations was high ($0.42 \leq Q_{ST} \leq 0.51$) for all traits related to leaf morphology, such as individual leaf area, petiole length and leaf shape, and its pattern was conserved in the two experimental sites. In contrast, $\Delta^{13}C$ exhibited a moderate level of metapopulation differentiation ($Q_{ST} \approx 0.25$) with significant changes of metapopulation clustering between the two sites. These results will be discussed considering the adaptive potential of black poplar to variations in environmental conditions.

Funding support: the study was carried out with financial support from the NOVELTREE project (FP7 – 211868). J.G. was supported by a PhD grant from the 'Conseil Régional, Région Centre, France'. The participation to the Sixth International Poplar Symposium is supported by a grant from the Foundation Dufrenoy.

S3 – O4

Key words:
aquaporins,
poplar, water
use efficiency

Aquaporins – tools to improve water use efficiency in poplar?

Katja Block[1], Zhen Bi[1], Juliane Merl[2], Stefanie Hauck[2], Ina Zimmer[1] and Jörg-Peter Schnitzler[1]*

[1]Research Unit Environmental Simulation, Helmholtz Zentrum München, Neuherberg, Germany [2]Core Facility Proteomics, Helmholtz Zentrum München, Neuherberg, Germany

Aquaporins are integral membrane proteins from a larger family of major intrinsic proteins (MIP) that form pores in the membrane of biological cells. They are water channel proteins that control the flow of water across cellular membranes and play vital roles in all aspects of plant-water relations.

Aquaporins in plants are separated into four main homologous subfamilies: Plasma membrane Intrinsic Protein (PIP), Tonoplast Intrinsic Protein (TIP), Nodulin-26 like Intrinsic Protein (NIP), and Small basic Intrinsic Protein (SIP). PIPs possess a uniform signature characteristic for highly specific water transport. The PIP subfamily in poplar consists of 15 members and can be furthermore divided into two groups (PIP1 and PIP2). It is suggested that the different PIP isoforms have functions in a number of cellular processes; however in poplar they have not yet been characterized in great detail.

In the presented project, we identified and characterized the entire PIP subfamily in grey poplar (*Populus* x *canescens*). Moreover, we developed transgenic *P*. × *canescens* lines in which PIP genes are down-regulated by RNA interference (RNAi) strategy to elucidate PIP specific functions. Under normal cultivation condition, GM poplars with reduced PIP content showed a distinct leaf phenotype with smaller total leaf area, but no general growth impairment. Physiological screening revealed differences in net CO_2 assimilation and transpiration rates compared to wild type plants pointing to modified H_2O and CO_2 conductance within the leaf. The application of fluorescence markers for the symplast and apoplast also supports changes in leaf water transport pathways.

Dependent on the role of PIPs in water metabolism, we will discuss the contribution of aquaporins to the water use efficiency in poplar. Additionally, since some previous findings from model herbaceous plants show that PIPs also can transport CO_2, we furthermore address the proposed function of poplar PIPs as CO_2 pores. Funding support: PRO-BIOPA, part of BMBF program 'Bioenergie2021

S3 – O5

Key words: drought, water use efficiency, $\delta^{13}C$, $\delta^{18}O$, QTL

Phenotyping for assessing genetic variation in water use across 474 willow (*Salix*) genotypes grown in contrasting water regimes in Italy and Sweden

Friderike Beyer, Martin Weih

Department of Crop Production Ecology, Swedish University of Agricultural Sciences, Uppsala / Sweden

To date, breeding of *Salix* increased its biomass yield by 60 %. However, the breeding history of willow is short and therefore there is great potential for crop improvements. Particularly in regard to global warming the drought tolerance of energy crops is becoming more important as well as the efficient use of nitrogen as both characteristics are directly linked to key physiological processes and biomass accumulation.

In the project “Salix Molecular Breeding Actions (SAMBA)” (www.samba-webb.se) we are analysing the drought tolerance of 474 genotypes (*Salix viminalis x S. schwerinii* cross) each replicated 6-fold in a field experiment in Sweden and Northern Italy, here with an irrigated and non-irrigated treatment. We analysed the water economy of the plants with a dual isotope approach. Three leaves at different plant heights were analysed for their $\delta^{13}C$ (water use efficiency) and $\delta^{18}O$ (transpiration rate) signatures. By calculating the ratio of the two ($\delta^{13}C/^{18}O$) we derived the assimilatory capacity of the leaves. The leaf water status is influenced by the total leaf area and thereby the productivity of the whole plant. We found high allometric relationships between total leaf area and diameter at breast height in a pilot study. Water economy traits were thus assessed at leaf level (isotope signatures) and whole plant level (stem diameter indicative of total leaf area) for individual genotypes field-grown in different environments (Sweden and Italy) and treatments (drought and well-watered). We will present data on the genotype and environmental variation in water economy traits at leaf and whole plant level across the 474 genotypes assessed in two locations. The data will also be used for QTL (quantitative trait loci) analysis to identify candidate genes.

The overall goal of the study is to support breeding development by identifying ecophysiological traits which are relevant for maintaining or increasing biomass yield at decreased water use. Funding support: Swedish Energy Agency (30599-3)

S3 – O6

Key words:
environment,
genotypes,
growth
dynamics,
QTL-based
model, Salix

Integrating new evidence into a sink-source interaction model to improve the selection efficiency for high yielding *Salix* species

Goetz M Richter, Marianna Cerasuolo, Benjamin Richard, Jennifer Cunniff, Steve Hanley, Angela Karp

Rothamsted Research, Harpenden, Herts., AL5 2JQ, United Kingdom

Quantitative, process-based models have contributed much to understand crop growth and the interaction between phenotypic properties of plants and their environment. These models are ideally suited to focus on those components that are related to key traits. The ambition is to narrow the phenotype and genotype gap and to enhance crop selection and productivity with model and marker assisted breeding. Traits and processes are dissected into their elements and related to the respective quantitative trait loci (QTL), and eventually, single or groups of genes are identified that control key processes.

A new sink-source interaction model is presented that was developed to simulate the growth of short rotation coppice willow. It describes pheno-morphological development, light interception and its conversion to dry matter, which is allocated depending on the *Salix* species. Different empirical and mechanistic models for the onset of growth (bud flush) are proposed. Partitioning is controlled by genotype-specific ratios, which determine the genotypic source and sink strength dynamically in dependence of various environmental factors. Phenotypic trait data from a dedicated trial and mapping populations were used to calibrate the model on the basis of *a priori* knowledge taken from the literature. Embedded in a stochastic simulation framework (global sensitivity analysis, Bayesian calibration) the model assists to identify key traits and assess their uncertainty.

We demonstrate the relationship between process simulation and QTL for two examples: (1) parameters that control bud flush in response to day length and temperature variation, and (2) partitioning of structural and reserve carbohydrate into above- and belowground organs during seasons and growth cycles in two different environments. The model enables us to conduct experiments on the computer (*in silico*) to develop and test hypotheses to design experiments and ideotypes.

Funding support: Institute Strategic Program Grant “Cropping Carbon” UK Biotechnology and Biological Science Research Council (BBSRC) and the project www.BSBEC-BioMASS.org within the BBSRC Sustainable Bioenergy Centre.

S3 – O7

Key words: biomass partitioning, climate, non-structural carbohydrates, reserves

Phenological dynamics of above and below ground biomass and non-structural carbohydrates in the perennial bioenergy crop willow *Salix* (spp.)

Jennifer Cunniff[1], *Sarah J. Purdy*[2], *Timothy Barraclough*[1], *March Castle*[1], *Ian Shield*[1], *Anne L. Maddison*[2], *Laurence E. Jones*[2], *Goetz Richter*[1], *John P. Andralojc*[1], *Angela Karp*[1]

[1]Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ [2]Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, Penglais, Aberystwyth, Ceredigion, SY23 3DA, UK

To reduce fossil fuel contributions to climate change perennial bioenergy crops are being developed for lignocellulosic derived fuels. One promising bioenergy crop is willow, and as part of the UK BBSRC Sustainable Bioenergy Centre we aim to increase willow yield through the identification and manipulation of key traits involved in dry matter production.

Currently, the dynamics of carbon assimilation and partitioning between above ground (shoots) and below ground (roots) is poorly understood. It is unknown whether there are differences in partitioning between willow genotypes and whether capacity exists to increase above ground harvestable biomass.

Four genotypes of willow were grown in replicated trials at Rothamsted Research (SE England) and Aberystwyth (West Wales). Total biomass and abundance of non-structural carbohydrates (NSC) were quantified in the above ground and below ground organs at key phenological stages. Meteorological conditions were monitored.

Biomass partitioning differed between sites and genotypes, with genotypes in Aberystwyth partitioning more biomass below ground. It is hypothesised that a climate of low PAR, temperature and importantly high winds is responsible for the phenotype. NSC peaked in above ground tissues during June, yet peak biomass was reached in September showing that soluble sugars were being converted to structural biomass in early autumn, whilst simultaneously being translocated below ground. In fact, translocation of resources below grounds begins early in the season, as soon as photosynthesis starts. Patterns of NSC dynamics were similar between sites and genotypes; however levels of NSCs were lower at Aberystwyth. Lower levels of reserves (belowground) are hypothesised to control stem numbers which were much lower in Aberystwyth. This study reveals differences in partitioning between willow genotypes presenting scope for manipulation of the trait and explores how environmental conditions influence the phenotype.

S3 – O8

Key words: nitrogen, water, drought, intra-specific variation

Intra-specific variation in poplars: growth responses to nitrogen fertilization and drought

Andrea Polle, *Dejuan Euring, Bai Hua, Dennis Janz*

Forstbotanik und Baumphysiologie, Georg-August Universität Göttingen, Göttingen, Germany

Poplar plantations can be used for sustainable biomass production serving as a feedstock for bio-energy. Most fast growing poplar species currently used for plantations are hybrids with a high demand for water and nutrients, whereas species of the section aspen (*P. tremula*), which are wide-spread in Europe, occur on marginal soils, in forests and on dry soil. These species must therefore be adapted to a large range of different environmental conditions. Commercial poplar clones have high productivity, but have higher resource demand for optimal growth in plantations. The aim of the present study was to investigate the intra-specific variation of growth, physiological performance, nitrogen use and gene expression of nitrate transporters (NRT) in response to N fertilization and drought and to analyze the nitrogen use efficiency of poplars in comparison with other potential biomass crops.

We found strong growth differences between different wild poplar progenies. The responsiveness of the progenies to drought and N varied strongly. Whole-plant analysis of N, C, and 15N-uptake revealed that plant with higher biomass production had a lower C/N ratio suggesting increased nitrogen use efficiency. N fertilization resulted in a shift of the C/N ratio indicating N storage rather than use for current growth. Based on previous analysis of the nitrate transporter family (with 79 members in poplar in three clades, Hua et al. 2013 Plos One 8(8): e72126), the most prominent NRTs were selected and studied in three contrasting demes. Principle component analysis pointed to distinct functions of NRTs in roots and leaves with consequences for N uptake and growth. The correlation between distinct NRTs and growth suggests that NRTs could be suitable targets to identify aspens with high biomass production and nitrogen use efficiency. Therefore these analyses were extended to commercial poplar clones in biomass plantations. The results of these studies will be presented.

Funding support: This work was supported by the Bundesministerium für Bildung und Forschung (BMBF) under project“Bioenergie Regionen Stärken”(BEST), the European Commission within the Seventh Framework Program for Research, Project Energypoplar (FP7-211917) and by the German Academic Exchange Service (DAAD) funding PhD scholarships to HB and DE

S3 – O9

Key words: transgenomics, *Populus euphratica*, salt-tolerance, functional genomics, *Arabidopsis thaliana*

An assessment of transgenomics as a tool for gene or gene cluster discovery in *Populus euphratica* Oliv.

Jing Zhou, Shu-Tang Zhao, Tie-Long Cheng, Meng-Zhu Lu

State Key Laboratory of Tree Genetics and Breeding, Research Institute of Forestry, Chinese Academy of Forestry, Beijing 100091, China.

Transgenomics becomes a member of omic family that introduce gene(s) from one organism into another in a genome-wide scale and then the transgenic recipients varied in phenotypes are screened. This strategy allows us to find gene or gene cluster involved in phenotypic differences between the transgenic plant genes and non-transgenic plant genes. It is particularly useful for woody plants that have a long life cycle, with a difficulty in manipulation at laboratory and limited molecular and genetic resources, etc. A large-insert BIBAC (binary bacterial artificial chromosome) genomic library of *Populus euphratica*, a stress-tolerance woody species, was constructed, comprising 55,296 clones with 127Kb inserts in average. And 1077 clones were transformed into Arabidopsis thaliana via *Agrobacterium tumefaciens* by floral dipping method. Fifty-nine transgenic lines have shown phenotypic changes represented by diverse aspects of plant form and development, 15 of which were repeatedly associated with the same phenotypic change. In addition, transgenic plants with clone 002A1F06 had shown increased salt tolerance comparing to the wild type. The 127,284bp insert in this clone harbors 8 genes reported to be involved in stress resistance. This study demonstrated that transgenomics is particularly useful to identify the functional genomics of woody plants and novel gene(s) or gene cluster responsible for economic traits, thus this tool could be used for validation of QTLs mapped by molecular markers.

Funding support: Special Fund on Essential Research for National Non-profit Institutions to Chinese Academy of Forestry (CAFYBB2011001)

S3 – O10

Key words: poplar, black cottonwood, cuticle, waxes, natural diversity

Cuticular waxes in *Populus trichocarpa* leaves: The diversity is within you

Eliana Gonzales-Vigil[1], *Charles Hefer*[2], *Michelle von Loessl*[1], *Nima Farzaneh*[2], *Carl J. Douglas*[2], *and Shawn D. Mansfield*[1]

[1]Department of Wood Sciences, University of British Columbia, Vancouver, British Columbia, V6T 1Z4 Canada [2]Department of Botany, University of British Columbia, Vancouver, British Columbia, V6T 1Z4 Canada

The cuticle is the extracellular lipid structure that covers all the aerial surfaces of land plants, acting as the interface between the plant and the environment. The cuticle is composed of a polymer matrix called cutin and a variety of aliphatic hydrocarbons, collectively called waxes. The objective of this study was to assess the diversity in cuticular wax components in *P. trichocarpa* as a first step towards identifying the genes involved in the biosynthesis and regulation of wax. To address this goal, the variation in wax load and composition was determined in the leaves of 37 genotypes of *P. trichocarpa*. From each tree, nine leaves were taken (plastochron index 2 through 10). This allowed us to obtain a dynamic view of the waxes with leaf development. The results show diversity in both qualitative and quantitative composition across trees. However, large changes in wax composition are observed in leaves from different plastochron index. Cluster analysis segregated the samples according to their developmental stage, with leaves of equivalent stage clustering together regardless of the genotypic origin. In addition, *P. trichocarpa* was found to produce large quantities of alkenes; compounds mainly found as minor components in the wax of other plants. Nevertheless, a small number of trees are deficient in the production of alkenes. We hypothesize that comparing the leaf transcriptome of trees producing and not producing alkenes could lead us to the identification of the enzymes responsible for the biosynthesis of alkenes in *P. trichocarpa*.

Funding support: Genome Canada (POPCAN project)

S3 – O11

Regulation of tension wood formation in *Populus*.

Key words: poplar, wood development, RNA sequencing, tension wood, KNOX transcription factors

Suzanne Gerttula[1]*, Matthew Zinkgraf*[1]*, Lijun Liu*[1]*, Andrew Groover*[1,2]

[1]US Forest Service, Pacific Southwest Research Station, Davis CA USA [2]Department of Plant Biology, University of California Davis, USA

In *Populus*, tension wood forms to counteract mechanical stress, such as the gravitational force on leaning stems or branches. Tension wood is characterized by a tertiary cell wall layer called the gelatinous (or G) layer, which is highly enriched in cellulose and is thought to generate the force that enables woody tissues to reorient and to “pull” the stem or branch. We are investigating the role of gene expression and hormone signaling in tension wood formation in *Populus*, and report here that the over-expression or miRNA-knockdown of two Class I KNOX transcription factors, ARK1 and ARK2, can alter the production of tension wood. In this study, four major findings emerged. First, using time-lapse photography we have found dramatic differences between our KNOX mutant genotypes vs. wild type *Populus* in both the rate and shape of stem bending. Second, histological examination of the zone of bending has identified differences in the production of secondary and tertiary cell walls of the mutant genotypes compared to wildtype. Third, RNA sequencing of stem cambial and developing xylem tissues after 48 hours of gravity induction showed extensive reprogramming of the transcriptome in both tension wood and opposite wood. Fourth, a network analysis of differentially co-expressed genes identified genotype specific gene modules in response to gravity and these modules contained transcription factors with hormone signaling and cell wall related enzymes. Taken together, this system serves as a model of regulation of the vascular cambium and can be used to better understand the intricacies and interplay of environmental stresses, genetic regulation and hormonal strategies involved in plant tropic responses. Ultimately, the ability to manipulate tension wood formation has potential application for *Populus* lumber, pulp, nanotechnology, and biofuels production.

Funding support: Agriculture and Food Research Initiative (AFRI), U.S. Forest Service.

S3 – O12

Key words:
target planting stock, plantation, seedling ecology, early establishment, nursery conditions

Assessing and manipulating planting stock quality in ***Populus***

Simon M. Landhäusser

Department of Renewable Resources, University of Alberta, Edmonton, Alberta, T6G 2E3, Canada

It is well understood that access to good quality planting stock is essential to achieve establishment success and promote early growth of planted trees. Generally, defining planting stock quality is difficult, as it is specific to the ecology of species and site conditions. In comparison to the vast amount of information that is available for the quality assessment of planning stock used for reforestation, relatively little knowledge exists on the characteristics of planting stock indicating their suitability for afforestation. In northern climates much of the nursery stock research has focused on the production of coniferous species, while much less research has been dedicated to the study of deciduous trees species, particularly those species with indeterminate growth strategies such as in the *Salicaceae*. In this presentation I will present results from a range of studies that explore the role of planting stock characteristics (such as size, growth strategy, root to shoot ratio, and nutrient and carbohydrate reserve status) as an assessment tool for stock quality and discuss their potential manipulation in a nursery setting. Further, since many afforestation areas have challenging initial site conditions, I will link these planting stock design techniques to specific site conditions.

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SESSION III

PHYSIOLOGY: INTEGRATING FORM & FUNCTION IN THE *SALICACEAE*

Poster Presentations

S3 – P1

Key words: abiotic stress-response, ABA-response, poplar species, histochemical analysis

Expression analysis on abiotic stress-response genes of poplar species

Xiang Yu[1], *Uenoyama Misa*[2], *Tokihiko Nanjyo*[3], *Misato Ohtani*[1,2], *Taku Demura*[1,2]

[1]RIKEN Center for Sustainable Resource Science, Japan [2]Graduate School of Biological Sciences, NAIST, Japan [3]Forestry and Forest Products Research Institute, Japan

Recent climatic problems have increased the demand of woody plants as key players in environmental protection and as a renewable resource for industry and energy. For future forestation, it is critical to decrease the influence from abiotic stresses, such as drought and salinity. However, it is difficult to taylor trees to tolerate abiotic stresses, since the knowledge on the response mechanism in trees is still limited. Here, we analyzed expression profiles of poplar (*Populus tirchocarpa*) genes during stress response. From microarray analysis data of *P. nigra*, we selected five genes that were up-regulated by salt and/or drought stress treatments. The five poplar genes were respectively named P1, P2, P3, P56 and P81 in this study. In the promoter regions of these genes, well-known cis-acting elements were related to dehydration- or ABA- induced expression. Quantitative RT-PCR analysis revealed the five poplar genes are mainly up-regulated in leaves and roots of *P. tirchocarpa* when the plants are exposed to abiotic stress or ABA treatment. In addition, we generated promoter::GUS reporter lines of poplar (*P. tremula* × *tremuloides*) for all five genes, and found that expression of these genes was induced in leaves undergoing dehydration. Altogether, expression of the five genes is commonly up-regulated in poplar species, *P. nigra*, *P. tirchocarpa*, *P. tremula* × *tremuloides*. These results suggest that the five genes might have fundamental roles in the abiotic stress-response mechanism of poplar species.

S3 – P2

Key words: poplar hybrids, drought tolerance, gene expression, global warming

Functional assessment of candidate genes involved in drought tolerance of *Populus*

Muhammad Arshad, Aine Plant, Jim Mattsson

Department of Biological Sciences, Simon Fraser University, Burnaby, BC, V5A 1S6

Populus species and derived hybrids are valued for their fast growth and are cultivated all over the Northern hemisphere, primarily for pulp, paper and board production. Fast growing poplar also has potential to be used for carbon sequestration as well as a feedstock for carbon-neutral production of energy. Many of the commonly used species and hybrids are, however, regarded as drought sensitive, which poses a problem for large-scale cultivation, particularly in light of climate change-induced drought spells in areas of poplar growth. While hundreds of drought-induced genes have been identified in poplar species, the contribution of individual genes to drought tolerance is unclear. We ranked nine commonly used poplar clones' ability to withstand drought based on a series of physiological and morphological responses, and used the least and the most drought tolerant clones to identify genes those expression levels correlated with drought tolerance. Among the identified candidate genes, a predicted positive regulator of drought tolerance was expressed at higher levels in the drought tolerant clone, and a predicted negative regulator was expressed at lower levels in the drought tolerant clone, relative to the drought sensitive clone. Over-expression of the predicted positive regulator, an NCED3-like gene potentially involved in ABA biosynthesis, resulted in Arabidopsis plants with elevated ABA levels and enhanced drought tolerance, whereas over-expression of the predicted negative regulator, a PP2C-like gene potentially involved in ABA signaling, resulted in plants with reduced drought tolerance, in line with their putative functions. Similarly, over-expression of the NCED3-like gene in transgenic poplar resulted in plants with moderately enhanced drought tolerance coupled with minimal effects on overall growth. Taken together, the obtained results validates our approach to identifying genes of particular relevance for drought tolerance in poplar, including two strong candidate genes for targeted improvement of drought tolerance in poplar hybrids.

S3 – P3

Key words:
drought,
Populus,
sucrose
transport

The tonoplast sucrose transporter of *Populus* facilitates plant-wide coordination of the drought response

Scott Harding[1,2], *Liang-Jiao Xue*[1,2], *Chris Frost*[1,2], *Chung-Jui Tsai*[1,2]

Shoot biomass distribution, water uptake and phytochemical response to drought stress are altered in poplar plants with plant-wide, uniformly reduced expression of a tonoplast sucrose efflux transporter, *PtaSUT4*. A transcriptomic investigation of plants exposed to a drought-recovery cycle was undertaken to identify protein sensors and mediators of systemic carbon and water use that respond to reduced subcellular sucrose trafficking. *SUT4* expression did oscillate during the treatment cycle thereby providing evidence of a role in plant dynamic responses to stress. In wild-type plants, *SUT4* transcript abundance exhibited tightly correlated expression changes in roots, xylem and bark throughout the drought-recovery cycle. Increased concentration of sucrose in the cytosol was therefore identified as a SUT4-mediated and systemically coordinated driver of water trafficking through symplastically connected tissues. This coordination was not observed in RNAi-SUT4 plants. Transcript abundances of water-trafficking aquaporins followed different stress-recovery response trajectories in wild-type and SUT4-RNAi plants, consistent with a prediction of altered hydraulic gradients. Roots were more negatively affected than other organs in drought-stressed SUT4-RNAi plants and this was linked to altered continuity of stem hydraulic conductivity during soil drying. Transcript abundance of sulfur assimilating proteins, bark storage and heat-shock proteins, among others, exhibited contrasting pre-drought, drought and recovery patterns in wild-type and RNAi plants. Overall, it appeared that RNAi inhibition of metabolic carbon and water trafficking not only promoted greater root stress, but enhanced the plant-wide, redox-related response to that stress. Drought response transcript networks were altered in transgenic roots consistent with a reduced sucrose component and enhanced ABA component compared to wild-type roots.

Funding support: The work was supported by the US Department of Energy, Office of Biological and Environmental Research grant no. DE-SC0005140.

S3 – P4

Key words:
glutamine synthetase,
transgenics,
nitrogen assimilation,
drought,
ecophysiology

Carbon allocation and plant water use efficiency in transgenic poplar with ectopic expression of pine cytosolic glutamine synthetase (GS1a)

Arman Yalcin, Karina VR Schäfer, and Edward Kirby

Department of Biological Sciences, Rutgers University, Newark, NJ 07102, USA

Glutamine synthetase (GS) is a key factor in the assimilation of nitrogen in plants. Hybrid poplar plants (*Populus tremula X P. alba,* INRA 717-1-B4) ectopically expressing pine GS display increased growth rates, increased nitrogen use efficiency, and resistance to drought. In order to assess mechanisms associated with observed drought tolerance of GS poplars, we studied the ability of the GS poplars to perform photosynthesis under control and water-limiting conditions, as well as their nitrogen and carbon isotope contents. Included in this study were carboxylation efficiency, maximum assimilation rate, quantum yield of photosynthesis, maximum electron transport rate, dark respiration, triose phosphate utilization, light compensation point, intrinsic water use efficiency, stomatal conductance, and nitrogen-use efficiency. Rooted cuttings (12-18 months) were grown in a growth chamber (24^{o} C; 16-hour photoperiod; approx. 330 µmol m^{-2} s^{-1}). Well-watered conditions were defined as a soil moisture value of over 0.40 $m^3 \cdot m^{-3}$, and drought conditions were defined as having a value of less than 0.20 $m^3 \cdot m^{-3}$. The results showed significant differences between the GS transgenic and wild type plants. Under drought and control conditions, GS poplars showed carboxylation efficiencies similar to the wild type grown under well watered and drought conditions. Photosynthetic capacities were not different regardless of growth condition or genotype. However, the GS poplars showed lower water use efficiency when compared to the wild type, but higher nitrogen use efficiency. These data suggest that, compared to the wild type, GS poplars display a tradeoff between water and nitrogen use efficiency and are characterized by slightly enhanced photosynthesis, even during drought stress.

Funding support: Consortium for Plant Biotechnology Research; ArborGen Inc.

S3 – P5

Key words: *Salix eriocephala*, AgCan*Salix*, phenology, ecophysiology, carbon and nitrogen isotopes

Genetic variation in phenology and ecophysiology among natural populations of *Salix eriocephala* Michaux.

Raju Y. Soolanayakanahally[1]*, Hamid A. Naeem*[1]*, William R. Schroeder*[1]*, Robert D. Guy*[2]

[1]Agroforestry Development Center, Agriculture and Agri-Food Canada, Indian Head, Saskatchewan, S0G 2K0 Canada [2]Department of Forest and Conservation Sciences, University of British Columbia, Vancouver, British Columbia, V6T 1Z4 Canada

Native willows (*Salix* spp.) occupying broad-ranges display strong local adaptation to differences in growing season length and climate. By making use of over 500 *Salix eriocephala* L. genotypes sampled from its natural range across Canada, we studied variation in seasonal phenology and growth rates planted into a common garden at Indian Head, Saskatchewan (50.33°N, 103.39°W). There were no differences in spring leaf-out dates among continental (Prairies) and coastal (Atlantic) Canadian populations, but autumn leaf discoloration varied by 2 weeks. Willow genotypes sourced from the Canadian prairies grew faster and accumulated higher stem biomass, while the Atlantic Canada genotypes grew much slower and accumulated less stem biomass. We observed large within-populations differences in foliar carbon isotopic compositions ($\delta^{13}C$) ranging between -25.4‰ to -31.1‰; while $\delta^{15}N$ varied almost by 15.5‰. The Prairie genotypes had higher leaf mass per area (LMA) and lower stomatal density. Natural infection by leaf rust was visually scored on a weekly basis from onset of autumn (Sep 21) with higher infection rates observed among Prairie genotypes on any given day. The outcomes from this study provide valuable insight into the levels of natural variation available in the Agriculture and Agri-Food Canada's AgCan*Salix* collection for use in the willow improvement program.

Funding support: Agriculture and Agri-Food Canada

S3 – P6

Key words:
mesophyll conductance,
balsam poplar,
leaf anatomy,
leaf structure,
liquid phase

Anatomical limitations to the mesophyll conductance of balsam poplar (*Populus balsamifera* L.)

Estefania Milla Moreno[1], *Robert D. Guy*[1], *Raju Soolanayakanahally*[2]

[1]Department of Forest and Conservation Sciences, University of British Columbia. 2424 Main Mall. Vancouver, BC. Canada. V6T 1Z4. [2]Science and Technology Branch, Agriculture and Agri-Food Canada, Indian Head, Saskatchewan, Canada. S0G 2K0

The mesophyll conductance (g_m) has been defined as a measure of the ease with which CO_2 may diffuse from substomatal cavities to the sites of carboxylation. g_m is inversely proportional to the pathway length in the gas phase and the structural resistances encountered in the liquid phase. Many of these attributes can be measured microscopically. There is evidence that within native balsam poplar populations, g_m may increase with latitude of origin as does leaf mass (LMA) and nitrogen per unit leaf area. A balsam poplar family consisting of 17 genotypes from a cross between a female tree of subarctic provenance (Kuujjuaq QC; 58°02′ N latitude - 68°65′ W longitude) and a polymix of pollen from three males of prairie origin (Carnduff SK; 49°18′ N latitude -101°83′ W longitude) was chosen to investigate anatomical limitations to g_m. Preliminary results of this study show that LMA is a good proxy for leaf thickness (r^2=0.93) and also for intercellular air space (r^2=0.74). However, N content per unit mass and cell wall thickness are not correlated with LMA (r^2=0.0005 and r^2=0.22, respectively) among genotypes. This implies that although an increase in leaf thickness may increase the diffusion path length (decreasing g_m) it may also increase the cell wall area available for liquid phase diffusion (improving g_m). Further work is being undertaken to ascertain which of these effects is dominant.

Funding support: Agriculture and Agri-food Canada. Science and Technology Branch.

S3 – P7

Key words: water use efficiency, varietal screening, hybrid poplar, $\delta^{13}C$, stable isotopes, biomass, bioenergy

^{13}C stable isotopes as a screening criterion for selecting water use efficient varieties of hybrid poplar for short rotation biomass plantations

Austin Himes, *Brian Stanton, Carlos Gantz*

GreenWood Resources Inc.

$\delta^{13}C$, the ratio of ^{13}C:^{12}C stable isotopes relative to a standard, was determined from leaf tissue samples of 134 hybrid poplar varieties representing three inter-specific taxa (*P*. *deltoides* × *P. maximowiczii*, *P*. ×*canadensis*, and the *P.* ×*generosa*) taken from a trial at GreenWood Resource's Boardman Research Site near Boardman OR, USA (45°50'04.82" N, 119°34'04.65" W). The trial was designed with single-ramet plots nested within taxon whole plots in six randomized complete blocks with 3.47 square meters per tree (3.05 x 1.14 m). The trial was managed with drip irrigation and at deficit rates (~75% of estimated plant demand) during the latter part of the second growing season just prior to sampling. Results show the taxon, genotype-within-taxon, and ramet-within-taxon effects account for 3.1, 34.2, and 62.7% of the variation in $\delta^{13}C$, respectively. Ignoring the taxon effect, clonal repeatability equals 0.63 indicative of good genotype selection opportunities. Eighteen (18) varieties composed of two from each taxa representing the extremes and mean of $\delta^{13}C$ values were selected to establish a validation test intended to verify the utility of $\delta^{13}C$ in predicting varietal growth per unit of water. The validation trial to be installed in April 2014 will employ a split plot design with three irrigation levels (60, 80, and 100% of estimated plant demand) as whole plots and $\delta^{13}C$ phenotype (high, average, low) as sub-plots. Additional phenotypic traits that may predict a growth response to water input will be measured, including leaf area index, mass per leaf area, specific leaf area, petiole length, stomatal conductance, and $\delta^{18}O$ of leaf tissue. The results of the initial study and the design of the new study will be presented at the "Physiology: Integrating Form & Function in the *Salicaceae*" poster session of IPS VI.

Funding support: USDA-NIFA SBIR grant program, 2012-00310

S3 – P8

Key words: *Populus*, water deficit tolerance, trade-off, breeding success

Assessing breeding success considering yield related traits and water deficit tolerance in new bred poplar accessions (*Populus* spp.)

Matthias Meyer[1], *Björn Günther*[2], *Gerd Helle*[3], *Alwin Janssen*[4], *Doris Krabel*[1]

[1]TU Dresden, Forest Botany, Molecular Tree Physiology Group, 01737 Tharandt [2]TU Dresden, Institute of Forest Utilization and Forest Technology, 01737 Tharandt [3]GFZ German Research Centre for Geosciences, Section 5.2, 14473 Potsdam [4]Northwest German Forest Research Institute, Department Forest Genetic Resources, 34346 Hann.Muenden, Germany

The domestication of Populus is a global process driven by the effort of tree breeders in many countries. In Germany, a densely populated country, plantation culture of poplars can coexist with annual crops only in competition for acreage. Agro-economic criteria, e.g. annual cash inflow, are most important in the German farmers' decision making process accounting for the higher importance of annual bio-energy crops. With that background, the breeding goals for Populus spp. are dominated by yield and resistance.

But the benefit of SRF results also from non-profit gain. Not all positive effects are appreciated, paid by a customer or even noticed by the public. For instance, the intensively managed farmlands can benefit from SRF by the reduced machine employment. Compared with annual bio-energy crops, sustainable SRF is much more energy-efficient.

One physiological issue for poplar improvement in Germany has to be seen in the context of water deficit tolerance and water use efficiency. The main reasons for the importance are (1) the relatively high water use of poplars and several yield-reducing drought adaptations, (2) the competition with other crops for acreage with appropriate water supply and (3) the necessity of protecting ground water resources under poplar cultures.

The data to be presented are derived from 3-yr field trials repeated on three sites, containing new bred clones (www.fastwood.org) and reference cultivars (> 30 clones). Evaluating the results on radial increment and biomass, the breeding success will be assessed. Applying LMM or correlation analyses between radial increment or biomass and adaptation-related wood anatomical traits (e.g. vessel lumen cross-sectional area) the xylem hydraulic characteristics of the new bred and the reference clones will be compared. It will be discussed if there is an undesirable trade-off relationship between improved yield and water deficit tolerance. Possible consequences on wood density will be shown up as well.

Funding support: German Federal Ministry of Food and Agriculture (www.bmel.de), German Agency for Renewable Resources (www.fnr.de), FKZ 22002911

S3 – P9

Key words:
willows,
flooding,
drought,
hydraulic
conductivity

Willow's response to alternate periods of drought and flooding stress

Guillermo Doffo[1], María Emilia Rodríguez[1], Silvia Monteoliva[2], Teresa Cerrillo[3], Virginia Luquez[1]

[1]Instituto de Fisiología Vegetal (INFIVE), CONICET – UNLP; CC 327, 1900, La Plata [2]Cátedra de Xilotectología, Dto de Ingeniería Agrícola y Forestal, Facultad de Ciencias Agrarias y Forestales, UNLP, CONICET, CC 31, 1900, La Plata [3]EEA Delta del Paraná, INTA, Paraná de las Palmas y Canal Laurentino Comas, 2804, Campana, Argentina

In the next years, climate change will increase the occurrence of flash flooding as a consequence of extreme rain events. In certain areas of Argentina, this fact will create alternate periods of drought and flooding during the growing season. To develop willow plantations in these areas, it is necessary to gain a better understanding of the physiology under these particular combinations of stresses. In this work, we explored the responses of two willow clones to different combinations of stress treatments: continuous flooding or drought for 6 weeks, or cyclic treatment of 2 weeks of stress separated by 2 weeks of normal watering.

The type of stress and the order of occurrence modified in different ways the growth in height, dry matter partitioning, gas exchange, leaf area dynamics and shoot hydraulic conductivity. Above ground growth was severely reduced by drought in both clones, either in continuous or cyclic treatment. A cycle of drought followed by flooding reduced growth in a lesser extent than the opposite situation. The clones responded to the flooding followed by drought combination in a different way. One clone experienced a marked reduction in shoot hydraulic conductivity that was ten times lower than in control plants, combined with and extensive defoliation. In the other clone, the reduction of hydraulic conductivity was not significant, and it experienced a lesser degree of defoliation. It is likely that the extensive defoliation is a consequence of the inability to maintain an adequate water supply to the leaves because of the occurrence of severe embolism. The higher susceptibility to embolism correlates with the occurrence of fewer vessels with a higher lumen.

The two willow clones analyzed in this work had different strategies to cope with the occurrence of drought episodes. One clone experiences an extensive defoliation and a rapid resuming of growth after the end of the stress, while the other maintained the leaf area at the expense of growth.

Funding support: This work was funded by a grant to V. Luquez (PIA 10007 - Sustainable Forest Plantations Module), from the Ministry of Agriculture, Stockbreeding and Fisheries (MAGyP), Argentina, with funds from the International Bank for Reconstruction and Development (IBRD 7520 AR).

S3 – P10

Key words:
poplar,
flooding,
reoxygenation,
AOS,
antioxidants

Characterization of the antioxidant response in two *Populus deltoides* clones with different sensitivity to flooding

María Emilia Rodríguez[1], *Fabio Germán Achinelli*[1,2], *Carlos Guillermo Bártoli*[1], *Virginia Luquez*[1]

[1]Instituto de Fisiología Vegetal (INFIVE), CONICET – UNLP, CC 327, La Plata (CP 1900), Argentina. [2] CIC Buenos Aires

Flooding causes anoxia or hypoxia in plants. When the stress episode ends, sudden oxygen exposure of tissues that have lacked oxygen can induce the generation of active oxygen species (AOS), causing oxidative stress damage. To prevent this, plants have systems of enzymatic and non-enzymatic antioxidants. There is no information on the impact of post-anoxic injury in the recovery of *Populus* plants after flooding, nor whether the degree of susceptibility is related to the antioxidant content. Our aim was to characterize the response of the antioxidant system in poplar during flooding and post-flooding period. Two *P. deltoides* clones with different response to flooding, Alton (tolerant) and ST67 (sensitive), were grown in pots in a greenhouse. Flooding was imposed by covering the pots with tap water up to 5 cm over the soil surface, for 28 days. Afterwards, pots were removed from the water and left to drain for one day. Leaf and root samples were taken on days 0 and 28 of the flooding period, and 0 and 24 hours after the end of flooding. Ascorbic acid (AsA), glutathione (GSH), peroxidase (PX) and ascorbate peroxidase (APX) were determined. In leaves and roots of flooded Alton plants, no change was observed in AsA, GSH and PX levels, and APX only had a transient increase immediate to reoxygenation. These results could indicate that antioxidants would be removing AOS generated during post-anoxic stress, without its detoxification capacity being exceeded. In leaves of flooded plants of ST67, AsA increased during recovery. In roots, AsA and GSH content were reduced, and APX activity was enhanced, both at the end of flooding and during recovery. The response of this clone suggests that antioxidants would be removing AOS generated both by flooding and post-anoxic stress. The greater antioxidant response of ST67 with respect to Alton, indicate a higher level of stress, which is consistent with its greater growth reduction during an episode of flooding. Funding support: this work was supported with funds from projects PICT 00487 (Agencia Nacional de Promoción Cientifica y Tecnológica) and PIP 0269 (CONICET) to V. Luquez.

S3 – P11

Key words: development, high temperature, poplar, RNA-seq

Moderately elevated day-time ambient temperature shapes architecture and regulates vegetative growth in poplar

Jin Zhang, Jianbo Li, Bobin Liu, Li Zhang, Jun Chen, Mengzhu Lu

State Key Laboratory of Tree Genetics and Breeding, Research
Institute of Forestry, The Chinese Academy of Forestry, Beijing, 100
091, P.R.China

Plants exhibit a range of different growth rates are known to be regulated both by genetic factors and by environmental conditions. In many plants, elevated ambient temperature induces heat stress and a variety of physiological consequences including a reduced vegetative growth rate, but the mechanism remains largely unclear. With the concern of the impact of global warming on tree growth, effects of a higher ambient temperature on tree growth need to be investigated.

In this study, a set of chambers were employed to conduct the elevated day-time ambient temperature treatment of a clone of hybrid poplar saplings 84K (Populus alba × P. glandulossa). And the first two internodes including apical meristematic tissue and the fifth internode undergoing active secondary tissue differentiation were separately harvested for transcriptome sequencing and analysis.

Long term (5 weeks) moderately elevated day-time ambient temperature (37°C) has profound effects both on architecture and on growth rate in poplar. In contrast to elevated temperature treatment of many plant species that leads to reduced vegetative growth rate, moderately elevated day-time ambient temperature obviously increased both longitudinal growth rate and lateral growth rate by three weeks treatment. In addition to increased vegetative growth rate, saplings acclimated to moderately elevated day-time ambient temperature were subjected severe architecture changes compared to control plants. Leaf elevation angle increased, and both leaf length and leaf width reduced in treated saplings. In order to further reveal molecular mechanisms involved, a genome-wide transcriptome analysis was performed and a set of transcripts and transcription factors was found to be dramatically changed during this process. Moreover, alternative splicing events in poplar were investigated during the development stage and heat treatment.

S3 – P12

Key words:
stress, *Populus*,
redox, defense

Growth-compatible hyperaccumulation of salicylic acid for improvement of stress tolerance

Liang-Jiao Xue[1,2], Wenbing Guo[1,2], Yinan Yuan[3], Batbayar Nyamdari[1,2], Chris Frost[1,2], Rhodesia Celoy[1,2], Scott Harding[1,2], Chung-Jui Tsai[1,2]

[1]School of Forestry and Natural Resources, University of Georgia
[2]Department of Genetics, University of Georgia [3]School of Forest Resources and Environmental Science, Michigan Technological University

The phytohormone salicylic acid (SA) regulates many aspects of plant growth, development and defense. Exogenous applications of SA have been shown to confer oxidative stress tolerance in agricultural crop species. However, increased SA accumulation leads to dwarfism in the model plant *Arabidopsis*. We developed a new strategy for engineering SA-mediated stress tolerance in agronomically important species without compromising growth. By over-expressing a *Yersinia* bi-functional SA synthase, we showed that *Populus* can accumulate up to 1000-fold higher levels of SA and SA-conjugates with normal growth. SA-hyperaccumulation induced partial stomatal closure and reduced photosynthesis. This was accompanied by considerable changes in transcript and metabolite abundance associated with altered carbon inputs, phenylpropanoid homeostasis and redox regulation. The responses overlapped substantially with those of heat-stressed wild types. Network analysis identified key drivers of the SA response, including receptor-like kinases and WRKY transcription factors previously implicated in defense. Of particular interest are members of the expanded *Populus* nucleoredoxin family which exhibited increased expression and increased network connectivity in response to elevated SA. The results are consistent with a sustained, growth-compatible oxidative response in SA-hyperaccumulating *Populus*. The extraordinary capacity of *Populus* to accumulate high levels of SA makes it an ideal model system for investigation of SA-mediate stress tolerance mechanisms.

Funding support: The work was supported by the US National Science Foundation Grants DBI-0421756 and 0836433, the Georgia Research Alliance, and the Warnell School’s Hank Haynes Forest Biotechnology endowment fund.

S3 – P13

Key words:
dry weight,
photosynthetic pigment, stress tolerance index,
glutathione reductase,
ascorbate,
nitrogen allocation

Positive and negative effects of elevated CO_2 and O_3 on the growth and physiological responses of *Populus alba × grandulosa*

Sim-Hee Han[1], *Gil Nam Kim*[1], *Nelly S. Aggangan*[2] *and Soo Jeong Shin*[3]

[1]Department of Forest Genetic Resources, Korea Forest Research Institute Suwon 441-847, Korea [2]National Institute of Molecular Biology and Biotechnology, University of the Philippines Los Baños, College 4031, Laguna, Philippines [3]Department of Wood and Paper Science, Chungbuk National University, Cheongju 361-763, Korea

The effects of elevated CO_2 and O_3 concentration in growth chambers (phytotron with artificial light control) on the growth and physiological responses of *Populus alba × grandulosa* were studied. The four treatments were: control, elevated CO_2 (750ppm), elevated O_3 (70ppb), and elevated CO_2 (750ppm) + elevated O_3 (70ppb). Both stem and root dry weight increased under the elevated CO_2. Under the elevated O_3, stem dry weight increased but root dry weight decreased. Particularly, the reduction of root dry weight was the highest under the elevated CO_2+O_3. Photosynthetic pigment content (chlorophyll and carotenoid) were also 1/2 of control under the elevated O_3 and the lowest under the elevated CO_2+O_3. Chl/N increased under the elevated CO_2 but decreased under the elevated O_3. Chl/N and stress tolerance index were the lowest under the elevated CO_2+O_3. In conclusion, the elevated CO_2 have positive effects such as the increase of leaf pigment synthesis and the stimulus of stem and root growth. In contrast, the elevated O_3 decreased stress tolerance index, increased GR (glutathione reductase) activity and ascorbate content as defensive responses, and brought about the reduction of nitrogen allocation to pigment synthesis. In addition, the elevated O_3 stimulated stem growth but inhibited root growth during short-term exposure. The effects were clearly observed under combination of the elevated CO_2 and O_3 as well as under the elevated O_3 treatment.

S3 – P14

Key words:
herbivory, near infrared spectroscopy, NIR, phenolic glycosides, condensed tannins

Vole and deer feeding preferences for clonal variants of poplar: a role for NIR spectroscopy in predicting consumption based on known and unknown compounds in foliage and bark.

Jeffery Kallestad[1], *Kennedy Rubert-Nason*[2], *Richard Lindroth*[2], *Austin Himes*[3] *Jen Vittetoe*[1], *Daniel Nyquist*[1], *Carri LeRoy*[4]

[1]Washington State University, Puyallup Research and Extension Center, 2606 W. Pioneer, Puyallup WA 98371. [2]University of Wisconsin-Madison, Dept. of Entomology, 237 Russell Labs, 1630 Linden Drive, Madison, WI 53706
[3]GreenWood Resources Inc.91151 Old Mill Town Road, Westport, OR 97016
[4]Evergreen State College, Olympia, WA 98505

Herbivory by voles, deer, elk, and moose present a production challenge and significant costs to short rotation coppice plantations. To mitigate potential loss in a low impact manner through genetic improvements, our focus has been to develop clone screening methods using near infrared (NIR) spectroscopy, and to employ selection criteria based on levels of naturally-occurring defensive compounds thought to reduce herbivory. We measured levels of condensed tannins and phenolic glycosides in a large pool of clones in 4 Populus species and 2 hybrid taxa for creating NIR calibrations. To verify our approach we selected clones from this population with high, medium, or low levels of total phenolic glycosides and presented stem segments to captive voles and whole plants to confined mule deer in cafeteria-style feeding preference experiments. In a related study we measured these same compounds in leaf samples collected from a replicated polyclonal trial that had been selectively browsed by wild deer. In all of these studies there were clear preferences for particular clones or taxa, but the amount of bark area consumed, leaf area consumed, or browse level ranking were not explained by the quantity of condensed tannins or total quantity of phenolic glycosides. However, in some experiments, certain low abundance phenolic glycosides were negatively correlated with consumption patterns. Taking a more direct approach, we developed partial least squares regression models that predict the level of vole or deer herbivory based the quantities of bark or leaves consumed from each clone with their respective NIR absorbance spectra. Cross validation R^2 range from 0.56 to 0.8. While this blind approach will not advance our understanding of the role of known “defensive” compounds in herbivore behavior, the capability to predict pest damage based on scans of bark or foliage highlight NIR spectroscopy as a robust technology for screening resistant varietals.

Funding support: USDA-NIFA-AFRI, agreement #2011-68005-30407

S3 – P15

Key words:
benzoic acid
pathway,
enzyme
analysis

Identification and characterisation of willow phenylpropanoid entry point enzymes

Femke de Jong, Michael H. Beale, Jane L. Ward, Steve Hanley, Angela Karp

Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, United Kingdom

The phenylpropanoid pathway is one of the major carbon users in plants. It not only produces lignins for the cell wall, but also hundreds of additional secondary metabolites such as flavonoids, alkanoids, and phenolic glycosides. Understanding how carbon is partitioned between these metabolites and how this is regulated is of major importance in the production of optimal biomass crops. Characterization of the enzymes of the entry point into the phenylpropanoid pathway gives information about the how the carbon flow is influenced by the enzyme efficiency of those enzymes. This information can be used in combination with gene transcription analysis to optimize biomass crop breeding. In this study we identified the willow enzymes for three of the reactions at the entry point of the phenylpropanoid pathway. 1) Phenylalanine ammonia-lyase (PAL), which catalyses the deamination of L-phenylalanine to cinnamic acid. 2) Trans-cinnamate 4-monooxygenase (C4H) which catalyses the reaction of cinnamic acid to coumaric acid, directing carbon flow into the lignin and flavonoid biosynthesis pathway. And 3) cinnamate-CoA ligase (CNL) which catalyses the reaction of cinnamic acid into cinnamoyl-CoA, directing the carbon flow into the benzonoid and phenolic glycosides biosynthesis pathway. Detailed characterization of the three willow PAL enzymes showed that there is up to 4 fold difference in activity and specificity between the best and worst performing PAL enzymes. A detailed kinetic analysis will therefore contribute to our understanding of how carbon flow influences plant growth and production of secondary metabolites.

Funding support: BBSRC

S3 – P16

Key words:
willow,
biomass,
wood
properties,
phenolic
compounds

Wood properties of different willow cultivars growing in contrasting environments

Nicolas Berthod[1], *Frédéric Pitre*[1], *Karine Pedneault*[2], *Michel Labrecque*[1]

[1]Institut de recherche en biologie végétale, Montréal Botanical Garden, Montréal, Québec, Canada. [2]Département des sciences de l'aliment et de la nutrition, Université de Laval, Québec city, Québec, Canada

Previous research has established that SRC willow rapidly produces a large quantity of biomass, thus reducing the footprint on land and minimizing production costs. Willow in SRC therefore has great potential for biofuel and bio-products markets. Here, we describe a first experiment in which we investigated how willow wood polymers (cellulose, hemicellulose and lignin) vary under different environmental conditions and between genotypes. In a second experiment, we examined wood extractible substances to identify high-value bio-molecules that could represent a secondary market for SRC willow. Among these substances, phenolic compounds are of particular interest, since they are already used in several industries. In 2010, eight sites in the province of Quebec were selected to represent contrasting environmental conditions. Five willow cultivars (including 5027, SV1, SX61, SX64 and SX67) were planted on each site (400 cuttings/site) and are being maintained following the SRC protocol. According to preliminary data, after two years of growth, the survival rate of willow on most sites is over 90%. The presence of pathogens and insects as well as growth parameters (diameter and height) have also been monitored throughout this period. Based on this data, four sites out of the eight were chosen to conduct the two above-mentioned experiments. These sites follow a west-east gradient along the Saint Lawrence River, and are located near Saint-Roch-de-l'Achigan, Beloeil, Saint-Siméon and La Pocatière. During the summer of 2013 (third year of growth), samples were collected for analyses. The cellulose, hemicellulose and lignin contents of the wood were determined by ASTM D1103-60, TAPPI T-212 and ASTM D1106-56 methods respectively. Using the same samples, 52 phenolic molecules were extracted, analyzed and characterized by LC/MS to identify those that are differentially present according to the various cultivars and environments tested. Analysis is in progress, and results will be revealed at the symposium.

Funding support: BIOFUELNET Canada

S3 – P17

Key words:
pretreatment,
wood chemistry,
hemicellulose,
O-acetylation,
cell wall
modification

Cell wall acetylation – a potential criterion for selecting putative poplar genotypes as a bioenergy crop

Amanda Johnson[1]*, Faride Unda*[1]*, Alex Skyba*[1]*, Francis Raiche de Araujo*[1]*, Carl J. Douglas*[2]*, Shawn D. Mansfield*[1]

[1]Department of Wood Science, Faculty of Forestry, University of British Columbia [2]Department of Botany, University of British Columbia

Non-cellulosic wood polysaccharides are acetylated in a species-dependent and spatially-regulated manner. Acetyl groups comprise approximately 5% of the dry weight of poplar wood. Biologically, acetyl groups increase xylan chain solubility and may therefore influence secondary cell wall formation by affecting the association of hemicellulose with the other cell wall components cellulose, lignin, and the pectic and proteinaceous constituents of the adjacent primary cell wall. Industrially, pretreatment process hydrolyzes acetyl groups releasing free acetic acid into the reaction media, where the acetic acid enhances the release of pentose sugars during this crucial first step of lignocellulosic biomass breakdown. This research examined the effect of acetyl groups on sugar release during lignocellulosic pretreatment, weighing hemicellulose release against the generation of undesired fermentation inhibitors, by assessing the relationships between wood composition and hemicellulose release following pretreatment of 15 black cottonwood (*P. trichocarpa*) genotypes with varying levels of acetylation. The results indicate that samples with higher acetyl content tended to release more hemicellulose, and also resulted in greater dehydration of xylose into furfural. However, these parameters were weakly correlated, which indicates that other factors, in particular the presence of neighbouring uronic acid substituents, may likely play a role in acetic acid and consequently hemicellulose hydrolysis. This research not only addresses the biology of cell wall synthesis and modification, but also highlights the impact acetylation may have on the large-scale industrial utility of plants.

S3 – P18

Key words:
poplar, short-rotation coppice, sylleptic branching, biomass, field trial

Performance of RAV1 transgenic poplars during the first year on field trial

Alicia Moreno-Cortés[1]*, Tamara Hernández-Verdeja*[1]*, M. Mario Sánchez-Martín*[2]*, Pablo González-Melendi*[1]*, Hortensia Sixto*[2]*, Isabel Allona*[1]

[1]Centro de Biotecnología y Genómica de Plantas UPM-INIA, Departamento de Biotecnología, Universidad Politécnica de Madrid, Campus de Montegancedo, E-28223 Madrid [2]Selvicultura y Gestión de los Sistemas Forestales, INIA-CIFOR, E-28040 Madrid, Spain

Populus spp, *Salix spp.* or *Eucalyptus spp.* are grown to produce wood in a short period. Poplars are cultivated with cycles of 15–18 years to obtain saw timber and peeler logs, but when grown as short-rotation coppice (SRC) for biomass production, cutting back/coppicing cycles are reduced to 3–5-years intervals. So far, however, breeding and research efforts have focused on the single-stem growth of poplars, and there is a need to identify traits and loci to generate improved SRC biomass-yielding genotypes. Biomass yield is a highly complex trait as it is the combined outcome of many other complex traits, each under separate polygenic control. Among profitable biomass yield-related traits are the amount of sylleptic branching and the length of winter dormancy. In poplar and in a few other Salicaceae species some lateral buds grow out sylleptically, the same season in which they form without the need of an intervening rest period. Sylleptic branching in poplar increases branch number, leaf area and general growth of the tree in its early years, and is a reasonable predictor of coppice yield. On the other hand, winter dormancy determines the extent of the growth period.

Our group has characterized the *RAV1* gene of *Castanea sativa* (*CsRAV1*), encoding a transcription factor of the subfamily RAV (Related to ABI3/VP1). *CsRAV1* expression shows a marked seasonal pattern, being higher in autumn and winter both in stems and buds. We generated transgenic lines of the hybrid clone *Populus tremula* x *P. alba* INRA 717 1B4 constitutively expressing CsRAV1. These CsRAV1-expressing poplars develop sylleptic branches a few weeks after potting and show an altered phenology when grown under controlled conditions. In 2012 we started up a field trial and we are currently assessing the performance of these transgenic trees during the first year, and its impact on the aerial biomass production.

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S3 – P19

Key words:
hybrid poplar,
polyclonal,
monoclonal,
biomass
allocation,
physiology

Effects of mixing clones on hybrid poplar productivity, photosynthesis and root development in northeastern Canadian plantations

Raed Elferjani, Annie DesRochers, Francine Tremblay

Forest Research Institute, University of Quebec in Abitibi-Temiscamingue, 341 Principale Nord, Amos, Qc, J9T2L8, Canada

Four hybrid poplar clones were planted in monoclonal and polyclonal blocks in three sites located in Quebec, Canada, to assess effects of clonal diversity on (i) aboveground biomass productivity, (ii) net photosynthesis and nutrient status of trees, and (iii) root spatial distribution. Stem growth was measured over five growing seasons, while root development, foliar nutrient concentrations and photosynthesis were measured during the fifth growing season. Results showed frequent but not general overyielding of trees in the polyclonal plots compared to monoclonal plots, after five years. Overall, stem volumes were 21% greater in the polyclonal (7.4 m^3 ha^{-1}) *vs* monoclonal (6.1 m^3 ha^{-1}) plots. Effects of clone mixing on growth were greater in sites where soil nutrients were more limiting. However, foliar macronutrient concentrations in trees growing in polyclonal plots were similar to those in monoclonal plots. Root development differed between the two plot layouts, with mean root**:**shoot ratio being greater in monoclonal (0.41:1) *vs* in polyclonal (0.35:1) plots. Mixing clones increased aboveground biomass allocation, which we attributed to reduced competition between individuals of different clones and could explain overyielding in the polyclonal plots. The root fraction most distance from the stem (≥ 60 cm) was greater in monoclonal (67% of total root biomass) compared to polyclonal (47% of total root biomass) plots, suggesting greater belowground competition in the former, which forced roots to extend further from the stems. Effects of plot layout on net assimilation rate (P_n) depended on site, with trees in polyclonal plots having greater P_n in two of the three sites. Root total non-structural carbohydrates were greater in the polyclonal (216 mg g^{-1}) compared to the monoclonal (159 mg g^{-1}) plots. Mixing hybrid poplar clones often resulted in greater aboveground growth, lower root:shoot ratios, and different spatial root distributions, when compared to clones planted in monocultures.

Funding support: NSERC, CRSNG-UQAT-UQAM Industrial Chair in Sustainable Forest Management, Norbord Inc.

S3 – P20

Key words:
competition;
mixed-species
plantation;
nitrogen-fixing
species

How does poplar / black locust mixture influence growth and functioning of each species in a short-rotation plantation?

Cécilia Gana[1,2]*, Daniel Epron*[1,2]*, Dominique Gérant* [1,2]*, Pascale Maillard* [1,2]*, Caroline Plain*[1,2]*, Pierrick Priault* [1,2]*, Nicolas Marron* [1,2]

[1]Université de Lorraine, UMR 1137 'Ecologie et Ecophysiologie Forestières' (EEF), F-54500 Vandœuvre-lès-Nancy [2]INRA, UMR 1137 'Ecologie et Ecophysiologie Forestières' (EEF), route d'Amance, F-54280 Champenoux, France

The introduction of a nitrogen-fixing species in intensively managed forest plantations is a solution already widely adopted in eucalyptus plantations of the South hemisphere in order to prevent soil fertility decline. Under temperate latitudes, the introduction of a leguminous species, such as black locust, in short rotation coppice (SRC) of poplar is much less developed and documented. The fact that the mixed plantation will produce, or not, at least as biomass as the monoculture depends on competition and complementarity processes between both species. Indeed, in poplar and black locust mixed-stands, changes in soil water and nitrogen status and intraspecific interactions (facilitation and complementarity for light and soil resources) could significantly (compared to pure plantations) modify carbon allocation between aboveground and belowground compartments. An instrumented SRC plantation of the poplar/black locust mixture is studied in central France. Our hypotheses are that, in the mixture:

- the relationships are facilitation and complementarity,
- the presence of black locust improves the nitrogen status of poplar,
- thus, the photosynthetic assimilation is improved,
- there is a stratification of aboveground and belowground organs between the two species, leading to a better capture of resources (light, water, nutrients)
- and the aboveground carbon flux is enhanced contrary to the belowground carbon flux.

Biomass production, soil and plant nitrogen contents, foliar gas exchanges, etc. are determined in order to test our hypotheses. After three growing seasons, our hypotheses begin to be verified. Notably, the impact of the interspecific competition seems to be in favor of the poplar trees, while black locust trees suffer from interspecific competition.

S3 – P21

Key words: short-rotation coppice, mortality, genotypic variation, coppice ability, self-thinning, biomass, POPFULL

Assessment of productivity and population dynamics during two consecutive two-year rotations of a poplar bio-energy culture (POPFULL)

Laura S. Broeckx, *M.S. Verlinden, S. Vanbeveren, R. Ceulemans*

University of Antwerp, Department of Biology, Research Group of Plant and Vegetation Ecology, B-2610 Wilrijk, Belgium

In April 2010, a high density (8000 cuttings per ha) short-rotation coppice culture was established with 12 different poplar genotypes on a former agricultural land in Lochristi (Belgium). The productivity and the resprout capacity of poplar genotypes were assessed during the two first two-year rotations, to evaluate the efficiency of this poplar bio-energy culture. At the end of each growing season, tree mortality, shoot diameter and the number of shoots per tree were assessed for all genotypes on the dormant winter habitus of the trees. Initial mortality, reflecting the establishment success, was high (genotypic averages ranging between 9 and 21%). But the additional tree mortality after the first coppice was negligible (on average 0.5%), confirming the high coppice ability of poplar. At the end of each two-year rotation, genotype-specific allometric relationships between stem diameter and above-ground woody biomass were established. Woody biomass production was estimated for each genotype on an area basis combining stem diameter measurements and effective planting density. The mean annual biomass production increased from an average of 4 Mg ha^{-1} y^{-1} during the first rotation to an average of 10 Mg ha^{-1} during the first year after coppice. Mean annual biomass production is being presented for all genotypes during the first and the second rotation after plantation establishment. During the first rotation, significant genotypic differences were found in the number of shoots per tree, although the majority of genotypes showed a single stem growth. After the first coppice, the average number of shoots per stool ranged between 6 and 18 (genotypic averages). The evolution of shoot number per tree and shoot diameter distribution is being presented, in relation to individual tree biomass. Genotypic variation in shoot density and in the degree of self-thinning due to competition were analyzed.

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S3 – P22

Key words:
adaxial-abaxial patterning, amphistomaty, *Melampsora*, stomatal conductance, GWAS

Association genetics of stomatal patterning in *Populus trichocarpa*, and links with geography and trade-offs in carbon gain and disease resistance

Athena D. McKown[1], *Robert D. Guy*[1], *Linda Quamme*[1], *Jaroslav Klápště*[1,2], *Jonathan La Mantia*[1], *Peter Constabel*[3], *Yousry A. El-Kassaby*[1], *Richard Hamelin*[1], *M. Shofiul Azam*[1]

[1]Department of Forest and Conservation Sciences, Faculty of Forestry, University of British Columbia, Forest Sciences Centre, 2424 Main Mall, Vancouver, BC V6T 1Z4, Canada [2]Department of Genetics and Physiology of Forest Trees, Faculty of Forestry and Wood Sciences, Czech University of Life Sciences, Prague, 165 21, Czech Republic [3]Department of Biology and Centre for Forest Biology, University of Victoria, Victoria, BC, V8W 3N5, Canada

We investigated functional adaptation in *Populus trichocarpa* by assessing the underlying genetics of leaf stomatal patterning and links with geography and other trait data. For this study, we measured numerous upper (adaxial) and lower (abaxial) stomatal traits from 452 *P. trichocarpa* accessions collected throughout much of the species range, grown in a common garden and genotyped with a 34K *Populus* SNP array. We calculated broad-sense heritability values (H^2) for stomatal traits using our SNP data. We also performed a genome-wide association study (GWAS) with correction for population structure to uncover genes underlying stomatal trait variation. H^2 values for stomatal traits were moderate to low (range=0.16-0.42). GWAS identified genes associated primarily to adaxial stomata, including polarity genes (e.g., *PHABULOSA*), stomatal development genes (e.g., *BRASSINOSTEROID-INSENSITIVE 2*) and disease/wound-response genes (e.g., *GLUTAMATE-CYSTEINE LIGASE*). We correlated stomatal trait data with available geoclimate, ecophysiology and severity of leaf rust (*Melampsora*) data for the same accessions. Clear latitudinal trends of increasing guard cell pore size and adaxial stomata (changing the adaxial:abaxial stomatal ratio) among northern genotypes was observed. This corresponded with higher stomatal conductance (g_s) and photosynthesis (A_{max}), faster shoot elongation, lower leaf tannins and greater *Melampsora* infection. By comparison, southern genotypes had lower gas exchange and lower *Melampsora* infection correlating with lower stomatal density, fewer adaxial stomata and smaller pore sizes. We suggest this points to an evolutionary trade-off in *P. trichocarpa* reflecting differing selection pressures across its range. In northern environments, increasing adaxial stomata and pore sizes reflect selection for higher carbon gain and faster growth during a limited growing season with less pressure from leaf pathogens. The opposite occurs in southern genotypes, which are not limited by growing season length, and have fewer stomata resulting in lower gas exchange but also reduced pathogen attack from fewer infection sites. Funding support: POPCAN: Genetic improvement of poplar trees as a Canadian bioenergy feedstock, Genome BC

SESSION IV – PESTS AND PATHOLOGY

Oral Presentations

S4 – O1

Key words: poplar breeding, *Populus nigra* L., *Melampsora larici-populina* Kleb., leaf rust resistance, partial resistance, strain-specificity

Exploration of *Populus nigra* L. genetic variation for partial resistance against the co-adapted pathogen *Melampsora larici-populina*

Catherine Bastien[1], *Redouane El-Malki*[1], *Arnaud Dowkiw*[1], *Patricia Faivre-Rampant*[2], *Elise Albert*[1], *Marc Villar*[1], *Patrick Poursat*[3], *José Almeida*[3], *Céline Ridel*[1], *Vanina Guérin*[1], *Brigitte Viguier*[1], *Marijke Steenackers*[4] *and Véronique Jorge*[1]

[1]INRA, UR0588 Amélioration Génétique et Physiologie Forestières, Orléans, France [2]INRA, URGV, Evry, France [3]INRA, UE0995 Génétique et Biomasse Forestières, Orléans, France [4]INBO-VLAGEW, Research Institute for Nature and Forest, Geraardsbergen, Belgium

Breeding poplars for more durable resistance to *Melampsora larici-populina* (*Mlp*) leaf rust is a real challenge, especially in an european context where *Mlp* populations exhibit high genetic diversity in black poplar-larch sympatric areas and where selective pressures from the domesticated interspecific host (*P. xeuramericana* and *P.xinteramericana*) are both rapid and strong.

Several approaches combining field evaluation under natural infection and artificial inoculation tests with control of the pathogen diversity were more recently directed at the study of genetic variation for partial resistance (QR), in the co-evolved parental species *P. nigra L*. Quantitative and molecular genetic studies of the genetic control of partial resistance were developed on a 4x3 factorial mating design, one enlarged F1 mapping pedigree and one association population composed of more than 800 clones and genotyped with more than 8,000 SNPs.

The existence of a large reservoir of variation for QR in different natural populations of *P.nigra* was demonstrated for several epidemiological parameters, with some evidence of geographical patterns. Significant variation for strain-specificity was observed at clonal level within a subset of high field-resistant natural poplar genotypes. From classical linkage analysis, a list of twenty QTL distributed over the poplar genome explained between 2% to 60% of the phenotypic variation observed in artificial inoculation tests. Most of these QTL were trait- or strain-specific. The genomic mining of the detected QTL regions revealed close vicinity to clusters of NB-LRR genes and to other

QTL associated to Salix resistance to *Melampsora larici-epitea*.

The characterization of selection pressures exerted by host resistance on pathogen population provides useful insights into the dynamics of host-parasite co-evolution processes and is crucial for the design of both breeding and deployment strategies. Preliminary experiments suggested local adaptation of *Mlp* populations to partial resistances present in *P.nigra* populations.

All these results will be discussed in the framework of poplar breeding strategies and poplar cultivar deployment.

Funding support: the study was carried out with financial support from the NOVELTREE project (FP7 – 211868) and from a phD grant for Redouane El-Malki from the FUTUROL project.

S4 – O2

Comparative association analysis of *Populus trichocarpa* resistance to *Melampsora* spp. and *Mycosphaerella populorum*

Key words: poplar leaf rust, *Mycosphaerella* stem canker, disease resistance, *Populus trichocarpa*, association analysis

Jonathan La Mantia[1], *Jaroslav Klápště*[1,5], *Yousry A. El-Kassaby*[1], *Robert D. Guy*[1], *Carl J. Douglas*[2], *Shawn D. Mansfield*[3], *and Richard Hamelin*[1,4]

[1]Department of Forest and Conservation Sciences, University of British Columbia [2]Department of Botany, University of British Columbia, Vancouver [3]Department of Wood Science, University of British Columbia, Vancouver [4]Natural Resources Canada, Laurentian Forestry Center, 1055 rue du P.E.P.S., Québec, G1V 4C7, Canada [5]Department of Dendrology and Forest Tree Breeding, Faculty of Forestry and Wood Sciences, Czech University of Life Sciences Prague, Kamýcká 129, 165 21 Praha 6, Czech Republic

Populus trichocarpa is a native natural resource to the Pacific Northwest. In addition to being an ecologically important species, it provides fast-growing woody biomass for utilization in wood and paper industries and conversion to biofuels. Hybrid poplar breeding and international trade threatens to introduce exotic pathogens to *P. trichocarpa*. Two pathogens; *Melampsora larici-populina* and *Mycosphaerella populorum*, are the causal agents of leaf rust and leaf spot and stem cankers on poplar plantations east of the Rockies. Moreover, a new hybrid rust; *M. ×columbiana*, has enhanced pathogenicity and has become the principal poplar leaf rust in the Pacific Northwest. Poplar rust pathogens are biotrophic. *M. populorum* is a hemi-biotroph, switching from biotrophic to necrotrophic early in the infection process. Resistance mechanisms to biotrophic pathogens are generally antagonistic to those of necrotrophic pathogens. In the

presented study, host resistance to all three species was elucidated through association analysis. A total of 423 *P. trichocarpa* genotypes were phenotyped via artificial inoculations of leaf disc cuttings. Association analysis with 29,355 SNPs covering 3,543 genes was used to compare host resistance mechanisms. A total of 14 SNP were significant at $P < 3.30 \times 10^{-5}$. SNP associations in genes regulating ROS, auxin signaling, and anthocyanin biosynthesis will be discussed.

Funding support: This work was supported by Genome British Columbia (103BIO) Applied Genomics Innovation Program and Genome Canada (168BIO) Large Scale Applied Research Project funding to YEK, RG, CD, SM, RH.

S4 – O3

Key words: poplar, *Septoria musiva*, leaf spot, *Septoria populicola*, RNA-seq

Molecular interactions between poplar and *Septoria spp.* during leaf spot infection

Adam J. Foster, Philippe Tanguay, Armand Séguin

Canadian Forest Service, Natural Resources Canada, Laurentian Forestry Centre, 1055 du P.E.P.S., P.O. Box 10380, Stn. Sainte-Foy, Quebec, QC G1V 4C7, Canada

Diseases of poplar caused by the fungal pathogen *Septoria musiva* and related species are of growing concern. *S. musiva* is able to cause infection on leaves, resulting in defoliation, and canker formation on stems. This pathogen is of particular concern for nurseries and could be a threat to hybrid poplars worldwide, potentially causing an increase in the overall cost of managing poplar production. To gain a greater understating of the different responses of poplar species to infection with their natural *Septoria* species, RNA-seq was conducted on leaves of *Populus deltoides*, *P. balsamifera* and *P. tremuloides* infected with *S. musiva*, *S. populicola* and a new unnamed species, respectively. Disease progression, pathogen growth and host response were recorded and differences were observed. Infected leaves were also histologically examined using fungal isolates expressing GFP markers in an attempt to correlate pathogen growth and behavior with gene expression. Very few individual genes were commonly regulated between the three species during infection, but metabolic pathways were commonly regulated. In all three species, genes associated with growth and development were down-regulated, while genes involved the phenylpropanoid, terpenoid and flavonoid biosynthesis were up-regulated. Poplar defensive genes were expressed early in *P. balsamifera* and *P. tremuloides*, but delayed in *P. deltoides*, which correlated with the

rate of disease development. Examination of the data by mapping to the *Septoria* spp. genomes confirmed the expression of many putative small secreted proteins. This data gives an insight into the large differences in timing and expression of genes between poplar species being attacked with similar pathogens.

Funding support: This work was supported by Natural Resources Canada's Genomics Research & Development Initiative Strategic Fund.

S4 – O4

Key words: *Septoria musiva*, molecular diagnosis, qPCR

Phenotyping of *Populus spp* by quantifying *Septoria musiva* using qPCR

Nivi Deena Abraham and Jared M. LeBoldus

Department of Plant Pathology, North Dakota State University, Fargo, ND 58102, USA.

Leaf spot and stem canker caused by *Septoria musiva* (≡ *Sphaerulina musiva*) is a limiting factor in the use of *Populus* spp. and their hybrids in agro forestry. These diseases are best managed through the deployment of disease resistant genotypes. The scoring of disease severity is a tedious and time consuming process involving the counting and measurement of necrotic lesions. The use of quantitative PCR (qPCR) for quantification of fungal biomass in host tissue following infection will greatly increase the speed and accuracy of disease resistance phenotyping. Here we report the use of a multiplexed Taqman qPCR assay to quantify *S. musiva* development in inoculated stems of two resistant and two susceptible genotypes of hybrid poplar. Stems were inoculated by the excision of fifth leaf from the shoot apex of each tree. Wounds were inoculated with sporulating mycelium from four different isolates. Species specific probes and primers were designed based on conserved gene sequences of, beta tubulin and eukaryotic initiation factor IV, for the pathogen and host respectively. The primers were highly specific with similar amplification efficiencies for the host (96 %) and the pathogen (97%). Canker disease severity was evaluated on a 1-5 scale 7 weeks post inoculation. Total genomic DNA was then extracted from each canker for the qPCR assay. The ratio of fungal DNA (beta tubulin) to host DNA (eukaryotic initiation factor IV) was used as a measure of resistance and correlated to the disease severity score. This assay showed significant differences in the level of resistance among the different genotypes. Funding Support: USDA-NIFA-RIPM (2012-34103-19771)

S4 – O5

Key words: disease etiology, perennial canker, leaf-spot disease, genome sequencing, RNA-seq

One fungus, two diseases: what makes a poplar canker pathogen

Braham Dhillon[1], *Nicolas Feau*[1], *Richard. C. Hamelin*[1,2]

[1]Department of Forest and Conservation Sciences, University of British Columbia, Vancouver, BC, Canada V6T 1Z4 [2]Canadian Forest Service, Natural Resources Canada, Ste-Foy, QC, Canada G1V 4C7

Co-evolved natural forest pathosystems tend to be at equilibrium, and deadly disease outbreaks are rare. With the introduction of exotic poplar species at the beginning of the 20th century and the intensification of hybrid poplar cultivation in North America, M. populorum, a native endemic pathogen of Aigeiros poplars, has emerged as a canker-causing pathogen, leading to branch and stem breakage. Its sister species M. populicola is only responsible for a leaf-spot symptom on trees with Tacahamaca parentage. We sequenced the genomes of these fungi to identify what is unique in the poplar canker pathogen. We estimated the divergence time between M. populorum and M. populicola to be ~6.4 Mya, which parallels the divergence time estimate between the endemic host species P. deltoides and P. balsamifera (6.8-7.8 Mya). In accordance with this recent divergence, the whole genome comparisons show a remarkably high macro-synteny between the two species. A consistent expansion of several pathogenicity-related gene families was observed in M. populorum, suggesting a role for gene-dosage in determining its ability to cause cankers. Genes encoding hemicellulose-degrading enzymes and peptidases were more numerous in M. populorum than in M. populicola. Transcripts encoded by these genes were up-regulated when M. populorum was grown on a medium amended with poplar wood-chips. This correlates with the ability of M. populorum to partially degrade lignocellulose compounds and grow in woody tissues. We also identified a secondary metabolite cluster unique to M. populorum that is activated following induction by poplar tissue-products. Phylogenetic analyses indicated that the cluster was probably inherited from a horizontal gene transfer from Ascomycete fungi associated with wood-decay. We hypothesize that M. populorum acquired and maintained hydrolytic enzymes and pathogenicity determinants resulting in adaptation to infect and grow in woody stems of poplar and cause tree mortality.

Funding support: Genome BC, Genome Canada

S4 – O6

Key words: *Septoria*, Stem disease, *P. trichocarpa*, riparian, British Columbia

Septoria musiva on poplar in the Fraser Valley of British Columbia

Harry H. Kope[1]*, Stefan Zeglen*[2]

[1]British Columbia Ministry of Forests, Lands and Natural Resource Operations, Victoria, British Columbia, V8W 1R8 [2]British Columbia Ministry of Forests, Lands and Natural Resource Operations, Nanaimo, British Columbia, V9T 6E9, Canada

Septoria musiva Peck (*Mycosphaerella populorum* G. E. Thomps.) on *Populus* species causes leaf blight but more importantly, necrotic lesions that often result in stem breakage. *S. musiva* is indigenous to North America and is found throughout much of eastern and central Canada and the United States. Since 2007 *S. musiva* has been repeatedly detected in leaf spots and cankers on hybrid *Populus* in the Fraser River of British Columbia (B.C.). How *S. musiva* was introduced into BC has not been determined, though movement of infested plant material is suspected. Some hybrid poplar clones are known to be highly susceptible, and in the Fraser Valley of 21 hybrid plantations assessed, 16 (76%) were infested (187 trees sampled, 105 infested; ~56% incidence). Inoculum spread from plantations could infect native black cottonwood (*Populus trichocarpa* Torr. & A. Gray), and this has occurred. Since 2008 *S. musiva* has been repeatedly isolated from leaf spots and cankers of *P. trichocarpa* in riparian areas along the Fraser River; although cumulative incidence was very low (1.2% from over 1000 trees sampled). A survey of a provenance trial, in close proximity to an infested hybrid plantation, containing 183 families of *P. trichocarpa* from western North America, found that 44 families tested positive for *S. musiva*. These results indicate that *S. musiva* under favourable conditions can infect multiple families of *P. trichocarpa*. The impact that *S. musiva* could have on *P. trichocarpa* growth and ecology is currently unknown.

S4 - O7

Key words:
Septoria canker,
Septoria,
Sphaerulina

Pathways analyses of the poplar pathogen, *Mycosphaerella populorum* using a population genomic approach

Monique L. Sakalidis, *Nicolas Feau, Richard C. Hamelin*

Department of Forest and Conservation Sciences, University of British Columbia, 2424 Main Mall, Vancouver, BC, V6T 1Z4 Canada.

One consequence of poplar domestication is that plantations of hybrid poplars are grown alongside native poplar. This can lead to the movement of pathogens from co-evolved poplar pathosystems into plantations, which may have catastrophic repercussions. Inevitably, the exchange of plant material for breeding increases the probability of pathogen spread. *Mycosphaerella populorum* Thompson (anamoph *Septoria musiva* Peck) is a leafspot and canker causing pathogen of poplar and is endemic and widespread amongst poplar in North America. Increased reports of this pathogen, including an ability to colonise and cause cankers in woody tissue coincided with the expansion of the poplar industry. We suspect that populations of *M. populorum* have complex and contrasting demographic histories that reflect migration during ancient time periods (post glaciation) and more recently, due to the exchange of plant material for commercial poplar culture. This pathogen was most recently reported in BC in 2006 and in Alberta in 2009. In order to manage disease spread, prevent further incursions and ensure the sustainability of breeding programs, we characterised pathways of pathogen movement by examining the genomes of 84 individuals that had been collected from planted poplar in five Canadian provinces and six American states. We found that *M. populorum* forms two broad groups in eastern and western North America and generally genetic clusters are linked to geographical populations. Furthermore, modeling based approaches suggest that *M. populorum* originated in the US and has repeatedly spread or been introduced into Canada. The most likely vector enabling the recent introductions of this pathogen may be the movement of poplar material for commercial poplar culture or breeding programs. This finding has significance for phytosanitary certification of plant material that relies only on observation-based pathogen screening as opposed to molecular based diagnostics.

Funding support: Genome BC and Genome Canada (grant #2112).

S4 – O8

Key words:
fungal leaf endophyte communities, *Melampsora* rust, *Populus trichocarpa*, plant defense, next generation sequencing

Fungal leaf endophytes alter *Melampsora* severity in *Populus trichocarpa*

Posy E. Busby[12], Joshua Miller[2], Shannon Fraser[2], Dylan Smith[3], Kabir Peay[3], George Newcombe[2]

[1]Biology Department, University of Washington, Seattle, Washington
[2]College of Natural Resources, University of Idaho, Moscow, Idaho
[3]Biology Department, Stanford University, California

Fungal leaf endophytes are nonpathogenic microbes that reside within land plants. Inoculation studies have demonstrated a range of disease outcomes when endophytes interact with pathogens of *Populus* (e.g., *Melampsora*, *Marssonina*), suggesting that they may play an underappreciated role in plant defense. For this to be true, strong interactions between endophytes and pathogens would have to be common across the geographic range of species of *Populus*. We used both high-throughput DNA sequencing and traditional culturing to characterize endophyte communities of *Populus trichocarpa* throughout the core of its range. Next, individual endophyte isolates captured by culturing were tested against *Melampsora* in greenhouse inoculation experiments. Endophyte community composition varied significantly among tree populations, yet strong interactions with *Melampsora* (both disease-enabling and disease-antagonizing) were common to most communities. Our findings suggest that strong interactions between fungal leaf endophytes and *Melampsora* are widespread and play a key role in disease outcomes.

Funding support: NSF SEES (PB), USDA AFRI (GN).

S4 – O9

Key words: herbivore functional groups: chewers, skeletonisers, miners, leaf rollers, sap feeders, gall makers

Herbivore association of different poplar species, hybrids and clones

Katalin Tuba, Ferenc Lakatos

University of West-Hungary, Institute of Silviculture and Forest Protection, 9400 Sopron, Hungary

We investigated the characteristics of herbivore associations developing on *Populus* trees of different European origin. Our aim was to determine which effect (e.g. the genotype or the geographic origin of a tree) has an influence on the herbivore insect association of the host plant. Various *Populus* taxa (species, hybrids and clones) were examined in a common-garden experiment in *Western* Transdanubia from 2009 to 2011. Damages caused by the insects were categorized into six functional groups: 1) chewers, 2) skeletonisers, 3) leaf miners, 4) sap feeders, 5) gall makers and 6) leaf rollers. Linkage between the damage caused by the different functional groups and the characteristics of the host plants were examined using various statistical methods.

Some of the functional groups (damage types) had closer linkage to the genotype and/or to the geographic origin of the host tree, while others hadn’t any. The genotype of the hosts affected the establishment of the functional groups significantly. The following linkages were identified: the rate of damage caused by the chewers; species composition and abundance of leaf miners. The damage caused by leaf rollers was closely related to the leaf size, especially for *Byctiscus populi*. The genetic relationship among the Poplar clones played an important role in the occurrence of gall makers and sap feeders. There was a geographic variation amongst the *Populus nigra* clones too.

The other functional groups didn’t show clear correlations with the various effects. E.g. no correlations were identified for the ratio of chewers, or for the damage caused by the skeletonisers and the geographic origin of the host tree.

Funding support: Our work was supported by the Network of Excellence EVOLTREE (contract no. 016322, 6th FP) and by the TÁMOP 4.2.2.A-11/1/KONV-2012-0013 project.

S4 – O10

Key words: pest insects, short rotation coppice, natural enemies, biological control

Economic importance and natural enemies of the red poplar leaf beetle (*Chrysomela populi* L.) in poplar short rotation coppice in Germany

Richard Georgi, Michael Müller

Department of Forest Sciences, Technische Universität Dresden, Tharandt, Germany

The red poplar leaf beetle (*Chrysomela populi* L.) is deemed to be the most important insect pest in poplar short rotation coppice (SRC) plantations in Germany. Both imagines and larvae damage young leafs through their feeding. As a consequence, cuttings newly sprouting shortly after plantation establishment and shoots growing from the stool in the first spring after harvesting are regularly damaged. Feeding on the apical leafs also serves to reduce the biomass yield. Given the ecologically-oriented management of SRC in Germany, the scope for the use of plant protection products is very limited. For this reason, biological pest control strategies are being studied as an important part of integrated pest management (IPM) for SRC in Germany. In a recent first step, the occurrence and influence of natural enemies of *C. populi* were studied. Imagines are frequently infected by the mite *Linobia coccinellea*, which can be found under the elytra feeding on haemolymph. Densities of up to 200 mites per imago are possible. The hoverfly *Parasyrphus nigritarsis* was found to be the most important antagonist during the egg stage, achieving predation rates of over 90%. In spite of a defensive secretion mechanism, stink bugs like *Arma custos*, *Picromerus bidens* and *Pinthaeus sangunipes* are common predators of the larval stage. With parasitation rates of between 30% and 70%, the chalcid wasp *Schizonotus sieboldi* has a great impact on the pupal stage. The common occurrence of the leaf beetle in combination with a high impact of natural enemies under normal outdoor conditions suggests that biological control agents may be used to control *C. populi*. Subsequent studies will focus on possible measures to enhance natural enemy densities through conservation and augmentation.

Funding support: German Federal Ministry of Education and Research.

S4 – O11

Key words:
pests,
pesticides,
control, poplar

Evaluating alternative pest control strategies in FSC certified hybrid poplar stands

R. Andrew Rodstrom[1]*, Alejandro I. Del Pozo*[2]*, Justin Skoczylas*[3]*,*
Tim D. Waters[3]*, John J. Brown*[3]

[1]GreenWood Resources, Inc. 1500 SW 1st Ave, Suite 1150, Portland, OR 97201 [2]North Carolina State University, Campus Box 7613, Raleigh, NC 27695-7613 [3]Washington State University, 404 W. Clark, Pasco, WA 99301 [4]Washington State University, Dept. of Entomology, Washington State University, Pullman, WA 99164, USA

Hybrid poplars are an attractive food source for native and non-native herbivores in many regions where they are grown. Pest control in certified (FSC, RSB, etc.) plantations can be a challenge based on the restricted use of conventional pesticides and their push for reduced pesticide use. As a result, alternative techniques and pesticides must be evaluated to determine their ability to control target pest(s) in the poplar system. We examined the efficacy of multiple pesticides and techniques to control two major pests (*Polydrusus impressifrons* & *Prionoxystus robiniae*) on the Boardman Tree Farm in eastern Oregon. *Polydrusus impressifrons* (Coleoptera: Curculionidae) is a European species of weevil that can cause mortality by defoliating cuttings during stand establishment when present in large populations. *Prionoxystus robiniae* (Lepidoptera: Cossidae) is a moth attacks larger trees and significantly degrades wood quality and tree integrity with their large larval galleries. Pesticide soaks and foliar applications were evaluated for their ability to control adult *P. impressifrons* during stand establishment, while a trap out strategy (mating disruption + attract-and-kill) was tested to manage populations of *P. robiniae* in high-risk stands. Cuttings soaked in a pesticide solution suffered less damage from adult *P. impressifrons* than those treated with a foliar application (ANOVA: $F = 78.42$; $df = 4, 194$; $P < 0.0001$). Populations of *P. robiniae* were significantly less in stands treated with the trap out than stands adjoining the treated areas (paired *t* test: $t = -3.45$, $df = 11$, $P = 0.0055$). Both cutting soaks and the trap out strategy were shown to be effective pest control options in the poplars. We would recommend the inclusion of these techniques in a growers integrated pest management program in systems that may require conformance to certification schemes.

Funding Support: GreenWood Resources, Washington State University, Washington State Commission on Pesticide Registration, Syngenta

SESSION IV – PESTS AND PATHOLOGY

Poster Presentations

S4 – P1

Key words: poplar, breeding, SRC, *Melampsora larici-populin*a, leaf rust

Tolerance characteristics of newly bred top performing black and balsam poplars against *Melampsora larici-populina*

Christina Fey-Wagner, Natalia Klippert, Alwin Janssen

Northwest German Forest Research Institute (NW-FVA), Department Forest Genetic Resources, Prof. Oelkers-Str. 6, D-34346 Hann. Muenden, Germany

There are numerous advantages of the production of biomass for energetic and material use by cultivation of fast growing trees such as poplars and willows in short rotation coppices (SRC). Therefore the importance of SRC with these fast-growing trees had been increased in Germany during the last few years. The reproductive material that is currently available on the market is based on a very limited number of suitable clones with a small genetic base. Therefore one focus of the joint research project “Breeding of fast-growing tree species for the production of renewable resources in short rotation coppices” (FastWOOD) is breeding, genetic characterization and evaluation of new black and balsam poplar as well as willow clones. The short rotation suitability of new varieties is controlled and evaluated not only on high yield but also by a continuous characterization of their tolerance characteristics under a broad range of rust infection and climate conditions as well as site properties on German wide test trials. First results from the test series show that newly bred varieties could double the yield of recently used clones. The tolerance behavior of these selected high yielding clones against the poplar leaf rust *Melampsora larici-populina* was frequently evaluated. The results of the yield potential were combined with the influence of rust infestation intensity. An overview of the development of leaf rust infections during the first three-year observation period will be given compared with the results of biomass yields at six black- and balsam poplar-plots established in the joint research project FastWOOD.

Funding support: German Agency of Renewable Resources (FNR) by order of the Federal Ministry for Food and Agriculture (BMEL)

S4 – P2

Key words:
poplar,
transplanting plants,
weakness pathogens,
endophytism,
transplant crisis

Pathogenic endophytic fungi in transplanting poplar plants

Naldo Anselmi[1], A Giorcelli[2], E. Rocco[1]

[1]Department for Innovation in Biological Systems, Agri-Food and Forestry (DIBAF), Università degli Studi della Tuscia, via S. Camillo de Lellis, 01100 Viterbo, Italy. [2]CRA (Agricultural Research Council) – PLF (Research Unit for Intensive Wood Production), Strada per Frassineto, 35, 15033 Casale Monferrato (AL), Italy

In the new poplar plantations, transplanted plants often die (crisis by transplant) for bark attacks of fungal weakness pathogens, such as (in Italy) Discosporium populeum, Cytospora spp., Phomopsis spp. and Fusarium spp. Recent studies showed that some of these fungi are able to live asymptomatically inside healthy tissues (endophytic pathogens).

The aim of this research was to evaluate the frequency of endophytic fungi on asymptomatic nursery poplar plants and to identify those that concur in crisis by transplant. The samplings were carried out in the years 2006-07 in 1 and 2 years-old Populus×canadensis ('I-214' clone) nurseries, both at Viterbo (Central Italy) and at Casale Monferrato (Northern Italy). In each nursery 10 plants were randomly selected and in vitro isolations from 20 twigs and 20 buds per plant were carried out. The fungal isolates were identified by their morphological and molecular characters.

Pathogenic and non pathogenic endophytic fungi were found in asymptomatic poplar tissues. Among latent bark pathogens it is remarkable the presence of D. populeum and Phomopsis spp. in samples from Casale Monferrato, Cytospora spp. and Phomopsis sp. in samples from Viterbo. This finding is in agreement with the high incidence on field trasplanted plants of Phomopsis and (rarely) D. populeum attacks in Northern Italy, and of Cytospora in Central and Southern Italy.

Our results showed that the aforesaid fungi can live asymptomatically in healthy tissues of nursery plants. In the new plantations they are probably induced to cause necroses by the stress connected with the transplanting. They also show a reduction in the incidence of D. populeum in Italy, probably a result of climate change. The importance of these results under a practical point of view is discussed.

S4 – P3

Key words:
Mycosphaerella populorum, *Populus trichocarpa*, real-time PCR

DNA-based survey of the incidence of the poplar canker pathogen (*Mycosphaerella populorum*) in plantations and natural stands of poplar in British Columbia

Padmini Herath[1]*, Nicolas Feau*[1]*, Harry Kope*[2]*, Stefan Zeglen*[2]*,*
Richard Hamelin[1]

[1]Department of Forest and Conservation Sciences, University of British Columbia, Vancouver, British Columbia, V6T 1Z4 Canada
[2]British Columbia Ministry of Forests, Land and Natural Resource Operations, P.O.Box 9513 Stn Prov Govt, Victoria, BC V8W 9C2, Canada

Septoria leaf spot and canker caused by *Mycosphaerella populorum* Peck is one of the most damaging poplar diseases in North America. Cankers can be especially devastating on susceptible trees, leading to tree death and reduced biomass in commercial plantations. The closely related species *Mycosphaerella populicola* Peck, causes only leaf spots on poplars of the Tacahamaca section (*P. balsamifera* and *P. trichocarpa*) without any significant impact on trees. Though it was originally thought that *M. populorum* was confined to eastern and central North America, in 2006 it was detected for the first time in the Pacific North-West in a hybrid poplar plantation in the Fraser Valley. The disease has so far only been reported in the Lower Fraser Valley in BC. In order to investigate the incidence and distribution of *M. populorum* from its point of introduction, four *P. trichocarpa* natural stands and 21 hybrid poplar plantations were surveyed from 2011-2013, by performing *Mycosphaerella* spp. real-time PCR assays directly on leaf spot symptoms. Only ~3% of the *P. trichocarpa* tested (natural populations and provenance tests) were diagnosed as positive for *M. populorum* while the majority of these trees (69-85%) were positive for the endemic species *M. populicola*. In contrast, *M. populorum* frequency was higher in the hybrid poplar plantations (56.1% of the sampled trees) whereas *M. populicola* occurrence was limited (4.8%), indicating that hybrid poplars are highly susceptible to *M. populorum*. Sixteen out of the 21 hybrid plantations surveyed were found to have at least one tree positive for *M. populorum*. These findings suggest that the occurrence of *M. populorum* in BC is likely linked to the planting of hybrid poplars. We suspect that the pathogen is spread via the dissemination of contaminated cuttings. We are currently testing protocols to detect the pathogen directly on dormant cuttings.

Funding support: British Columbia Ministry of Forests, Genome Canada, Genome BC

S4 – P4

Key words:
genomics,
disease
resistance,
RPM1
interacting
protein 4,
poplars, natural
selection,
evolution

Strong positive selection on the innate immunity regulator RIN4 in poplars and its significance to disease resistance evolution in poplars

Subinoy Biswas[1], *Armando Geraldes*[1], *Charles A. Hefer*[1], *Nima Farzaneh*[1], *Daisie Huang*[1], *Carl J. Douglas*[1], *Shawn D. Mansfield*[2], *Quentin C. B. Cronk*[1]

[1]Department of Botany, University of British Columbia, Vancouver, British Columbia, V6T 1Z4 Canada [2]Department of Wood Sciences, University of British Columbia, Vancouver, British Columbia, V6T 1Z4 Canada

Resequencing data from different poplar species offers a means to survey the evolutionary dynamics of genes integral to plant innate immunity. As an example, we report evidence that RPM1 interacting protein 4 (RIN4), an important negative regulator of plant immunity, is under strong purifying selection within species, but strong diversifying selection between species. This conclusion is supported by (i) a significantly raised proportion of non-synonymous to synonymous substitutions between species relative to within species, (ii) a significantly lower ratio of polymorphism to fixed differences in exons relative to non-coding regions, and (iii) model-based phylogenetic tests of diversifying selection. This evolutionary dynamic suggests that episodes of exceptionally strong positive selection have shaped the plant immune system in poplars. In Arabidopsis, RIN4 is known to have compatible and co-evolved interacting partners. In contrast, RIN4 and its interacting partners are incompatible in some hybrids (e.g. lettuce), where autoimmune effects can occur, such as leaf-spot necrosis. This immune phenomenon has even been suggested as a species isolating mechanism. Autoimmune effects are currently not known in poplars, but merit investigation, particularly between more distant hybrids.

Funding support: Genome Canada

S4 – P5

Key words:
Salix,
Melampsora,
rust resistance,
BAC library,
gene
expression

Development of molecular markers for rust resistance genes in bioenergy willow

Berit Samils[1], Tom Martin[2], Ann-Christin Rönnberg-Wästljung[2] and Jan Stenlid[1]

[1]Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences, Uppsala, Sweden [2]Department of Plant Biology, Swedish University of Agricultural Sciences, Uppsala, Sweden

A major challenge in willow cultivation is to avoid leaf rust epidemics as willow cultivars are under constant threat of new virulent races that can overcome resistance. One of the most important breeding goals in willow is therefore to obtain a durable rust resistance. The aim of our work is to characterize resistance genes in the current breeding material and develop molecular markers for a variety of resistance genes to be used in marker-assisted selection (MAS). Pyramiding of more than one resistance gene in a cultivar will make it more difficult for the rust fungus to overcome the resistance.

Some hybrids between *Salix viminalis* and *S. schwerinii,* an exotic species introduced from Siberia, are highly resistant towards the willow leaf rust caused by *Melampsora larici-epitea* in Sweden and have become some of the most cultivated cultivars in short rotation coppice for bioenergy purposes. A major QTL (quantitative trait locus) responsible for several components of rust resistance in a hybrid backcross *S. viminalis* x *S. schwerinii* has been identified with QTL mapping. By screening a BAC library constructed for the hybrid parent and sequencing of positive BAC clones we could identify a putative rust resistance gene with a TIR-NBS-LRR domain structure. The gene has been tested in an expression study where infected and uninfected resistant and susceptible leaf tissue was analysed to compare expression levels of the candidate gene at different time points.

Funding support: Swedish Energy Agency, Swedish University of Agricultural Sciences, Lantmännen SW seed AB.

S4 – P6

Key words:
light interception, leaf area, rust, clonal variability

The effect of rust on light interception depends on canopy structure

Silvia Cortizo[1,2], Nora Abbiati[3], Corina Graciano[4,5], Juan José Guiamet[5]

[1]E.E.A. Delta del Paraná (INTA), Argentina [2]Facultad de Agronomía, Universidad de Buenos Aires, Argentina[3]Facultad de Ciencias Agrarias, Universidad Nacional de Lomas de Zamora, Argentina [4]Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata [5]Instituto de Fisiología Vegetal (INFIVE), CONICET, Argentina.

Growth and productivity of trees depend largely on photosynthetic surface area, provided primarily by the leaves. To assess the effect of crown architecture and rust on light interception we established a factorial experiment, with two clones (Populus deltoides ‘Australiano 106/60’ and ‘Onda’) and two levels of disease (without rust, sprayed with 25.8 g ai/ha of Tebuconazole, and with rust, no sprayed), in a completely randomized design with three replications. Incident light and leaf area were evaluated monthly since the appearance of the first pustules until the start of the fall defoliation at different heights within the canopy. The results indicate differences in the transmission of photosynthetically active radiation (PAR) between the clones and between treatments that were changing during the evaluated period. Rusted plants, which lost part of their foliage, transmitted more light than healthy plants, but the amount of transmitted light varied with the height and the date differently depending on the clone. In early summer, the healthy plants of ‘Australiano 106/60’ transmitted less light than the ones of ‘Onda’, but this difference was gradually reduced as the growing season progressed and in late April both clones intercepted a similar proportion of light. The differences were even greater between rusted plants, because while the ‘Australiano 106/60’ plants intercepted around 50-60% of the incident radiation, the ‘Onda’ plants intercepted only 35-40%. From March onwards the light transmitted differences between clones lost importance and only the differences between treatments and between heights remained significant. Changes in leaf area did not fully explain light transmission differences. Other variables such as chlorophyll and nitrogen content and crown architecture, also modified by rust, could have affected light interception without significantly modifying the leaf area. The inclusion of such variables would improve the light interception estimation.

Funding support: INTA, MAGyP, Carabelas Group.

SESSION V – ENVIRONMENTAL APPLICATIONS OF POPLAR & WILLOW

Oral Presentations

S5 – O1

Key words: waste water, rhizosphere, nitrogen, denitrification, year-round

Field-scale year-round waste water treatment in poplar rhizosphere - Lessons learned

Louis A. Licht[1], C.J. Just[2], H Ausland[2]

[1]Ecolotree® Inc., North Liberty, IA. USA [2]Civil and Environmental Engineering, The University of Iowa, Iowa City, IA USA

Port of Morrow (POM) at Boardman OR, USA creates average 21 million liters (5.8 million gallons) waste water per day discharged by food processors. The effluent has an average 114 ppm nitrogen applied by center pivot irrigation on crops as protein and ammonia nitrogen. Waste water is legally irrigated during the growing season at agronomic rates to provide both water and nitrogen on 2,300 hectares (5,000 acres) of food crops. Oregon State University has established nitrogen agronomic rates for all commodity crops irrigated with reclaimed waste water. Agronomic rate serves as the basis for a land application discharge permit issued by Oregon Department of Environmental Quality.

Poplar and willow are an important core plant in the EBuffer® phyto concept where agronomic nitrogen uptake by plants is supplemented by denitrification loss in the rhizosphere. A demonstration EBuffer is installed on 23 hectares (60 acres) of sandy soil to adequately treat winter waste protein nitrogen in effluent. The demonstration EBuffer is planted with poplar supplied by Ecolofarm Nursery and Greenwood Resources. In the field, irrigation water and subsoil moisture content are tracked. Nitrogen dosage is tracked and monitored by both soil sampling and lysimeters. These field data are coupled with poplar rhizosphere research at The University of Iowa. In a controlled lab held at 4.5°C, soil and established poplar roots create a dormant-tree rhizosphere reactor. The field and laboratory data sources combine to better quantify nitrogen transport and cycling dynamics when POM irrigates waste water.

Early research test cell data indicate increased nitrogen retention in a poplar rhizosphere during winter when there is no crop uptake. Phyto treatment and cropping is an alternative to aerobic mechanical waste water systems which cost more capital, require more operating energy and emit more greenhouse gases.

S5 – O2

Key words:
POPFULL, soil greenhouse gas fluxes, soil microbial communities, root biomass

Environmental controls versus microbial community composition as drivers of soil greenhouse gas flux dynamics in a poplar bioenergy plantation (POPFULL)

Carolyn-Monika Görres, R. de Man, J.T. Weedon, G. Berhongaray, R. Ceulemans

University of Antwerp, Department of Biology, Research Group of Plant and Vegetation Ecology, B-2610 Wilrijk, Belgium.

The cultivation of short rotation woody crops (SRC) is receiving a lot of attention as a means to replace greenhouse gas (GHG) emissions from fossil fuels. However, the true GHG mitigation potential of SRC remains largely unknown due to limited knowledge on the amount of GHG released from the soil during cultivation. The aim of this study is to identify drivers of the spatial variability in soil GHG fluxes in a poplar SRC plantation located in Lochristi (Flanders, Belgium; POPFULL). In 2010, the unfertilized plantation has been established, partly on former pasture and partly on former cropland. This offers the unique opportunity to study soil GHG flux dynamics with respect to their dependency on former land-use type under identical climate and management conditions. Furthermore, spatial differences in soil organic carbon input are created within each former land-use type by alternating row spacings between the poplars, allowing a more detailed study of the role of the SRC crop in regulating the soil carbon and nitrogen cycle. Thus far, harvesting events have taken place in February 2012 and February 2014. Since summer 2013, simultaneous monitoring of soil CO_2, CH_4 and N_2O fluxes has been conducted with a custom-made automated chamber system accompanied by soil gas concentration profile measurements. Preliminary results show that former land-use type as well as inter-row spacing seem to affect soil CO_2 concentrations and thus also soil CO_2 fluxes. Significant N_2O fluxes are observed after heavy rain events, but the spatial variability is high. No significant CH_4 fluxes have been monitored thus far. The soil GHG flux data are being linked to data on root biomass, on soil microbial community composition and on soil microbial biomass from different developmental stages of the plantation. This research results in new significant insights into the importance of environmental controls versus microbial community composition for soil GHG flux dynamics in SRC.

Funding support: ERC Advanced Grant agreement (# 233366) POPFULL under the EC 7th Framework Programme (FP7/2007-2013), Flemish Hercules Foundation as Infrastructure contract # ZW09-06, and the Methusalem Program of the Flemish Government.

S5 – O3

Key words
slope erosion, pastoral land, tree effectiveness, tree size, roots

Effectiveness of conservation poplar and willow trees in reducing slope erosion following a storm event

Ian McIvor[1], *Kerry Clarke*[1] *and Grant Douglas*[2]

[1]Plant & Food Research, Private Bag 11600, Palmerston North 4442
[2]AgResearch, Private Bag 11008, Palmerston North 4442, New Zealand

On the 25th of April 2011 a severe storm in coastal Hawke's Bay, New Zealand, caused major slipping, infrastructure damage, and stock and pasture losses. The effectiveness of soil conservation plantings of poplar and willow in reducing slipping in the affected areas was evaluated during the period January – May 2013. Eleven of the farms that suffered severe slipping were visited. For each farm, sites with trees and comparable sites without trees were identified from aerial maps. The sites were visited and mapped for slipping, tree spacing and distance of slips from the nearest tree. Other data collected were aspect of the site, mean slope, number of trees, species of tree and tree size (trunk diameter). Calculations were made of the area of protection extended by the trees and the effectiveness of the conservation trees was linked with tree size, tree species, and tree spacing.

Data were collected from 86 sites with trees (treed sites) and 25 sites with pasture only (control sites). The control sites were close to a treed site. Numbers of trees at treed sites were usually between 3 and 8, but ranged from 1 to 14.

Slipping was reduced by 78% on treed sites compared with control sites. Mature plantings of groups of both poplar and willow reduced slipping within a zone of ~10 m of the trees to almost zero. Where plantings had a mean DBH of <20 cm, their effectiveness was reduced dependent on spacing. For trees with a mean DBH of ~10 cm effectiveness was negligible regardless of spacing. Despite this, trees with DBH of ≥10 cm were generally able to withstand shallow slipping without being totally dislodged. Where trees were planted in gullies the same criteria applied for their effectiveness in halting the advancement of the gully upslope.

Funding support: Funding was contributed by New Zealand Ministry for Primary Industries, New Zealand Poplar & Willow Research Trust and Beef + Lamb NZ

S5 – O4
Key words: poplars, willows, phytoremediation, pollution, trangenics, environmental contaminant

Enhancing phytoremediation of a complex contaminated soil system

Raymond Oriebe Anyasi, Harrison Ifeanyichuku Atagana
Department of Environmental Sciences, Institute for Science and Technology Education, University of South Africa, 1 Preller St, Muckleneuk Ridge, Pretoria, South Africa

Extensive human activities have lead to the release of enormous amount of toxic compounds into the soil. These compounds that include heavy metals, metalloids as well as organic pollutants causes threats to the environment as a result of its serious health effects to human, animals and plants. Phytoremediation has been generally accepted as the proven technology for the removal of such contaminants from soil due to its minimal energy requirement and the beauty of the technique. However, the efficient nature of phytoremediation technology suffers serious limitations that have affected its field application. Plants are autotrophs hence lack the necessary enzymes needed for a complete xenobiotic removal from the soil. In contrast, heterotrophs which include microbes and mammals possess such metabolic enzymes that could be used to mineralize organic molecules. Therefore catabolic genes from heterotrophs can be used to complement the remediation capabilities of plants. Although genetically modified plants have been extensively employed in metal sequestration from soil, such implication have not been employed In a complex contaminated soil system (soil containing heavy metals, high molecular weight PAHs and PCBs) as can be found in nature. This study aimed to explore the effects of genetically modified plants (poplars and willows) in the remediation of compounds that constitute a complex contaminated soil system and the interaction between those compounds. Poplars and willow plant engineered with human *CYP2C19* and *γ-ECS*, were grown in microcosm containing soil which has been pre-treated with 30,000 ppm of Aroclor 1260, pyrene, and lead salt. The experiment was monitored for 90 days and the plant harvested and analyzed. Genetically modified plants showed much tolerance to the contaminants than the control plants which was free from the genes. At the end of the 90 days of growth of the transgenic poplars and willow in the contaminated soil, the residual concentration of the mixed contaminants was reduced to between 47-91% while control only reduced by 12%. This has shown that transgenic phytoremediation could be implemented in soil containing mixture of environmental contaminants.

S5 – O5

Key words: forest restoration, eastern cottonwood, conservation capitalism

Advancement of an eastern cottonwood afforestation system from research to natural capitalism that promotes forest restoration in the Lower Mississippi Alluvial Valley, USA

Emile S. Gardiner[1], *Theodor D. Leininger*[2], *Chander Van Voorhis*[3]

[1]Center for Bottomland Hardwoods Research, Southern Research Station, USDA Forest Service, Stoneville, MS, USA [2]Center for Bottomland Hardwoods Research, Southern Research Station, USDA Forest Service, Stoneville, MS, USA [3]GreenTrees, LLC, The Plains, VA, USA

Deforestation has claimed more than 70% of forests in the 10 million hectare Lower Mississippi Alluvial Valley (LMAV). Efforts to develop forest restoration practices applicable to economically marginal agricultural land in this region date to the 1940s. Early afforestation was accomplished with single-species plantations established under low intensities of site preparation and stand maintenance. Federal legislation passed in the 1980s began providing landowners with incentive to remove marginal land from production. This led to a realization that existing afforestation practices were limited in their ability to meet a diversity of landowner objectives while facilitating restoration of forest functions. In 1994, scientists and collaborators at the Center for Bottomland Hardwoods Research in Stoneville, Mississippi began investigating a prototype afforestation system designed around stand development patterns observed in natural eastern cottonwood (*Populus deltoides*) forests. In 2005, research findings led to changes in federal policy that has enabled landowners to employ cottonwood-hardwood interplanting to restore forests on marginal agricultural land. More recently, this cottonwood-based afforestation system is being applied by a private conservation company pursuing “natural capitalism”. Specifically, carbon sequestered by cottonwood–hardwood interplantings managed by private landowners in the LMAV is marketed to industrial emitters of CO_2 operating in other regions. Currently, more than 8,000 hectares of cottonwood–hardwood interplantings are contributing to the greatest source of afforestation derived carbon credits in North America. This presentation will chronicle advancement of this novel afforestation system from initial research, to demonstration of application on economically marginal agricultural land, to informing federal policy, and through its eventual use to secure private investment capital for forest restoration in the LMAV.

S5 – O6

Key words:
Glomus,
*Gigaspor*a,
tissue
culture,
microplants,
peat-perlite-vermiculite

Screening arbuscular mycorrhizal fungi for tolerance to arsenic by *Populus alba x glandulosa* transgenic clone Cd26c2

Nelly S. Aggangan[1], *Young-Im Choi*[2], *Eun Woon Noh*[2] *and Sim-Hee Han*[3]

[1]National Institute of Molecular Biology and Biotechnology, University of the Philippines Los Banos, College, Laguna 4031 Philippines [2]Biotechnology Center, Korea Forest Research Institute, Suwon 441-847, Korea; [3]Department of Forest Genetic Resources, Korea Forest Research Institute Suwon 441-847, Korea

This experiment was conducted to determine the effect of mycorrhizal inoculation and arsenic on the growth of *Populus alba x glandulosa* transgenic clone Cd26c2. Cd26c2 is one of the preferred fast growing and easily propagated transgenic clones at the Biotechnology Center, Korea Forest Research Institute (KFRI), Suwon, Korea for rehabilitation of mine tailings. One-month old microplants were inoculated with mycorrhizal fungi from mine site in Bonghwa, South Korea (coded as AMM4, AMM6, AMM11 and AMM12) and that from the Philippines: *Glomus etunicatum*, *Glomus fasciculatum* and *Gigaspora margarita*. The control microplants received sterilized soil inoculant. The microplants were planted in plastic cups filled with autoclaved peat perlite vermiculite medium and incubated under growth room conditions. After one month, seedlings were transferred into bigger (9.14cm top diameter x 7.14cm bottom diameter x 10.84cm height) pots filled with non-autoclaved peat perlite vermiculite (150g dwt pot^{-1}) medium. Two levels of arsenic [0 and 1.75g As (as $Na_2HAsO_4.7H_2O$) kg soil^{-1}] were mixed thoroughly in the soil medium prior to seedling transfer. Pots were watered to field capacity and incubated for one week prior to transplanting the treated seedlings.

After three months, height, stem diameter and plant dry weight were significantly affected by mycorrhizal inoculation and arsenic. There was also a significant interaction between mycorrhiza and arsenic treatments. Irrespective of arsenic treatment, *G. margarita, G. fasciculatum* and AMM4 increased (p<0.001) plant height. Highest stem diameter and total dry weight was obtained from plants inoculated with *G. fasciculatum*. All the Bonghwa AM fungi except for AMM6, significantly promoted plant dry weight. AM12 promoted the highest increase (42%) over the control. Arsenic significantly reduced the total plant dry weight of treated plants with reduction ranged from 12 to 33%. The lowest reduction was obtained from *G. margarita* inoculated plants and the highest was observed in AM11 inoculated plants.

Funding support: UP System International Conference Travel Fund/Commission on Higher Education International Conference Travel Grant

S5 – O7

Key words:
Salix,
phytoremediation, plant
physiology,
organic
compounds,
soil

Effect of organic compound contamination on biomass production and physiological responses of 11 *Salix* clones in southern Quebec.

Vanessa Grenier[1], Werther Guidi[1,2], Frédéric Pitre[1], Michel Labrecque[1]

[1]Institut de Recherche en Biologie Végétale, 4101 Sherbrooke East, Montreal, Québec, Canada, H1X 2B2 2Department of Plant, Soil and Environmental Science, University of Florence, Piazza di San Marco, 4, 50121 Florence, Italy

This research project is part of a larger multidisciplinary initiative called GenoRem (www.genorem.ca). GenoRem brings together scientists from the Université de Montréal and McGill University in a quest to improve bioremediation techniques through molecular and biological processes involved in willow-microbe-soil interaction. Eleven willow (*Salix*) cultivars were studied, primarily to identify those that produce high biomass yields, demonstrate capacity to establish under harsh environmental conditions and may tolerate the presence of PAHs, PCBs and C10-C50. A high-density field experiment was undertaken during the summer of 2011 on a former petrochemical industrial site in southern Quebec, Canada. Cuttings of the selected willow genotypes were planted following a complete randomized experimental design on an area covering ~5000 m². Trees were monitored over two growing seasons by recording a series of growth parameters and physiological measurements (specific leaf area, chlorophyll content, stomatal conductance and nutrient composition) in order to assess and compare growth performance of each genotype on polluted soils. Statistical analyses (two-way ANOVA) revealed that *S. sachalinensis* (SX61) and *S. miyabeana* (SX67) achieved the highest biomass production, while *S. nigra* (S05) and *S. acutifolia* (S54) had the gestest photosynthetic capacity. While the cultivars *S. dasyclados* (SV1)*, S. purpurea* (Fishcreek) and *S. caprea* (S365) seemed negatively affected by the presence of contaminants, the establishment and development of *S. nigra* (S05)*, S. eriochephala* (S25) and *S. purpurea X S. miyabeana* (Millbrook) appear to have been much less influenced by soil pollution. This comparative analysis of physiological responses of willow on contaminated soil may improve plant selection processes and bioremediation practices in future phytoremediation projects.

Funding support: Génome Québec, Genome Canada

S5 – O8

Key words: biomass productivity, carbon storage, field tests, phytotech-nologies, *Populus*

Ecosystem services of poplar at long-term phytoremediation sites in the Midwest and Southeast, United States

Ronald S. Zalesny Jr. [1], *William L. Headlee*[2], *Gayathri Gopalakrishnan*[3], *Richard B. Hall*[2], *Dennis W. Hazel*[4], *J.G. Isebrands*[5], *M. Cristina Negri*[6], *Elizabeth Guthrie Nichols*[4], *Donald L. Rockwood*[7]

[1]Institute for Applied Ecosystem Studies, U.S. Forest Service Northern Research Station, Rhinelander, Wisconsin, 54501 [2]Department of Natural Resource Ecology and Management, Iowa State University, Ames, Iowa, 50011 [3]Space Science Institute, Boulder, Colorado, 80301 [4]Department of Forestry and Environmental Resources, North Carolina State University, Raleigh, North Carolina, 27695 [5]Environmental Forestry Consultants, New London, Wisconsin, 54961 [6]Argonne National Laboratory, Lemont, Illinois 60439 [7]School of Forest Resources and Conservation, University of Florida, Gainesville, Florida, 32611

Short rotation woody crops (SRWCs) such as *Populus* species and their hybrids (i.e., poplars) are ideal for incorporating biomass production with environmental applications such as phytoremediation. In addition to being phreatophytes (i.e., having fast growth, extensive roots, and elevated water usage), poplars exhibit tremendous genetic variability that allows for selection of superior clonal material based on genetic- and site-related factors, as well as how specific genotypes respond to such stimuli. One of the most challenging responsibilities for traditional tree improvement programs, and most recently for phytoremediation applications, is the commitment and ability to continue measurements and monitoring throughout the rotation. To address this need, during 2012 and 2013 we sampled 15 poplar plantings from 11 long-term phytoremediation systems located in the Midwest (Illinois, Iowa, Wisconsin) and Southeastern United States (Florida, North Carolina). In total we tested 54 clones belonging to 10 genomic groups. For each site, we measured diameter at breast height (dbh), estimated individual-tree biomass according to Netzer et al. (2002), determined total biomass from planting density and survival, and calculated mean annual increment (MAI). In addition, based on a regional carbon study, we used an aboveground woody carbon percentage of 47.15% to estimate total and annual carbon per ha. Overall, total biomass ranged from 7.3 to 183.0 Mg ha^{-1} and MAI values were 1.5 to 19.8 Mg ha^{-1} y^{-1}. Associated carbon estimates were 3.4 to 86.3 Mg C ha^{-1} and 0.7 to 9.3 Mg C ha^{-1} y^{-1}.The variability was not surprising given that the trees ranged in age from 5 to 15 years old and the contaminants of concern were inorganic and organic, ranging in complexity from salts to petroleum hydrocarbons. Our presentation will focus on these differences within the context of provisioning (e.g., biomass, water) and regulating (e.g., carbon, soil quality, erosion control) ecosystem services and the need for a cleaner environment during times of accelerated ecological degradation.

S5 – O9

Key words:
Salix,
agroforestry,
windbreaks,
shelterbelts,
transportation

Shrub-willow living snow fences show potential for snow trapping and reduced drift length shortly after planting, increasing their function and feasibility

Justin P. Heavey, Timothy A. Volk

State University of New York College of Environmental Science and Forestry, Syracuse, NY 13224

Living snow fences are an alternative application for shrub-willows that can mitigate blowing snow problems on roadways by trapping and storing snow in drifts before it reaches the road. Living snow fences reduce the cost of highway maintenance, increase road safety, and provide numerous environmental benefits. Shrub-willows are an ideal choice for living snow fences because of their rapid height growth, high branch density, and relatively low cost of installation compared to other vegetation types. Research studies on living snow fences are limited and educational publications subsequently lack precise design protocols. This study measured height and optical porosity, the two vegetation characteristics most essential to snow trapping, on ten shrub-willow fences of various ages, as well eight additional fences of various vegetation types across an eleven year chronosequence. Vegetation data was combined with data on site characteristics to model snow trapping potential using established equations. The height of the living snow fences increased linearly and porosity decreased linearly over the eleven year chronosequence investigated. Changes in height and porosity were more rapid and predictable for shrub-willow than other vegetation types. Estimated snow trapping capacity of fences exceeded the quantity of blowing snow at each site just three years after planting, much earlier than the seven to twenty years commonly reported. Storage capacity at least15 times larger than snow transport at a given fence/site was strongly correlated with predicted downwind drift lengths of 10 m or less, which occurred as early as five years after planting. This influence of capacity/transport ratio on drift length is not accounted for in most publications, and existing setback recommendations range from 30 - 180 m. Results and regression equations produced in this study can improve the design, analysis, function, and feasibility of living snow fences.

Funding Support: New York State Department of Transportation (NYSDOT) and the United States Department of Transportation (USDOT) Research and Innovative Technology Administration (RITA)

S5 – O10

Key words : phytoremediation, *Salix*, heavy-metals, mono or polymetallic constraint

Potential use of three different *Salix* genotypes for the phytostabilisation of metal contaminated soils

Domenico Morabito[1], Sylvain Bourgerie[1], Daniel Auguin[1], Sylvain Bart[1], Florie Miard[1], Ladji Idrissa Diabagate[2], Emmanuel Joussein[3], Maryline Soubrand[3], Bashar Qasim[2], Mikael Motelica-Heino[2]

[1]Université d'Orléans, Laboratoire de Biologie des Ligneux et des Grandes Cultures, EA.1207, INRA-USC1328 [2]Université d'Orléans, Institut des Sciences de la terre d'Orléans, UMR 7327 CNRS-Université d'Orléans [3]Université de Limoges, Groupement de Recherche Eau Sol Environnement, EA. 4330, France

In the context of the ecological restauration of contaminated soils, it is crucial to acquire knowledge about the behavior of metal(oids) in different abiotic and biotic compartments and to define the mechanisms which condition their transfers, bioaccumulation and ultimately, their toxic and ecotoxicological effects at different levels of biological integration to assess further phytomanagement options.

The aim of this study was to assess the capacity of three salix genotypes (*Salix viminalis*, *Salix purpurea* north-west and *Salix purpurea* south-east) to develop a root system and to grow under a mono-or a polymetallic constraint. The mono-metal stress (Cd, Co, Pb, Hg, Ni) was obtained by adding at the beginning of a 55 days period of growth a mono-metal solution of 0, 16, 32, 64, 128 and 256µM to a sandy substrate. The three salix genotypes demonstrated the ability to produce a root system which was mainly affected by the highest metal concentration, whereas no significant differences in terms of biomass production was observed in the upper part of plants among the different metal concentrations. At the end of the treatment, plants samples were divided into initial cutting and roots, stems and leaves entirely formed during the treatment in order to evaluate the metal repartition. We observed a significant absorption of heavy metals in the different genotypes, while the accumulation of heavy metal was contrasted in the different organs. Additionally we studied in mescosms experiments the speciation, reactivity and transfer of metal(loid)s in a soil-water-plant continuum obtained from mining soils containing up to 15000 mg Pb.kg^{-1}, 83000 mg As.kg^{-1} and 1100 mg Sb.kg^{-1}. We demonstrate the possibility to grow *Salix* in such conditions when mixing the contaminated mining soils to a control garden soil at different ratios in mescosms. We concluded that these *Salix* genotypes can be proposed to stabilize metal contaminated soils.

S5 – O11

Key words: biomass, carbon sequestration, clones, environmental services, *Populus*

Aboveground biomass production and carbon sequestration of 12 hybrid poplar genotypes harvested from 17 sites in the north-central USA

William L. Headlee[1], *Ronald S. Zalesny Jr.*[2], *Richard B. Hall*[1], *Edmund O. Bauer*[1], *Bruce A. Birr*[2], *Jesse A. Randall*[1], *Adam H. Wiese*[2]

[1]Department of Natural Resource Ecology & Management, Iowa State University, Ames, Iowa, 50011 [2]Institute for Applied Ecosystem Studies, U.S. Forest Service Northern Research Station, Rhinelander, Wisconsin, 54501

Genetic improvements have resulted in tremendous gains in the biomass productivity of poplars in the north-central region and around the globe. The suite of clones produced by these efforts have shown considerable variation in their adaptability to different types of sites, demonstrating that genotype × environment interactions are important factors to be considered for successful and efficient deployment. Genetic and between-site variability in the carbon content of poplar wood is also an important consideration for carbon sequestration efforts, but the topic has received comparatively less attention than that of productivity. Therefore, the purpose of this study was to evaluate the impacts of site, clone, and their interactions on both aboveground biomass productivity and carbon content of 12 hybrid poplar genotypes harvested from 17 sites in Iowa, Minnesota, Wisconsin, and Michigan, USA. Preliminary results indicate that individual site × clone combinations varied in mean productivity from 2.2 to 25.9 dry Mg ha^{-1} yr^{-1} and in carbon content from 45.9% to 48.5% on a dry weight basis. Though *Populus deltoides* clones had similar productivity as interspecific hybrids (*P. deltoides* × *P. nigra*; *P. nigra* × *P. maximowiczii*; [P*. trichocarpa* × *P. deltoides*] × *P. deltoides*), the pure *P. deltoides* clones typically had lower carbon content. Among the interspecific hybrids, the clones having the highest and lowest carbon content varied between sites, thus further highlighting the importance of genotype × environment interactions when selecting clones to maximize carbon sequestration at a given site.

Funding support: U.S. Forest Service Research and Development Washington Office Woody Biomass, Bioenergy, and Bioproducts Program; U.S. Forest Service Northern Research Station Climate Change Science Council and the Institute for Applied Ecosystem Studies (RWU-NRS-13).

S5 – O12

Key words: aquatic-terrestrial linkages, community genetics, ecosystem dynamics, *Populus trichocarpa*

Exploring the genetic architecture of intraspecific variation and the consequences for aquatic-terrestrial linkages

Gregory M. Crutsinger[1], Seth M. Rudman[1], Mariano A. Rodriguez-Cabal[1], Athena D. McKown[2], and Takuya Sato[3]

[1] Department of Zoology, University of British Columbia 4200-6270 University Blvd. Vancouver, B.C. Canada [2] Department of Forest and Conservation Sciences, University of British Columbia, Vancouver [3]Department of Biology, Graduate school of Science, Kobe University, 1-1 Rokkodai, Nada-ku, Kobe 657-8501 Japan.

Ecologists are increasingly recognizing that individual variation, especially genetic variation, plays a crucial role in the assembly of communities and functioning of ecosystems. Yet, considerable gaps remain in our understanding of the underlying genetic basis of intraspecific trait variation and the extent to which the ecological consequences spill over the boundaries of different ecosystems. Here, we present an interdisciplinary study that integrates a common garden containing different genotypes of *Populus trichocarpa* and aquatic mesocosms to determine how intraspecific variation in leaf litter alters aquatic communities and ecosystem functioning. Moreover, we incorporate data from trait screening and genome-wide association studies to estimate the heritability and genes associated with litter traits. We found that tree genotypes varied considerably in the timing, quality, and production of leaf litter, which contributed to variation in phytoplankton, zooplankton, and mayfly abundances, as well as nutrient dynamics and light availability in aquatic mesocosms. Litter traits differed in their heritability values and the underlying genetic architecture of leaf litter traits was complex. Our results posit that genetic variation is an important component of aquatic-terrestrial linkages, while challenging the utility of associating community or ecosystem responses to the actions of one or a few genes.

SESSION V – ENVIRONMENTAL APPLICATIONS OF POPLAR & WILLOW

Poster Presentations

S5 – P1

Key words:
Glomus,
Acaulospora,
transgenic,
mine tailing
site,
cadmium,
lead, zinc,
arsenic

Arbuscular mycorrhiza versus ectomycorrhizal fungus *Pisolithus tinctorius* on growth and nutrient status of *Populus alba x glandulosa* in heavy metals amended soil

Nelly S. Aggangan[1] *and Sim-Hee Han*[2]

[1]National Institute of Molecular Biology and Biotechnology, University of the Philippines Los Banos, College, Laguna 4031 Philippines
[2]Department of Forest Genetic Resources, Korea Forest Research Institute, Suwon 441-847, Korea

Two concurrent experiments were conducted to determine the effectiveness of arbuscular mycorrhiza (AM) or ectomycorrhizal (ECM) fungus *Pisolithus tinctorius* in promoting growth and nutrient uptake of two (PCP301CG0R4 and PABC21) transgenic clones of *Populus alba* x *glandulosa* grown in non-autoclaved peat perlite vermiculite medium amended with three levels of heavy metals (HMs) (cadmium, lead, zinc and arsenic). AM soil inoculant was a mixture of unidentified species of *Glomus* and *Acaulospora* collected in mine tailing site in Bonghwa, Korea while ECM was vegetative mycelia of *P. tinctorius*.

After three months in a glasshouse, height increment, shoot and total dry weight of PCP301CG0R4 were reduced by the addition of HMs. Without HMs, AM promoted higher total plant dry weight than by ECM. The control plants had the lowest total dry weight. In HMs amended soil, both ECM and AM promoted similar plant growth and dry weight which were significantly higher than the control. In terms of nutrient uptake, AM inoculated PCP301CGoR4 plants took the highest N, K and Na than the ECM inoculated plants. AM and ECM plants had similar P, Mg, Cu and Zn uptakes. Without HMs, AM promoted higher N and K than ECM while in HMs amended soil, both ECM and AM plants had similar P, Mg, Cu and Zn which were significantly higher than in the control plants. The latter had the lowest nutrient uptake. In PABC21, height increment, diameter increment, shoot, root and total dry weight of AM inoculated plants were similar with the control plants. On the other hand, ECM inoculated plants had significantly lower shoot, root and total dry weight than the control. AM promoted the higher P and Na uptakes than ECM. Control and AM plants had comparable N, Ca, Mg, Cu and Zn uptakes. By contrast, ECM plants had lower N, P, Ca, Mg, Na, Cu and Zn uptakes as compared with the control. Funding Support: UP System International Conference Travel Fund/Commission on Higher Education International Conference Travel Grant

S5 – P2

Key words:
Populus nigra,
Castor fiber,
genetic
ressources,
management

Study of Salicaceae x European beaver (*Castor fiber*) interactions following modification of *Populus nigra* resources by fluvial maintenance operations in the mosaic of islands of Mareau-aux-près (Loire, France)

Anthony Dubois[1], Benjamin Dimouro[2], Marc Villar[1]

[1]INRA, UR588 Amélioration, Génétique et Physiologie Forestières (AGPF), Centre de Recherche d'Orléans [2]UE0995 Génétique et Biomasse Forestières (GBFOr), Centre de Recherche d'Orléans, France

The European beaver is a 'keystone species' of Loire riverine ecosystem and present in the islands of Mareau-aux-prés (Loiret, France) since 1982. Our study is located in a mosaic of five islands (13 hectares) within the National Nature Reserve of St-Mesmin (Loiret, France), constituting the first Conservation Unit of the national programme of conservation of genetic resources of *Populus nigra*. The central island constituted the main source of food, with young shoots coming from stumps of black poplar (*Populus nigra*). In september 2012, the vegetation of such island was uprooted and the bar level lowered in order to maintain the flow capacity of the river.

Modification of such feeding habitat is at the origin of our study, with a particular view of the consequence on genetic resources of *Populus nigra,* especially on mature trees located in the other four islands. Scientific questions focuss on (i) what were the feeding habits of the beaver family (4 to 6 individuals) before maintenance operation? (ii) how have they changed their feeding habits after such fluvial work, and what will be the impact on other vegetated islands? (iii) what is the impact of foraging activity by beavers on the genetic diversity of black poplar?

To answer these issues, we have recorded data on *Salicaceae* vegetation (species, circumference, density of trees), presence of beaver (slides, lodges) and herbivory pressure (fresh or old traces on the bark, girdling percentage of these trees, cut trees). All of these data are georeferenced and were recorded before and after fluvial works (in september 2012). The results will be presented and discussed in terms of species selectivity, circumference of the tree, density of poplar and willow stumps acting as barrier to the consumption of adult stems, feeding tracks and forest composition and structure (in competition with underneath non-preferred *Acer negundo*). Funding support : project 'BioMareau', ERDF - EU Operational programme and Région Centre, within the 'Loire River Interregional Programme'

S5 – P3

Key words: poplar, willow, eucalyptus, irrigation, plantation

Determination of some fast growing species for plantation along the irrigation canals and field edges in Southeast of Anatolia

Hüseyin Karatay[1]

[1] Southeast Anatolia Forest Research Institute Directorate, Elazığ, Turkey

This study applied Şanlıurfa-Harran District in South East of of Turkey. The trial operation were taken in order to determine some of the fastest growing leafy species which can be grown in the buffer zone between the agricultural fields and widely used irrigation trunkings in the region. In the experiment established whit 1 years old seedlings which are 9 species including 3 origins of Poplar and Willow, 1 Eucalyptus origin (7046 numbered of *E. camaldulensis*), 1 origins of Euphrates poplar (*Populus euphratica* Oliv.: Şanlıurfa-Birecik origins) and White poplar (*Populus alba* L. - Tunceli origins) 5 years development process observed. On the experimental design, the saplings were planted by two rows within 5x5 m. distance. The plantation are carried out 4 replicated. In the experiment 5th. year of measurement and observation were evaluated. In the evaluation, diameter, height, body form, insect damage, and survival percentages measurements were used. As a result of the trial period, 7046 numbered origins of Eucalyptus (*E. camaldulensis*), Kocabey clones of Black poplar (P. nigra) and Birecik origins of Euphrates poplar (*P. euphratica* Oliv.) were successful in the region according to diameter, height, body form and the insect damage. In the same way, the origins and clone of this tree species showed considerable success in terms of survival rate also. Of these, Euphrates poplar that is used the first time in the trial work in the South East Anatolia Project region, performed more than other species in terms of survival performance with 100 % percentage in addition to have a good diameter and hight development. The clone and origins of these trees species can be used in the afforestation activites in the irrigated areas in in Southeast of Turkey especially taking into considreation the risk of frost that can be seen in the eraly years for Eucalyptus.

S5 – P4

Key words: biomass productivity, ecosystem services, *Pinus strobus* L., *Populus* spp.

Comparing aboveground, stand-level carbon storage potential of intensively-managed poplar with plantation-grown eastern white pine in the North Central United States

Ronald S. Zalesny Jr. [1], *William L. Headlee*[2]

[1]Institute for Applied Ecosystem Studies, U.S. Forest Service Northern Research Station, Rhinelander, Wisconsin, 54501 [2]Department of Natural Resource Ecology and Management, Iowa State University, Ames, Iowa, 50011, USA

Although both are grown in plantations and share similar silvicultural needs during establishment, it is well known that basic dendrological traits greatly differ among poplars (*Populus* spp. and their hybrids) and eastern white pine (*Pinus strobus* L.). From a biomass production standpoint, poplars exhibit much faster growth rates but have lifespans that are a fraction of their white pine counterparts. As a result, few comparisons highlighting potential ecosystem services across the groups have been made. To address this knowledge gap, our overall objectives are to integrate the results from two independent regional studies and to test for differences among poplar and white pine in their aboveground carbon storage potential. Comparisons are being made at 10 and 20 years after planting, representing a commonly-accepted poplar rotation age in the North Central United States (10 years) and an age when most poplar plantations lose substantial vigor and begin to die in the region (20 years). For poplar, stand-level carbon is being estimated from individual-tree biomass (measured), carbon concentration (measured), and stocking of 10 clones growing at 15 sites (4 × 10 y; 11 × 20 y) in Michigan, Wisconsin, Minnesota, and Iowa. For white pine, similar estimates are being developed from integrating growth ring measurements at 10 and 20 years (measured) with published biomass equations to obtain individual-tree biomass (calculated), carbon concentration (literature-derived), and stocking of 13 provenances growing at 4 sites in Michigan and Wisconsin. Both within and between genera, stand-level results will be highlighted at the conference according to specific genotypes, sites, and their interactions. In addition, the results will be described in the context of provisioning (i.e., biomass) and regulating (i.e., carbon) ecosystem services as defined in the 2005 Millennium Ecosystem Assessment. Funding support: Northeastern States Research Cooperative (Theme 3); U.S. Forest Service Research and Development Washington Office Woody Biomass, Bioenergy, and Bioproducts Program; U.S. Forest Service Northern Research Station Climate Change Science Council and the Institute for Applied Ecosystem Studies (RWU-NRS-13).

S5 – P5

Key words: poplar, land availability, biomass production, biofuels

Poplar as a feedstock for biofuels: land availability and biomass production potential in Italy

Giuseppe Scarascia Mugnozza[1]*, Antoine Harfouche*[1]*, Riccardo Salvati*[1]*, Maurizio Sabatti*[1]*, Francesco Fabbrini*[1] *and Piermaria Corona*[2]

[1]Department for Innovation in Biological, Agro-food and Forest Systems, University of Tuscia, Via S. Camillo de Lellis, Viterbo 01100 [2]Research Centre for Silviculture, Agriculture Research Council, Viale S. Margherita 80, 52100 Arezzo, Italy

A major constraint to the development of biofuels to reduce our reliance on fossil fuels is the availability of suitable land for growing crops for biofuel production without compromising land currently being used for food, feed, fiber, and ecosystem services. By focusing on the use of lignocellulosic feedstocks such as poplar, it should be possible to overcome this constraint. However, pending implementation of cost-effective technologies to convert lignocellulosic biomass to liquid fuels and a continuous supply of high-yielding, low-input biomass feedstocks will be needed. In this study GIS-based land suitability and land availability assessment are used for a number of dedicated energy crops. Hybrid poplars are among the fastest growing trees in the world, and thus a very promising feedstock for biofuels and high-value bioproducts. To estimate the country's supply of biomass from lignocellulosic feedstocks, we developed multiple scenarios where these plantations should not conflict with food production and not impact ecosystem services. This study also examines how much of Italy's liquid fuel consumption could rely on lignocellulosic biofuels. In addition, it explores several biotechnology scenarios that could further increase biomass yield, overcome biomass recalcitrance, and consequently decrease the amount of land required for biofuel production. Likewise, if marginal lands are considered for planting low-input biotech energy crops, less agricultural and forest land will be required. Moreover, this study uses GIS and maps to examine the spatial distribution of input resources in combination with proximity to infrastructure considered favorable to the production of lignocellulosic biofuels to determine which regions in Italy might be best suited for the possible location of biorefineries. The results provide a basis for future research and will likely offer a useful tool for investment framework for lignocellulosic biofuels and biorefineries development. Funding support: Our research is supported by grants from the European Community's Seventh Framework Program (WATBIO FP7 - 311929)

S5 – P6

Key words:
Salix, C sequestration, SOC, fine roots, ground vegetation

Carbon sequestration in willow plantations – the first rotation from theory to practice

Rose-Marie Rytter[1,2], *Lars Rytter*[2], *Lars Högbom*[2]

[1]Rytter Science, Backavägen 16, SE-268 68 Röstånga, Sweden,
[2]Forestry Research Institute of Sweden, Ekebo 2250, SE-268 90 Svalöv, Sweden, [3]Forestry Research Institute of Sweden, Uppsala Science Park, SE-751 83 Uppsala, Sweden

Atmospheric concentrations of CO_2 have increased by 40% since pre-industrial time and the effect on the global climate is significant. This increase is attributed primarily to fossil fuel emissions, and secondarily to land use changes and deforestation. Afforestation of abandoned arable land therefore has a positive impact on carbon (C) sequestration and the mitigation of high CO_2 concentrations. Today, fast-growing willows are of high interest as producers of biomass for fuel, but also as C sinks. The average rates of C sequestration in willow plantations on arable land in Sweden have been estimated to 3.5 and 0.4 Mg C ha^{-1} yr^{-1} in woody biomass and soil organic carbon (SOC), respectively, over the first 22 years. To increase the accuracy of such estimates, there is a need to understand how the different pools in the C cycle respond to climate and management. A tree species trial, including willows (*Salix* spp.), with emphasis on biomass production and soil effects was established on former agricultural sites along a north-south gradient in Sweden. The rate of C sequestration was calculated for willow plantations during the first rotation period on five different latitudes. Calculations of the annual accumulation of C in woody biomass and SOC were based on actual stem production and present knowledge on C allocation above and below ground in willows. Special emphasis was put on estimating fine-root production and decay, as well as the contribution of fine-root and leaf litter to the SOC-pool. The outcome of those calculations was compared with measured changes in the SOC-pool from pre-plantation conditions until the end of the first rotation period. The contribution to SOC from the ground vegetation was also included. Deviations and similarities in outcomes from the theoretical approach and the field samplings were discussed and further research areas to fill the knowledge gaps were suggested.

Funding support: The Swedish Energy Agency

S5 – P7

Key words:
decomposition, biomass productivity, mycorrhizal communities, *Salix*, competition

Integrating plant breeding and ecosystem science: Linking plant traits to ecosystem processes in a willow biomass production system

Stefanie Hoeber[1], *Martin Weih*[1], *Stefano Manzoni*[1], *Petra Fransson*[2]

[1]Department of Crop Production Ecology, Swedish University of Agricultural Sciences, SE- 750 07 Uppsala [2]Uppsala BioCenter, Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, SE- 750 07 Uppsala, Sweden

Salix is an energy crop with favorable economy, energy balance, and production potential, which is also promising regarding provision of important ecosystem services. Breeding programs are under way to improve crop performance of *Salix* through selection and modification of desirable traits from a production perspective. At present, we have a poor understanding on how adding individual genotypes of an energy crop like *Salix* to ecosystems create, maintain, and change biological diversity and ultimately affect ecosystem processes relevant for productivity, nutrient cycling and carbon sequestration. In the current project (www.slu.se/ecolink-salix), we examine how the diversity of four *Salix* genotypes in mono and mixed culture affect (1) ecosystem productivity; (2) nutrient use and nitrogen accumulation; (3) decomposition rate; and (4) abundance and structure of mycorrhizal communities. In a parallel pot experiment, we assess the water use and productivity of two genotypes of *Salix* with contrasting phenologies and functional traits. Species differ in their demand for nutrient and water use; to compete they must be able to either acquire a greater proportion of nutrients and/or water or use nutrients and water more efficiently for biomass production. These strategies are often complementary, as there are inherent tradeoffs that do not allow having both high resource use intensity and efficiency. Apart from a better understanding of the functional links between individual plant traits and ecosystem processes, the gained knowledge will help us to evaluate the consequences of introducing new plant genotypes with different characteristics into biomass production systems. This research has implications for future bio-energy concepts, as new genotypes of energy crops (e.g. *Salix*) generated by plant breeding must comply with the sustainability requirements (e.g. biodiversity, C sequestration) for a carbon-neutral and environmentally friendly energy source.

Funding support: Swedish Energy Agency (36654-1)

S5 – P8

Key words: backyard planting, phytogeographical regions, poverty, *Salix,* social forestry

Backyard planting of willow – A vital income-generating activity in north-east India

Surendra Kumar Sharma

Department of Environmental Sciences, Carman Residential and Day School, Dehradun 248007, India

Backyard planting is an age-old practice in India. Habitants of north-east India have been raising, willow *(salix)* and bamboos in their backyard. It is the most rewarding component of social forestry to reduce poverty in an otherwise backward area where the country' developmental benefits have not reached fully. The temperate zone of The Himalayas ranging in altitude from 1600 to 3500m representing one of the phytogeographical regions of India, considered best suited for backyard planting of different varieties of willow (large to small trees) grown by branch cutting, namely, *salix alba (bis)* – large tree used for making cricket bats, matchwood, tool-handles, fuel and fodder, *Salix babylonica (majnu*) - large tree used for fodder, fuel and ornaments and *Salix daphnoides (bhashi, bashroi)* – small tree suitable for basket making, fuel, fodder. The study examined how willow planting in north-east region of the country ties to wider national agendas, including those associated with poverty reduction. Best practices, constraints and opportunities for establishing effective linkages were identified through interviews with representatives from civil society organizations The study revealed that the backyard planting, as a part of social forestry in northeast, is a labor – intensive activity and large scale adoption of this program would help generate income through employment and through the sale of surplus goods and services thus, enhancing their contribution to the reduction of poverty.

The activity of raising willow and bamboos in their backyard and their marketing, has not only taken employment to the very door-steps of the unemployed but also contributed to the reduction of poverty in the poor areas of north-east India.

S5 – P9

Key words:
shoot elongation,
temperature,
adaptation,
vegetation period

Height increment development in three-year-old hybrid aspen (*Populus tremula* L. x *Populus tremuloides* Michx.)

Martins Zeps, Aris Jansons, Juris Rieksts Riekstins, Liga Purina, Kaspars Gailitis, Janis Smilga, Arnis Gailis

Latvian State Forest Research institute “Silava”, Latvia

Superior productivity of hybrid aspen clones has been attributed both to longer growth period and higher growth intensity. Formation of height increment is adapted to local environmental conditions. In order to understand the possible impact of predicted climate change on the growth of hybrid aspen and thus the selection of clones, the aim of our study was to assess the pattern of hybrid aspen’s shoot elongation and factors affecting it.

Weekly height measurements were taken for 19 hybrid aspen clones during their 3rd growing season in two sites (Mazirbe 57o40' N, 22o19' E and Vecumnieki 56o36'N, 24o30'E) and hourly data of meteorological conditions collected.

Tree height at the beginning of the vegetation period was a significant factor influencing the length of total height increment (R2=0.89-0.95), indicating accumulation of height differences over time.

Relationship between height growth intensity and climatic variables in both sites were statistically significant ($p<0.05$, R2=0.22 and R2=0.73 in Mazirbe and Vecumnieki, respectively) and positive, indicating a potential to increase productivity of plantations with the rise in temperature.

Significant differences ($p<0.05$) among clones were found both for the total length of the height increment (ranging from 59 to 249 cm in Mazirbe and from 100 to 224 cm in Vecumnieki) and for the average growth intensity (from 3.2 to 15.6 mm day-1 and 3.4 to 9.8 mm day-1, respectively). Ranking of the clones remained unchanged between sites, suggesting that selection of clones for a relatively short rotation period (20 years) is efficient also in the predicted rate of climatic changes.

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S5 – P10

Key words:
POPFULL,
specific leaf area,
genotypic variation,
direct LAI,
indirect LAI

Evolution of leaf area index during four years (two rotations) of a SRC poplar plantation (POPFULL)

Stefan Vanbeveren, L.S. Broeckx, M.S. Verlinden, I. Sarzi Falchi, T. Zenone & R. Ceulemans

University of Antwerp, Department of Biology, Research Group of Plant and Vegetation Ecology, B-2610 Wilrijk, Belgium

In April 2010 a large-scale Short Rotation Coppice (SRC) plantation with 12 poplar genotypes was established in Lochristi (Belgium) at a planting density of 8000 cuttings per ha. The plantation was neither fertilized nor irrigated. A first coppice was realized in February 2012, i.e. after two years, and a second coppice in February 2014. Leaf Area Index (LAI) was measured indirectly and non-destructively at frequent intervals with a Li-Cor-2200 Plant Canopy Analyzer since the establishment of the plantation and over two complete rotations. Indirect LAI measurements were validated annually at the end of the growing season by direct leaf area measurements using leaf litter collection and conversion from leaf dry mass into leaf area. The evolution of LAI during the growing season and over both rotations showed significant yearly and genotypic variations. The mean LAI of the plantation reached 1.11 and 2.38 in the first, respectively the second year of the first rotation; mean LAI further increased to 5.74 in the second rotation. The highest LAI of all genotypes (9.16) was reached by genotype Hees (*Populus deltoides* x *P. nigra*). In terms of length of the growing season genotypes Hees and Skado (*P. trichocarpa* x *P. maximowiczii*) had the longest growing season during the first rotation. Genotypes Hees, Skado and Bakan (*P. trichocarpa* x *P. maximowiczii*) had the longest growing season in the second rotation. LAI increased much faster at the onset of the growing season after the coppicing as compared to the establishment year. In terms of LAI genotypes Hees and Skado showed the highest LAI in the first rotation and genotypes Bakan, Hees and Skado showed the highest LAI in the second rotation. Seasonal evolution of LAI during both rotations could be linked to shoot biomass and was explained in relation to the seasonal evolution of air temperature, precipitation and incoming radiation.

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S5 – P11

Key words; Indo-Gangetic, clones, agro-forestry, carbon sequestration, livelihoods

Clones of *Populus deltoides;* their journey from Texas (USA) to Indo-Gangetic plains; and their uncanny role in forest conservation and rural livelihoods in India

Gulshan Ahuja Kumar

Indian Forest Service, Department of Forests, Haryana State, Panchkula, India

Populus deltoides Bartr. ex. Marsh. Var. deltoides, the typical eastern cottonwood from USA is a species of great choice for agro forestry in the Indo-Gangetic plains in India. The species has a robust market playing an important role in farm economy and rural livelihood generation besides occupying a center-stage in National Forest and Agricultural policies of the country. Coming to India mainly from Brazos County in Texas and Southern Mississippi river source in USA, it has best adopted the Indo-Gangetic plains 28 degrees N latitude. A large number of clones introduced in India since 1950 have been screened for better growth and yield under Indian conditions and suitable clones have been multiplied for mass propagation under agro- forestry. Realizing the potential of *P. deltoides* clones, an All India Coordinated Project on Poplar Improvement (AICPPI) started in late nineties has yielded good results. *P. deltoides* is a fast growing species producing soft wood for multifarious uses, viz. plywood, pulp, matchwood, veneer, light furniture and packing cases. Its growth characters like straight stem, thin crown, deciduous nature, short rotation and remunerative economic returns have made it a farmer's first choice for growing on farm land under various agro forestry models. Through its use in agro-forestry, it has played an important role in restoration of soil health and water resources in depleted agricultural fields developing into a sustainable land use system. The poplar trees grown under agro forestry system have also been instrumental in saving precious natural forests by providing alternate source of industrial wood besides playing an important role in carbon sequestration. This paper traces the journey of *P. deltoides*, an exotic, in development of suitable clones through breeding programs pursued in India, its role in strengthening rural economy through development of wood based cottage industries and also in forest and environmental conservation.

S5 – P12

Key words: biomass, *Populus alba*, irrigation deficit, SRF

Populus alba clones and their hybrids growing under water deficit conditions for biomass production in short rotation forestry

<u>Hortensia Sixto</u>, Mario Sanchez, Maria de la O Sanchez, Isabel Cañellas

Centro de Investigación Forestal (CIFOR) del INIA. Ctra. de la Coruña Km 7,5, 28040, Madrid, Spain

Water supply is important for poplar growth under Mediterranean conditions. Two constraints need to be considered, i) climatic change may necessitate more restrictive irrigation and ii) competition for land use with food production may force plantations into marginal areas. Therefore, it is of particular interest to explore the variability of the genus in order to attain greater resilience. *Populus alba* L., with a highly variable response to salt stress, may offer interesting possibilities in this context. Therefore, the aim of this work is to characterize and analyze the response of autochthonous *P.alba* clones and their hybrids under two irrigation scenarios: optimal and restrictive, growing in high density and short rotation.

A trial was established at high density (10.000 cuttings·ha^{-1}), which included two autochthonous *P. alba* clones and two *P. deltoides* x *P. alba*, using the Italian *P. alba* clone ‘111’ as reference. Four blocks were watered to field capacity and in another four, half the irrigation was applied. Parameters related to growth (total height, Ht; number of shoots, NS; diameter at 10 and 130 cm, D_{10} and D_{130}), tree architecture (% stem occupied by living branches, %SLB; stem straightness, SS; angle, BA; branch aptitude, BAP, syleptic branches, SYLB and total branch number, TBN), morphology (specific leaf area, SLA), phenology or functional variables (intrinsic water use efficiency, IWUE) were recorded during the second growing season. The duration of rotation period will be three years.Significant differences in growth (diameter and height) were detected in the second year between the irrigation rates applied as well as between the genotypes tested. NS were not significantly different under the two irrigation regimens. However, both SYLB and TBN were significantly affected by the water regime and differed between genotypes. Growth was significantly correlated with TBN and with %SLB. IWUE differed significantly under the two irrigation scenarios and this is linked to stomatal closure. No correlation was detected between growth and IWUE, which could facilitate bidirectional selection. SS, BA and BAP were similar under both irrigation scenarios. As regards the genotypes tested, the patterns of these variables were very similar, with only SS variable differing slightly. The genotypes tested grow well under water deficit conditions. Under such conditions, variability exists in the response of the genotypes, allowing the identification of the most suitable material for use in marginal conditions under semi-intensive management in the Mediterranean area.

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S5 – P13

Key words:
Salix,
survival,
flooding,
clones,
selection

Flooding tolerance in willows (*Salix* spp.) germplasm for the Paraná River Delta, Argentina: evaluation in the field and the greenhouse

Teresa Cerrillo[1]*, Guillermo Doffo*[2]*, María Emilia Rodríguez*[2]*, Flavia Olguín*[2]*, Fabio Achinelli*[2,3]*, Virginia Luquez*[2]

[1]EEA Delta del Paraná, Instituto Nacional de Tecnología Agropecuaria (INTA). Paraná de las Palmas y Canal Laurentino Comas, (2804) Campana, Buenos Aires [2]Instituto de Fisiología Vegetal (INFIVE), CONICET-UNLP, CC 327 (1900) La Plata [3]CIC, Buenos Aires, Argentina

Willows (*Salix* spp.) plantations cover an extensive area in the Paraná River Delta, especially in flood- prone sectors. According to climate change scenarios, the risk of flooding will increase in the next decades. Therefore, it is important to include flood tolerance as a selection criteria for breeding programs aimed for the Paraná Delta area.

In this work, we present results of two kinds: field trials that experienced a natural episode of flooding during the 2009-2010 growing season, and a controlled flooding experiment carried out on plants growing in pots in a greenhouse during 2013-2014.

In the field trials, 68 experimental clones were evaluated, these clones represent a wide range of the germplasm included in the willow breeding program, including *Salix alba* L*, S. babylonica* L., *S. bondplandiana* H.B.K., *S. matsudana* Koidtz, *S. nigra* Marsh, and *S.X argentinensis* Ragonese *et* Rial Alberti.

The clones were planted in 2 field trials, one was a two year old plantation flooded for 6 months, and a 3 months old plantation flooded during 4 months. The water level fluctuated between 1 and 1.5 m in both sites. Survival was evaluated at the end of the growing season. The most tolerant clones had a survival between 73% and 90% (average height=5.8 m), these group includes six experimental genotypes and 4 recently commercially liberated clones.

In the greenhouse experiment, the 3 best individual of 7 families were included, with different combinations of the species *S. matsudana*, *S. nigra*, *S. alba*, *S. amygdaloides y S. x argentinensis.* The plants were flooded for two months, and left to recover afterwards for one month. Several morphological and physiological variables were measured; the aim is to correlate them with flood tolerance and eventually use these traits as an early selection criteria.

By combining the information of both field and greenhouse trials, we can develop a more fast and accurate way to identify flood tolerance in new willow genotypes.

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S5 – P14

Key words:
Salix spp., oilsands, end-pit lakes, stabilization, shoreline

Assessing the potential of willows for shoreline stabilization of end-pit lakes in the Alberta oilsands- Tolerance of willow clones to process affected water

Richard Krygier

Natural Resources Canada, Canadian Forest Service, Northern Forestry Centre, 5320 122 St, Edmonton, Alberta, T6H 3S5, Canada

In the Alberta oil sands, end-pit lakes are part of the overall reclamation plan after open-pit mining activities have been completed. Their use as a method to treat fluid fine tailings containing water produced during processing of the oil sands (process affected water) and not captured by other treatment processes is also being investigated. Water quality in end-pit lakes may be an issue until biological processes eventually break down naphthenic acids and other compounds which are naturally occurring substances in bitumen.

As end-pit lakes are man-made structures constructed within the mined landscape from mined and reclaimed materials, their shorelines take some time to stabilize to the same point found with natural lakes. In the interim, these shorelines and riparian slopes are subject to erosion from wind and wave action, and to fluctuating water levels.

Given these lakes will be long-term features on the landscape, a natural, self-sustaining method of shoreline stabilization is being sought that can cope with the challenges mentioned above. Willows planted within the riparian area of these lakes may provide the stability required provided they can tolerate the process affected water.

Over 80 hybrid and native willow clones were tested for their tolerance to 50:50 and 25:75 process affected water:reservoir water mixtures, and a control using aeroponics chambers in the greenhouse. Several hybrid willow clones used for biomass production and native willow clones collected across Alberta were found to be tolerant. Tolerance varied not only between but within native species. If greenhouse results are confirmed by field trials to be established in 2014, the potential exists to collect, screen and utilize native willow clones for shoreline stabilization of end-pit lakes in the oil sands.

S5 – P15

Key words: wastewater, nursery, poplar, wilting

Response of poplar nurseries to irrigation by domestic wastewater

Dinesh Kumar

Silviculture Division, Forest Research Institute,Dehradun, Uttarakhand, Pin 248 006, India

Domestic wastewater is one of the principal sources of polluted water in rural areas of India. It presents danger to public health and environment. There are two types of domestic wastewater: blackwater (wastewater from toilets) and graywater (wastewater from all sources except toilets). In areas of water scarcity, a few nursery growers are tempted to use domestic wastewater for irrigating poplar nurseries.

In order to examine the feasibility of using graywater for irrigating poplar nurseries, a study was carried out in Punjab, India. Plots in a poplar nursery were irrigated from February (after planting of cuttings) to October (cessation of growth) at four-day intervals. Graywater and underground water irrigations were alternated as follows: (a) one irrigation with graywater after every three irrigations with underground water, (b) one irrigation with graywater after one irrigation with underground water, (c) three irrigations with graywater after one irrigation with underground water, (d) all irrigations with graywater, and (e) all irrigations with underground water i.e. control. Data were recorded in December of the same year.

There was no significant difference in treatments (a) and (e) although shoots of plants in treatment (a) developed chlorosis within first week of planting. This chlorosis could be kept under check by frequent application of nitrogenous fertiliser. About 20 to 32 per cent of the cuttings in treatments (b) and (c) wilted within three months of planting, while those surviving developed chlorosis and possessed smaller height and diameter at the end of the year. On the other hand, 67 per cent cuttings of treatment (d) died within three months of planting. The surviving plants tended to recover during monsoon rains (July to September) but remained too small to meet the outplanting standards.

S5 – P16

Key words: SRC, willow, *Salix* (spp), landfill leachate, bioremediation

The use of Short Rotation Coppice Willow for the bioremediation of landfill leachate

Sian M. Farrar[1,2], *Alistair R. McCracken*[1], *John P. Quinn*[2]

[1]Sustainable Agri-Food Science Division, Agri-Food & Biosciences Institute, 18A Newforge Lane, Belfast, BT9 5PX, N. Ireland, UK
[2]School Biological Sciences, Queens University Belfast, Medical Biology Center, 97 Lisburn Road, Belfast, BT9 7BL, N. Ireland, UK

Short Rotation Coppice (SRC) Willow is grown as a source of biomass for the production of renewable energy. Many newer commercial genotypes e.g. Olaf, Tora, Resolution or Endeavour are fast growing and high yielding and may have potential for the bioremediation of wastewater streams, including landfill leachate. In the Ireland / Northern Ireland border region there are several older, often closed landfill sites producing large volumes of very polluting leachate, currently being treated through Waste Water Treatment Works, which is both expensive and challenging. A selection of 120 genotypes was screened for their response to a single concentration (equivalent to 45 N ha^{-1} yr^{-1}) of leachate. Height, above ground biomass, below ground biomass, chlorophyll content and mortality were measured. Genotype response was varied with Olaf, Nimrod and 970111 producing significantly more biomass when treated with leachate. A sub-set of 8 genotypes was treated with a range of leachate concentrations (equivalent to 0, 75,150, 225 and 300kg N ha^{-1} yr^{-1}) and their response measured. Six of these genotypes showed high yields at 225 and 300kg N ha^{-1} yr^{-1} and were further treated with a higher concentration (600kg N ha^{-1} yr^{-1}). Plant tissue (leaves and stems) and the soil were analysed (using ICP Emission Spectroscopy) for key nutrients (N, P, K, Ca, Mg, S, and C). There were significant differences in nutrient levels in the tissues when plants were treated with higher leachate concentrations, indicating that certain genotypes may be more tolerant to leachate that others. Further work is being conducted on the effects leachate on the root health and development. A greenhouse trial is currently underway to study the factors influencing the amount and composition of the secondary leachate and over ground run-off, particularly following a rainfall event.

Funding support: This ANSWER (Agricultural Need for Sustainable Willow Effluent Recycling) project is part funded by the European Union's European Regional Development Fund (ERDF) through the INTERREG IVA Cross-border Programme, managed by the Special EU Programmes Body (SEUPB).

S5 – P17

Key words: SRC, willow, *Salix* (spp), wastewater, bioremediation

Effects on plants, soil and water quality of irrigating Short Rotation Willow Coppice with municipal effluent

Michele Garofolo[1,2], Alistair R. McCracken[1], John McGrath[2]

[1]Sustainable Agri-Food Science Division, Agri-Food & Biosciences Institute, 18A Newforge Lane, Belfast, BT9 5PX, N. Ireland, UK
[2]School Biological Sciences, Queens University Belfast, Medical Biology Centre, 97 Lisburn Road, Belfast, BT9 7BL, N. Ireland, UK

A set of 132 willow genotypes were initially screened to select the most promising genotypes which could be used for bioremediation of effluents. Plants were irrigated with primary municipal effluent, and the effect on willow growth was assessed by measuring shoot and root biomass along with leaf chlorophyll to evaluate the level of stress. *Salix spp.* RR06202, RR06141, RR05196, RR06070, 00279, 01211, 00187 and *Salix eriocephala* had the greatest increased above ground biomass ($p>0.01$) in response to effluent. Fourteen genotypes, representing a range of responses, were selected from the previous screening. They were irrigated using three different levels of effluent to give the equivalent of 0,150,300 kg N ha^{-1} yr^{-1}. Genotypes RR06002, RR06070 and *Salix eriocephala* produced significantly ($p>0.01$) more above ground biomass, in contrast to genotype 00129 which had more root biomass ($p>0.01$). Plant tissues (stems and leaves) were analysed (using ICP Emisson Spectroscopy) for key nutrients (N, P, K, Ca, Mg, S, and C) all of which were higher in leaves than in stems ($p>0.01$). Most of genotypes irrigated with 300 kg N ha^{-1} yr^{-1} showed toxic effects after three months. When the same 14 genotypes were treated with very high effluent levels (equivalent to 0, 300, 450, 600 kg N ha^{-1} yr^{-1}) there was generally an increase in height and biomass, although as the treatment progressed all genotypes died at the highest level. It is probable that this was due an excessive build up of nutrients. Genotype 00129 once again had the highest root production ($p>0.01$). In a field trial irrigated with primary sewage effluent soil samples were collected before, during and after irrigation at two weekly intervals over a six month periods in 2012 and 2013. Soil samples from 2013 are still being analysed, however, results from the previous year did not indicate any significant changes in soil composition between the irrigated and non-irrigated plots.

Funding support: This ANSWER (Agricultural Need for Sustainable Willow Effluent Recycling) project is part funded by the European Union's European Regional Development Fund (ERDF) through the INTERREG IVA Cross-border Programme, managed by the Special EU Programmes Body (SEUPB).

S5 – P18

Key words:
BVOCs emission, bioenergy crops, air pollution, short rotation forestry, greenhouse gas balance

Impact of poplar bioenergy cultivation on ozone and volatile organic compound emissions in Europe

Terenzio Zenone, Reinhart Ceulemans

University of Antwerp, Department of Biology, Research Group of Plant and Vegetation Ecology, B-2610 Wilrijk, Belgium

The need for renewable energy sources to meet EU Directive 2009/28/EC might lead to a considerable expansion of the planting of dedicated fast-growing biomass crops managed as Short Rotation Coppice (SRC) across Europe. Among them poplar (*Populus* spp) is currently the most widely planted SRC species and thus an increase in large-scale SRC poplar plantations might be expected. Poplars are characterized by a considerably high isoprene emission coefficient, and are susceptible to ozone pollution. In this project we will – for the first time – simultaneously measure the fluxes of Biogenic Volatile Organic Compounds (BVOCs), ozone (O_3), and NOx emissions in combination with the fluxes of greenhouse gases (CO_2, CH_4 and N_2O). All these flux measurements will be made in an SRC poplar plantation located in Lochristi (Belgium) using the eddy covariance technique. The primary objectives of the project are: (i) to quantify the NOx, BVOC and O_3 emissions at leaf and ecosystem levels, and (ii) to identify the environmental variables that drive these fluxes. The data obtained will be used to parameterize the CTM LOTOS-EUROS and the CTMs Chemical Transport models. Model simulations will reveal the potential impact of large-scale biomass plantations on isoprene emissions, and consequently on ozone air pollution at the European level. The study will also quantify the global warming potential of poplar SRC and the carbon offset that could be achieved with the bioenergy produced.

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SESSION VI

MANAGEMENT AND APPLICATION: PUTTING BIOLOGY INTO PRACTISE

Oral Presentations

S6 – O1

Key words: willows, short rotation willow coppice, annual yield, marginal lands

Two decades of research on short rotation willow crop in Quebec, Canada

Michel Labrecque[1], Werther Guidi Nissim[2], Nicolas Bélanger[3], Mario Fontana[3], Benoit Lafleur[3]

[1]IRBV/Montreal Botanical Garden, 4101 Sherbrooke East, Montreal, Québec
[2]Department of Plant, Soil and Environmental Science - University of Florence
[3]Centre d'étude de la forêt, Université du Québec à Montréal, 141 Président-Kennedy, Montréal, Québec, Canada

Over the last 20 years, extensive research has been conducted in Quebec on short rotation willow crop (SRWC). In some cases, experimental fields have even been successfully scaled up to large-scale commercial willow nurseries. The potential productivity of willow in diverse pedoclimatic regions has been evaluated through these studies. We have highlighted the importance of selecting the appropriate cultivar in order to maximise productivity and maintain yield over several rotations. Some productive cultivars have been found to perform less well after a few rotations; others, selected from among indigenous species, have shown promise for future development, mostly in the northern regions of the province. For many reasons, the use of marginal lands for SRWC has been a focus, and this is why several of our experimental studies aimed to develop strategies to enhance growth and yield in these specific conditions. For example, the use of biosolids or wastewater has produced excellent results and often led to very high annual yields, up to 20 odt per hectare. Conversely, inoculation with mycorrhizal fungi has been used in poor sandy conditions with mitigated results. Research has also been conducted to investigate changes in soil carbon stock and dynamics, upon establishment and throughout exploitation. After multiple rotations, it has been shown that the sequestration of carbon in the soil increased notably at depths of 20-40cm, confirming the potential of SRWC as a carbon sink. The experimental data cumulated over the last two decades confirm the huge potential of this crop's production as a green energy source with multiple environmental benefits. Expanding the technology will require development of an active market as well as political willingness to support such initiatives.

Funding support: Natural Resources Canada, Ministry of Municipal Affairs, Regions and Land Occupancy (Quebec), Fonds de recherche du Québec - Nature et technologies (FRQNT), Conseil pour le développement de l'agriculture du Québec (CDAC)

S6 – O2

Key words: POPFULL, energy balance, energy efficiency, greenhouse gas balance

System analysis of a short-rotation bio-energy plantation: full greenhouse gas balance, energy balance and environmental life cycle assessment (POPFULL)

Reinhart Ceulemans, N. Arriga, G. Berhongaray, L.S. Broeckx, M. Camino Serrano, T. De Groote, O. El Kasmioui, C.-M. Görres, S. Njakou Djomo, S. Vanbeveren, M.S. Verlinden, T. Zenone

University of Antwerp, Department of Biology, Research Group of Plant and Vegetation Ecology, B-2610 Wilrijk, Belgium.

Two issues need to be addressed before the efficacy of bio-energy for carbon mitigation can be conclusively assessed: (i) a quantification of the global warming contribution of short-rotation woody crops (SRC), incl. their mitigation potential; and (ii) an assessment of the energy efficiency of the system. We therefore monitored – within the ERC 'POPFULL' project – an operational, large-scale SRC plantation (18.4 ha) for the production of bio-energy from the establishment of the plantation to the production of green electricity and/or heat. The high-density plantation in East-Flanders (Belgium) is managed in two-year rotation cycles and uses poplar as the planted crop species. Eddy covariance techniques monitor net fluxes of all greenhouse gases (GHG) between the SRC plantation and the atmosphere. For the energy accounting, the global warming contribution and the energy efficiency assessment, we used life cycle assessment (LCA) over the two rotations from the cultivation of the plantation to the conversion of the harvested chips into electricity and/or heat. The collected information on the GHG and the energy inputs of the two-year rotations was used toward predictions and simulations of the net reduction of fossil GHG emissions of SRC. During two rotations the SRC plantation supplied woodchips – ca. 10 tons of dry mass ha^{-1} yr^{-1} – for decentralized bio-energy operators in Belgium. The CO_2 balance of the plantation – until the farm gate – was positive, i.e. we quantified a larger carbon uptake than was released from the plantation. But the overall GHG balance was slightly negative, i.e. taking other GHG beside CO_2 into account the plantation released GHG. This was explained by N_2O peaks from the (previously agricultural) soil during wet periods. The ratio of energy output to energy input was 6, indicating a net return of energy. Compared to fossil fuels a low-input SRC plantation is energy efficient and has considerably lower GHG emissions.

Funding support: ERC Advanced Grant agreement (# 233366) POPFULL under the EC 7th Framework Programme (FP7/2007-2013), Flemish Hercules Foundation as Infrastructure contract # ZW09-06, and the Methusalem Program of the Flemish Government.

S6 – O3

Key words:
SRWC,
bioenergy,
trees,
feedstocks,
plantations

Supplying potential bioenergy markets with SRWCs in North Carolina, USA

Dennis William Hazel[1], *Elizabeth Guthrie Nichols*[1], *Shawn Dayson Shifflett*[1], *and Jeff Wright*[2]

[1]Department of Forest and Environmental Resources, North Carolina State University, Raleigh, NC USA [2]Arborgen, LLC, Summerville, SC, USA

Woody biomass will be a major feedstock for energy production, including biofuels, to secure the future of domestic energy production in the United States. Fast-growing, purpose-grown tree plantations, or short rotation woody crops, are one of the most promising methods to meet future woody biomass demands. Whereas considerable research has been done in the U.S. on SRWCs, relatively little has been done in North Carolina. Over the last two years, we have begun a research and extension program to develop an understanding of potential SRWC production in North Carolina in order to meet immediate woody biomass market demands for wood pellets and potential biofuel demand for an ethanol producer. Our programmatic objectives are to develop recommendations of species or clones for specific site conditions; to develop planting, management; and harvesting guidelines for site conditions; to estimate growth rates, harvest yields, and feedstock characteristics; to estimate production and harvesting costs on various lands; to understand potential pest and disease issues; and to evaluate wood chemistry of species and clones specific to energy wood use. We will report on performance results from randomized-block establishment trials at coastal to mountainous sites for select clones of *Populus,* native hardwoods, and eucalyptus. Site trials vary by land type from cropland, managed forests, degraded lands, and marginal lands.

Funding Support*:* The former Biofuels Center of North Carolina, North Carolina Department of Agriculture, ArborGen, and GreenWood Resources

S6 – O4

Key words: vegetative regeneration, biomass production, growth dynamics, thinning strategies

Productivity and thinning effects of hybrid aspen (*Populus tremula* × *P. tremuloides*) root sucker stands

Rebecka Mc Carthy[1,2] *and Lars Rytter*[1]

[1]The Forestry Research Institute of Sweden (Skogforsk), Ekebo 2250, 268 90 Svalöv [2]Swedish University of Agricultural Sciences (SLU), Southern Swedish Forest Research Centre, Box 49, 230 53 Alnarp, Sweden

The interest for woody biomass is increasing due to, among other things, the Nordic vision to become independent of fossil fuel by 2050. One way to approach this vision is to use fast-growing tree species.

This study investigated how hybrid aspen performs in the second generation of root suckers. Four sites that were clear-felled in different years were studied, and diameter, height and dry weights were followed and measured on root suckers between 1 and 12 years old.

After two growing seasons, the four sites had a mean stand density of 64,600 living stems ha^{-1}. The range among the sites was 46,800 to 76,500 stems ha^{-1}. At this age the mean standing biomass, including living and dead stems, was 14.5 ton dry matter (DM) ha^{-1} (ranged between 8.8 and 22.5 ton DM ha^{-1}). The mean annual increment (MAI) was after two years 7.3 ton DM ha^{-1}.

Additionally, thinning effects were studied for three different treatments: 1) no thinning, 2) corridor thinning, and 3) cross corridor thinning. The harvests from the two thinning regimes were 2/3 and 8/9 of the area, respectively, and performed after the 2-year measurements. On the site that had been growing for 12 years, the mean number of stems in unthinned parcels was 12,000 stems ha^{-1}, holding a biomass of 102.5 ton DM ha^{-1}. Corridor thinned parcels held 12,100 stems ha^{-1} and 121.0 ton DM ha^{-1}, while cross corridor thinned parcels contained 8,700 stems ha^{-1} and 100.7 ton DM ha^{-1}. The MAI after 12 seasons were: 8.5 ton DM ha^{-1} in unthinned treatment, 10.9 ton DM ha^{-1} in corridor thinned treatment, and 9.7 ton DM ha^{-1} in cross corridor thinned treatment. No significant differences of thinning effects on standing biomass were found. Nor did the accumulated biomass, including the harvest at age 2, differ significantly between the treatments.

This study shows that the second generation of hybrid aspen can contribute significantly to the biomass supply, and that different treatment strategies in short rotation forestry can be applied.

Funding support: Swedish Energy Agency, Nordic Energy Research

S6 – O5

Key words: hybrid poplar, competition, vegetation management, nutrient availability

Above- and belowground effects of four establishment systems on hybrid poplar tree performance, understory vegetation and resource availability

Jeannine Göhing[1]*, Ellen S. Macdonald*[1]*, Edward W. Bork*[2]*, Barb R. Thomas*[1,3]

[1]Department of Renewable Resources, University of Alberta, Edmonton, AB, T6G 2H1 [2]Department of Agricultural, Food & Nutritional Science, University of Alberta, Edmonton, Alberta, T6G 2P5 Canada [3]Alberta-Pacific Forest Industries Inc., PO Box 8000, Boyle, Alberta, T0A 0M0, Canada

Hybrid poplar plantations are an important source of fiber products with potential environmental benefits including carbon sequestration. Conventional establishment protocols strive for maximum weed control leading to intensive and costly management practices. The objective of this study was to test alternative strategies that could optimize weed control, increase tree growth and survival, while offering environmental benefits compared to current (business-as-usual) establishment systems. We assessed the effect of four establishment systems on early aboveground growth of two hybrid poplar (*Populus* spp.) clones (‘*Walker*’ and ‘*Okanese*’), understory vegetation and above- and belowground resource availability. The experiment was established in a strip-plot design replicated in fifteen blocks on formerly mixedwood boreal forest sites converted to agronomic perennial forages, located in central Alberta, Canada. Establishment systems included: (1) a full year of mechanical and chemical fallow prior to planting; (2) sowing of a cover crop mixture into conventionally fallowed fields between tree rows; (3) planting of poplars into untilled fields following localized vegetation suppression using herbicide; and (4) business-as-usual as the control treatment using conventional repetitive cultivation and herbicide spraying. Tree growth, survival and damage, herbaceous vegetation cover and composition, soil nutrient availability, soil water content, soil temperature and light availability were measured over two years (2012-2013). Results showed that treatment (1) increased tree performance significantly, with effects on understory including reduced cover in the first season and a high percentage of annuals. Treatment (4) resulted in moderate tree performance and understory cover, while both treatments (2) and (3) resulted in low tree performance with moderate to high vegetation cover and a high percentage of perennials, suggesting strong competition for resources.

Funding support: Natural Sciences and Engineering Research Council Collaborative Research and Development Grant with Alberta-Pacific Forest Industries Inc. (Al-Pac)

S6 – O6

Key words:
nitrogen,
phosphorous,
water use
efficiency,
biomass

Increasing growth and drought tolerance using symbiotic microorganisms

Sharon Lafferty Doty, Andrew W. Sher, Neil D. Fleck, Zareen Khan, Jenny Knoth, Amy Baum, and Thomas H. DeLuca

University of Washington, Seattle, WA 98195-2100

The use of symbiotic microorganisms rather than chemical fertilizers could offer a more environmentally sustainable means of increasing poplar and willow biomass production. Poplar and willow do not have root nodules and are not considered as N_2 fixing trees although they are often found in N-limited environments across the Northern hemisphere. Their ability to grow in low-nutrient substrates is usually attributed to nitrogen use efficiency (NUE); however, when there is little to no organic material available, NUE cannot account for the rapid growth rates and high biomass attained by poplar. We previously reported the presence of endophytic *Rhizobium tropici* from hybrid poplar grown in greenhouses. A variety of diazotrophic (N_2 fixing) endophytes were subsequently isolated from wild poplar at a natural riparian area dominated by cobble. These endophytes were shown to be mutualistic symbionts by adding them to other plant species, including grasses, corn, and a variety of crop plants for improved growth and health in nutrient-limited conditions. When the endophytes from wild poplar were added to hybrid poplars under greenhouse conditions, there was a significant increase in nitrogen fixation. We will report the first definitive evidence of N_2 fixation within poplar plants from a natural stand of riparian poplar. Not only do the endophytes from wild poplar fix N_2, they also produce plant hormones, solubilize phosphate, and increase drought tolerance. By using symbiosis with natural microbial partners of wild poplar on cultivated poplar, substantial increases in biomass can be attained with reduced inputs.

S6 – O7

Key words:
Chrysomela populi, ASPIK, short rotation coppice, IBM, pest monitoring

Forecasting the abundance of the red poplar leaf beetle at different stages in its life cycle in poplar short rotation coppice using individual-based modelling

Martin Schubert, Uta Berger

Department of Forest Sciences, Technische Universität Dresden, Tharandt, Germany

Poplar short rotation coppice (SRC) plantations are established with a low number of varieties and using genetically identical plants. This low diversity and the growing cultivation of SRC benefit the spread, adaption, and gradation of biotic pests. Recent research identified the red poplar leaf beetle (*Chrysomela populi* L.) as being one of the most damaging pest insects in poplar SRC in Germany, capable of suppressing the regrowth of harvested plants and decreasing yields over the subsequent rotation. As yet there is no standard approach for the estimation of pest abundance or the planning of plant protection measures. We developed a simulation tool to forecast the relative beetle abundance using daily temperature sums. The tool core is based on the individual-based model 'ASPIK'. It simulates the complete bivoltine life cycle of the beetles during one growing season. Reproduction, development and mortality of the different beetle stages (egg, larvae, pupa and adult) are controlled by daily temperature sums. The quantity for each stage is observed on a daily interval. The model was parameterized using data from the literature and from laboratory studies, and was validated with field data collected from SRC plantations in Saxony and Brandenburg from 2011 to 2013. From April 2014, the simulation tool will be available to end users – farmers and agricultural consultants – as front-end through ISIP, a German association for decision-making in plant production (http://www.isip.de). Utilizing current data sourced from meteorological stations, the tool calculates (1) a map marking the current development stages of the beetle and (2) a detailed forecast of the abundance ratio of adult, egg and larvae stages. This enables the end user to coordinate monitoring and plant protection measures. ASPIK was built with a free and easy to use software (NetLogo). Consequently, it is a generic tool that can easily be applied in other countries and for species with similar life cycles. Funding support: German Federal Ministry of Education and Research (BMBF)

S6 – O8

Key words:
cuttings, *Salix*,
willow, weed
competition

Establishment and early growth of *Salix* at different weed competition levels

Stina Edelfeldt, Theo Verwijst and Anneli Lundkvist

Department of Crop Production Ecology, Swedish University of Agricultural Sciences (SLU), P.O. Box 7043, SE-750 07 Uppsala, Sweden

A successful and even establishment of plant stands is important for high biomass production when Salix is grown for bioenergy purposes. However, establishment may be disrupted by weeds competing for resources such as light, water and nutrients. As the stand grows and competition increases, this may lead to gaps in the stand and result in production losses at harvest.

The effects of weed competition on growth of Salix were assessed in an outdoor bucket experiment conducted in central Sweden in 2011. Four Salix-weed mixtures, two model weed species (white mustard and spring barley), two fertilizer levels (30 and 90 kg N ha^{-1}), and 12 replicates were used in the experiment. Salix cuttings from the same original rod were planted in each bucket. Mixtures were created by planting weeds at three different occasions (15, 26, and 30 days after initial Salix planting) plus a pure Salix stand. The experiment was harvested after nine weeks. Total Salix biomass and height were measured.

The results showed that mixture, fertilizer level and cutting diameter had significant effects on biomass production. Compared to pure Salix stands, less biomass was produced only when weeds were planted 15 days after the Salix cuttings. Larger cuttings and higher fertilizer level produced significantly more biomass compared with small cuttings.

Analyses of shoot height showed that only mixture was significant. The effects were similar to the results from biomass production analyses.

For the given high experimental planting density (78 cuttings m^{-2}), weed control the first two-three weeks is important for Salix establishment and growth. Weed control after this critical period is of less importance as the Salix plants by then have grown large enough to withstand weed competition.

S6 – O9

Key words:
plantation termination, salix, weed development, yield of subsequent crops

Effects of termination method in willow cultivation on the need of weed control and yield in subsequent crops

Nils-Erik Nordh, Anneli Lundkvist, Monika Welc, Theo Verwijst

Department of Crop Production Ecology, Swedish University of Agricultural Sciences

In Scandinavia, a willow plantation is considered to have a productive durability of 20-30 years. The willow growth then declines considerably and the plantation needs to be terminated. To succeed in the establishment of a subsequent crop (willow or other agricultural crops), it's important to use effective methods for fracturing willow stubs and concurrent soil cultivation for a subsequent crop. Additional measures for weed control after a willow crop also may need to be done.

In a field experiment, a 26-year-old willow cultivation in central Sweden was terminated by spraying the plantation with herbicides and then using two different methods (shallow or deep soil cultivation) for fracturing the willow stubs and soil cultivation. Assessments were made on (1) labor time and fuel consumption for each method, (2) the amount of coarse willow roots and stubs left in the field and how they affected soil cultivation in the subsequent crops (willow and cereals), and (3) the need for weed control.

Shallow cultivation was significantly faster and cheaper compared with deeper cultivation. No differences in (i) establishment and growth on subsequent crops, (ii) the amount of remaining root parts three years after termination of the willow cultivation, and (iii) amount of weeds were detected between the methods.

We conclude that shallow cultivation is the most appropriate method for termination of old willow plantations. A winter cereal can be sown in the same season, directly after termination. A fast decay of stubs and roots does not hinder consecutive annual cropping. A new willow plantation may be established the year after termination.

Funding support: The Swedish Farmers' Foundation for Agricultural Research

S6 – O10

Key words:
cell wall chemistry, biofuel feedstock, wood ultrastructure

Wood phenotyping of balsam poplar for biofuel feedstock development

Faride Unda[1], *Alex Skyba*[1], *Francis De Araujo*[1], *Foster Hart*[1], *Raju Soolanayakanahally*[2], *Carl J. Douglas*[3], *Robert D. Guy*[4], *and Shawn D. Mansfield*[1]

[1]Wood Science Department, University of British Columbia, Vancouver, British Columbia [2]Science and Technology Branch, Agriculture and Agri-Food Canada, Indian Head, Saskatchewan, Canada [3]Department of Botany, University of British Columbia [4]Department of Forest and Conservation Sciences, University of British Columbia, Vancouver, British Columbia, Canada

In Canada, *Populus balsamifera* is an ecologically important species, but is also seen as an ideal candidate for breeding programs to produce woody biomass feedstock for bioenergy applications on the Canadian landscape. Balsam poplar is a dominant north temperate to boreal hardwood species, with a vast range covering most of forested Canada, Alaska and the northern USA. As part of a large-scale project, we evaluated the impact of growing location on 15 traits associated with the chemical composition and ultrastructure of wood from 4-year-old balsam poplar trees clonally replicated in two common gardens in Saskatchewan. In this study we analysed 35 genotypes, represented by ~400 trees planted at Indian Head (IH) and Prince Albert (PA). Phenotypic correlation analysis shows that the main cell wall components (cellulose and lignin) are negatively correlated on both sites. Generally, the trees planted in PA had slightly higher cellulose and hemicellulose content than those planted on IH. Additionally, as expected, the insoluble lignin content was lower in the PA trees. Furthermore, the monolignol composition was comparable across both locations. We found positive correlations for density, fiber length/width and monolignol composition between sites. The complete analysis and results will be discussed. The ultimate goal of the project is to associate the changes in phenotype with genomic variation and identify novel targets for genetic improvement of the lignocellulosic biofuel feedstock.

Funding support: Genome Canada

S6 – O11

Key words:
willow,
recalcitrance,
transcriptomics,
reaction wood,
lignocellulose

Reaction wood, a readymade toolkit for major alterations to angiosperm cell walls - Escaping the gravity well

Nicholas JB Brereton[1], *Michael J Ray*[2], *Steven J Hanley*[3], *Richard J Murphy*[4], *Angela Karp*[3]

[1]Department of Life Sciences, Imperial College, London, SW7 2AZ, UK
[2]Department of Chemistry, Imperial College, London, SW7 2AZ, UK
[3]Department of AgroEcology, Rothamsted Research, Harpenden, AL5 2JQ, UK
[4]Faculty of Engineering & Physical Sciences, Centre for Environmental Strategy, University of Surrey, Guildford, Surrey GU2 7XH, UK

Biomass cell wall recalcitrance to deconstruction varies greatly in angiosperms, yet the source of this variation remains unclear. Here, in eight genotypes of the lignocellulosic bioenergy crop willow (*Salix* sp.), variability of reaction wood (RW) response and its impact on cell wall recalcitrance was explored. RW formation is an innate physiological response by trees to counteract environmental stimuli, such as gravity, resulting in altered tissue and cell wall development.

A pot trial was designed to test if RW response varies between genotypes and contributes to recalcitrance. Biomass composition was measured via mass closed wet chemistry and used with enzymatic saccharification to assess recalcitrance. Glucose release from pot-grown control trees did not significantly correlate with field-grown trees. However, when RW was induced in pot-grown trees by *tipping* at 45°, glucose release strongly correlated with mature field-grown trees (Corr. Coeff. 0.96, $p < 0.001$). Further field studies revealed up to 5-fold increase in glucose release from a site with potentially natural high RW inducing conditions (Orkney Islands, exposed to high wind speeds).

This demonstrates that RW response both varies significantly between genotypes and can explain almost all variation in cell wall recalcitrance of willow. Current work has revealed further new findings by exploring variation in RW phenotype using novel 3D X-Ray Computational Tomography as well as carbohydrate structural analysis of specific cell wall layers (the g-layer) isolated using sonication. In conjunction with this, RNAseq has been used and genetic mapping strategies are planned to uncover some of the underlying genetics behind the variation. Results will be used to drive selective breeding strategies and provide novel targets for genetic modification for improved biofuel feedstocks.

Funding support: BBSRC Sustainable Bioenergy Centre (BSBEC), working within the BSBEC BioMASS (http://www.bsbec-biomass.org.uk/) Programme of the centre (Grant BB/G016216/1).

SESSION VI

MANAGEMENT AND APPLICATION: PUTTING BIOLOGY INTO PRACTISE

Poster Presentations

S6 – P1

Key words: bioremediation, SRC willow, regulations

ANSWER (Agricultural Need for Sustainable Willow Effluent Recycling): an EU funded project to encourage the use of SRC willow for bioremediation

Alistair R. McCracken[1], *Chris Johnston*[2], *Linda Walsh*[3]

[1]Sustainable Agri-Food Sciences Division, Agri-Food & Biosciences Institute, 18a Newforge Lane, Belfast BT9 5PX [2]Sustainable Agri-Food Sciences Division, Agri-Food & Biosciences Institute, Large Park,Hillsborough,Co. Down,BT26 6DR [3]Sustainable Agri-Food Sciences Division, Agri-Food & Biosciences Institute, Loughgall, Armagh, BT61 8JB, Northern Ireland, UK

The ANSWER (Agricultural Need for Sustainable Willow Effluent Recycling) project has seven partners including water utilities, local government councils, higher education colleges and science organisations from both sides of the Ireland / N. Ireland border. The total value of the project is around £2m ($3.3m). Ireland has numerous small rural inefficient Waste Water Treatment Works (WWTWs) which are too expensive to upgrade but are non-EU compliant and are discharging potentially damaging pollutants into the local waterways. This project has enabled 'proof of concept' schemes to be established using SRC willow to treat primary sewage effluent. A major output from the project is the growing of ~45 ha of SRC willow, which will be irrigated with municipal effluent in spring 2014. The process to get to this point has been complex. Initially WWTWs which would benefit from this technology were to be identified; there were then often protracted negotiations with potential land owners willing to grow willow and to enter long term contracts to allow irrigation; at all stage the relevant Environmental Protection Agencies were involved to insure compliance with all discharge regulations – the scientific data was really important to give the EPAs confidence to proceed with a technology with which they had little or no experience; in two cases willow had to be established, in the third case an already mature plantation was used; the irrigation system had then to be designed, built and commissioned. The plantations were divided into zones which are irrigated in sequence at a rate of approximately 1mm day^{-1}, ($10m^3$ ha^{-1}, 3,250 m^3 ha^{-1} yr^{-1}) although there are soil moisture feed-back probes which prevent irrigation during times of wet weather. A further aspect of the project has been to investigate using SRC willow to manage landfill leachate. The cap on closed landfill site will be planted with willow and when established will be irrigated with leachate. EPA regulation around this scheme has been very challenging.

Funding support: ANSWER is part-financed by the European Union's European Regional Development Fund through the INTERREG IVA Cross-border Programme managed by the Special EU Programmes Body.

S6 – P2
Key words: *Populus nigra*, genetic diversity, spatial structure, management

Small-scale spatial genetic structure of *Populus nigra* 2013 seedlings on a new sandy-gravelly bar appeared after fluvial maintenance operations in the Loire River (France)

Sara Marin[1]*, Véronique Jorge*[1]*, Vanina Guérin*[1]*, Coraline Wintenberger*[2]*, Marc Villar*[1]

[1]INRA, UR588 Amélioration, Génétique et Physiologie Forestières (AGPF), Centre de Recherche d'Orléans, CS 40001 Ardon, F-45075 Orléans [2]University François Rabelais of Tours, E.A 6293, GéoHydrosytèmes Continentaux, UFR Sciences et techniques, Parc de Grandmont, F-37200 Tours, France

Black poplar is a key species of the Loire riverine ecosystem, and in addition to its important genetic intra and interpopulation diversity, represents one biological marker of the sediment morphodynamics and fluvial metamorphosis of the river. This species is threatened by human impacts, notably because of fluvial management works. The main objective of this work is to study small-scale spatial genetic structure of *Populus nigra* 2013 seedlings on a new sandy-gravelly bar appeared after fluvial maintenance operations in the Loire River (France). Our study focuses in a mosaic of islands (13 hectares) constituting the Conservation Unit Mareau/St-Mesmin of the national programme of conservation of genetic resources of *P. nigra*. In september 2012, the vegetation (black poplar) of the central island was uprooted and the bar level lowered in order to maintain the flow capacity of the river. During 2013 spring and summer, the fresh sediments of the new island has been regularly surveyed to identify and geocode successive regeneration zones. In September 2013, 31 seedlings of 50 different zones were collected for genetic analysis using 11 microsatellites DNA markers (total 1550 seedlings). Genetic diversity was expressed in terms of : percentage of polymorphic loci, observed and effective number of alleles, observed and expected heterozygosity and genetic differentiation (Fst). Although significantly different from zero, Fst within the 50 regeneration zones is very low and level of genetic diversity is comparable to putative parental populations (mature trees on the river banks). The low level of structure was further investigated using Structure software. The results will be presented and discussed in terms of microtopography, water level fluctuation, phenology of seed dehiscence and sediment texture. Such research work will lead to recommendations for future management of the Loire river, in terms of genetic diversity of *Populus nigra* populations.

S6 – P3

Application of phyto-recurrent selection: poplars grown in biochar-amended soils

Key words:
biomass, clonal selection, *Populus*, plant uptake, phytotechnologies

Ronald S. Zalesny Jr.[1], *William L. Headlee*[2], *Deborah S. Page-Dumroese*[3]

[1]Institute for Applied Ecosystem Studies, U.S. Forest Service Northern Research Station, Rhinelander, Wisconsin, 54501 [2]Department of Natural Resource Ecology and Management, Iowa State University, Ames, Iowa, 50011 [3]Forest and Woodland Ecosystems, U.S. Forest Service Rocky Mountain Research Station, Moscow, Idaho, 83843, USA

Phyto-recurrent selection (PRS) is a method used in phytotechnologies to select workhorse species and genotypes that improve environmental conditions while moving from ecosystem degradation to remediation and restoration. Similarly, PRS can be used to aid the selection of genotypes grown in soils amended with bioenergy byproducts, such as biochar. For example, Headlee et al. (2013) reported that biochar provided similar benefits to hybrid poplar clone ‘NM6’ in terms of nutrient availability and growth when substituted for vermiculite in greenhouse production. The overarching objectives of the current study were to use PRS to identify poplar genotypes that exhibited greater survival and growth when grown in biochar-amended soils versus those containing vermiculite or no soil amendments, and to test whether effects from greenhouse production remained when trees were outplanted to the nursery. Using three PRS cycles, we reduced a base population of 70 genotypes (cycle 1) to 30 (cycle 2) in the greenhouse and 10 clones (cycle 3) in the field. Our specific objectives were to test for differences among: 1) soil treatments (100% peat, 75% peat + 25% vermiculite, 75% peat + 25% biochar) for chemical properties (pH, CEC, ECEC) and nutrient content (total N, P, and exchangeable K, Ca, Mg, and Na), 2) poplar genotypes for survival, growth and biomass, and uptake of nutrients (N, P, K, Ca, Mg, Na) into roots, stems, leaves, and cuttings, and 3) their interactions. Overall, PRS was a viable genotype identification method, and field survival and nutrient effects were consistent with short-term greenhouse results (i.e., greenhouse testing can be used to predict outplanting success). Also, vector analysis was conducted to diagnose the biological significance of tree responses to the soil treatments, and improved availability/uptake of some nutrients with biochar and vermiculite treatments was demonstrated.

S6 – P4

Key words: willow, soil erosion, RUSLE2

Characterizing shrub willow for soil erosion modeling using RUSLE2 across a chronosequence of age classes

Danielle Proulx Kloster, Timothy Volk

State University of New York, College of Environmental Science and Forestry, Syracuse, New York, USA

Perennial bioenergy crops have the potential to provide environmental benefits, as well as energy. One of these benefits is mitigation of soil erosion potential, especially on the marginal land that is being targeted for production. Willow biomass crops are perennial crops that are less intensively managed than annual crops, minimizing soil disturbance. Shrub willows close canopy in the second year of production and produce a diffuse network of roots, reducing the erosivity of rainfall and the erodibility of the soil. The Revised Universal Soil Loss Equation 2 (RUSLE2) is used widely by the USDA and other organizations to assess erosion potential. However, the current vegetation file for shrub willow is based on a study from 2000 and accounts for only four age classes of one clone of willow, with no accounting for the first year of establishment. The objective of this study is to develop a more thorough vegetation file, including the first year of establishment, and to model the potential soil erosion from sites under consideration for willow production in the northeastern United States. Data, including leaf area index (LAI), canopy fall height, ground cover, and root biomass, were collected over the 2013 growing season at five sites in New York and Vermont, representing six age classes of willow. Three clones were studied: SX64, 9882-34 (Fish Creek), and SV1. We hypothesize that soil erosion will be most substantial during the first year of growth, before the willow has closed canopy and established an extensive root system. Based on initial modeling, the average annual erosion over the course of the willow growth cycle is likely to be very low, especially compared to annual crops, making it an option for soil erosion mitigation on marginal agricultural lands. We will present the changes in the parameters studied over the lifetime of a willow plantation, as well as the results from soil erosion modeling using RUSLE2.

S6 – P5

Key words:
Salix, collection, phenology, morphology, Iran.

Expansion and completion of a willow collection in Iran

Farhad Asadi[1], *Mohsen Calagari*[2], *Seyed Ehsan Sadati*[1], *Ali Bozorgmehr*[3], *Syamak Hanife*[4], *Ali Samadzadeh*[5]

[1]Agriculture and Natural Resources Research Center of Mazandaran Province [2]Research Institute of Forests and rangelands, Tehran [3]Agriculture and Natural Resources Research Center of North Khorasan Province[4]Agriculture and Natural Resources Research Center of west Azarbayjan Province [5]Agriculture and Natural Resources Research Center of Ardebil Province, Iran

A collection establishment project of willow clones has been started in the Research Institute of Forests and Rangelands (RIFR) in Iran since 2001. The main purpose of this project was to provide a data bank of *Salix* species and clones to investigate the morphological, phenological and growth characteristics and controlled crossing among of species of willow. The present research is in continuation of the earlier collection project. In 2010, several species and clones were planted in six stations in six different provinces of Iran. After identification of natural willow trees and labeling of elite mother trees, their cuttings were planted in nurseries at each research station. These practices have been repeated for three years (2011-2013). Then a clonal archive and an arboretum were established. In this collection from each species and clones, nine two -years- old seedlings were planted in 3 by 3 meter spacing. Fifteen *Salix* species and their clones including *Salix acmophylla, S. alba, S. babylonica, S. carmanica, S. caprea* (*S. aegyptiaca*), *S. elborsensis* (*S. purpurea*), *S. excelsa, S. fragilis, S. pycnostachya, S. purpurea, S. triandra, S. wilhelmsiana* (*S. angustifolia*), *S. zygostemon, and S. matsudana* were identified and collected in six stations from north to west of Iran. These accessions and species were phenotyped based on the phenological and morphological characteristics in each station. During each growing season, irrigation and weed control were done. The results showed that active growth period in the trees starts between late February (in Karaj station) to late March (in Ardebil station) and end between early December until late December in the same places, respectively.

S6 - P6

Key words: shrub willow, genotype by environment, biomass composition

Variation in first-year biomass growth and wood composition among 23 shrub willow genotypes across a range of environments

Eric S. Fabio[1], *Fred E. Gouker*[1], *Armen R. Kemanian*[2], *Felipe R. Montes*[2], *John E. Carlson*[3], *Raymond O. Miller*[4], *Timothy A. Volk*[5], *and Lawrence B. Smart*[1]

[1]Department of Horticulture, Cornell University, Geneva, NY [2]Departments of Plant Science or [3]Ecosystem Science and Management The Pennsylvania State University, University Park, PA [4]Forest Biomass Innovation Center, Michigan State University, Escanaba, MI [5]Department of Forest and Natural Resources Management, SUNY College of Environmental Science and Forestry, NY USA

The successful development of commercial short rotation shrub willow production grown on marginal lands will require testing a wide range of genotypes in environments that are typically challenging for traditional crop production. Tracking growth in years prior to harvest will aid in the prediction of final yield estimates and help to determine site variables that influence growth patterns. In addition, little is known about changes in biomass quality (specific gravity, cellulose, hemicellulose, lignin, ash) across time. We report the growth and biomass traits of 23 genotypes, including commercial and pre-commercial cultivars, on contrasting environments over the first year of a three-year rotation. Yield trials were established in 2012 on marginal agricultural lands in New York, Pennsylvania and the Upper Peninsula of Michigan in a randomized complete block design with four replicates of each genotype. First year post-coppice growth was measured and stem segments from border plants in each plot were collected and analyzed for biomass composition using thermogravimetric analysis. Growth was greatest in the New York trial, with a fair degree of correlation in rank position among cultivars. Correlation of growth and biomass composition with soil characteristics, soil microbiome composition and climate data will be analyzed for determining their relative importance in predicting biomass production and will be related to modeled potential growth for an unstressed willow canopy. These data will be part of a larger network of yield trials located across the northeastern and upper Midwest US. Growth and yield data from previous trials will be explored for the usefulness of first year growth in predicting third year biomass yield and quality. These data will be helpful in validating models of shrub willow yield on regional scales and evaluating the genotype-by-environment interactions that are likely to exist in short rotation woody crop production. Funding support: This work was made possible through funding from the Northeast Sun Grant Center and a USDA NIFA CAP project.

S6 – P7

Key words: genotype, parameterisa-tion, *Salix*, water stress, yield

Identifying key environment-specific traits through process-based modelling for improvement of short rotation coppice willow

Benjamin Richard[1]*, Marianna Cerasuolo*[2]*, Salvador Girbau*[1]*,*
Jennifer Cunniff[1]*, Sarah Purdy*[3]*, John Clifton-Brown*[3]*, Goetz Richter*[1]

[1]Rothamsted Research, Harpenden, AL5 2JQ [2]Department of Mathematics, University of Portsmouth, Portsmouth, PO1 3HF
[3]Aberystwyth University, Aberystwyth, SY23 3FL, UK

Biomass production from perennial crops could help decrease dependency on fossil fuels and mitigate climate change. Yield and carbon partitioning are key to sequestering carbon and providing a carbon-neutral fuel. Our poster demonstrates how process-based modelling can be used to identify key traits for selection in breeding crops for environments differing in water availability.

Identical experiments were carried out in Wales (Institute of Biological Environmental and Rural Sciences, IBERS; 1038 mm/year) and south-east of England (Rothamsted Research, RRES; 747 mm/year) using four short rotation coppice (SRC) willow genotypes. A process-based willow growth model (Cerasuolo et al., 2014), implemented in a water and energy balance model, was first calibrated for a first growth cycle at IBERS (2010-2011; no water stress) and then evaluated using two successive growth cycles at RRES (2010-2014; water stress).

We observed some common, genotypically stable parameter values (e.g. stem elongation rate) whilst other traits (e.g. assimilate allocated to root growth) varied according to site. The parameterisation of water uptake and stress response model was also informative with regard to belowground processes which are less supported by evidence. E.g. the validation under water stress highlighted the importance of rooting depth of plants to adjust and avoid water stress.

Sensitivity and uncertainty analysis using this new model allowed simulation of scenarios of willow genotypes in different environments and development of hypotheses with regard to key traits. Our next step will be to develop a more mechanistic process understanding by integrating our model calibration with trait information gathered for mapping populations. Eventually, we will develop a QTL-based approach in order to narrow the gap between genotypic and phenotypic information.

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S6 – P8

Key words: wood properties, poplar, clone

Poplar wood properties: Characterization of performances of current cultivars and evaluation of possible early prediction

Alain Berthelot[1], *Alain Bouvet*[1], *Didier Reuling*[1], *Jérôme Moreau*[2], *Louis Denaud*[3], *Eric Paillassa*[4], *Pierre Nougier*[1], *Catherine Bastien*[5]

[1]Institut Technologique FCBA, 10, avenue de Saint-Mandé 75012 Paris
[2]Ecole Supérieure du Bois, Atlanpôle, BP 10605, 44306 Nantes [3]LABOMAP, Arts et Métiers ParisTech, rue porte de Paris, 71250 Cluny
[4]IDF-CNPF 6, parvis des Chartrons, 33075 Bordeaux [5]INRA UR AGPF, Centre de recherche d'Orléans, Orléans Cedex 2, France

For ten years, numerous studies have been carried out in France on the properties of poplar wood, both by research institutes, technical centers, and in close collaboration with industry.

Thus, the wood of 23 cultivars, representing the most cultivated poplars in France, has been characterized from a large and structured sample of mature trees (multi-sites and multi-trees per site). Characterization focused on the general properties of logs and wood, as well as major industrial uses of wood. The mechanical characteristics of the sawings and their drying behavior, suitability for peeling and characterization of the plywood, pulping and the shape of the fibers have been described. The results are presented in two publications widely available*, either with a "cultivar" entry describing all the characteristics measured on a cultivar or with an entry "characteristics of wood" where performances of the different cultivars are compared between them.

Furthermore the GIS Peuplier (French scientific consortium) studied the properties of 55 clones of poplar (*P. deltoides* and *P.* x *canadensis*) from samples of juvenile wood (2 and 4 years) and mature wood (21-27 years). Correlations between variables were investigated for different ages of wood. The best correlations with adult performances concerned basic density and fiber width, and to a lesser extent, shrinkage of the wood samples measured at 4-yrs. These results should enable breeders to include these wood properties predictors in the first steps of their breeding program.

Funding support: French Ministry of Agriculture, France-Bois-Forêt, Conseils Régionaux de Bourgogne, Aquitaine, Champagne-Ardenne, Midi-Pyrénées, Nord-Pas-de-Calais

*http://www.peupliersdefrance.org/qualites-du-bois-des-nouveaux-cultivars-de-peuplier-401542.html; http://www.peupliersdefrance.org/referentiel-qualites-du-bois-des-cultivars-de-peuplier-755801.html

S6 – P9

Systems modeling for short rotation woody crops

Key words: systems modeling, woody crops, short rotation coppices, system dynamics, SRWCs

Huy T. Nguyen[1], *Evan G. R. Davies*[1], *Miles Dyck*[2], *Martin Blank*[3], *Richard Krygier*[3]

[1]Department of Civil and Environmental Engineering, University of Alberta [2]Department of Renewable Resources, University of Alberta [3]Natural Resources Canada, Canadian Forest Service, Canadian Wood Fibre Centre

Short rotation woody crop (SRWC) systems are complex and involve numerous interactions and feedbacks among different elements: climate, wastewater and biosolids characteristics, irrigation, soil characteristics, woody crop establishment and growth, energy content, environmental regulations, and economics, for example. It is thus critical to have a comprehensive, systems-based tool to understand these interactions and feedbacks. In this research, “system dynamics” – a widely-used method for representing and modelling complex systems – is used to develop an integrated decision-support tool for planning viable, sustainable SRWC systems. The first step in developing a systems model is to construct causal loop diagrams (CLDs), from which stock and flow diagrams (SFD) and decision-support tools (DSTs) are developed.

This presentation will describe CLDs for SRWC systems: a single comprehensive CLD for the entire SRWC system is first presented, followed by a set of seven CLDs that provide greater detail on 1) biomass production, 2) irrigation supply, 3) environmental quality, 4) bioenergy, 5) land use, 6) production and 7) SRC policy. The author will then describe a simulation model for SRWC system at Whitecourt, Alberta, and present some validated results. Based on 8 years of data (2006 to 2013), the comparison between model-predicted and observed values show that the model matches biomass production (R^2=0.98) and tree height (R^2=0.91) data well. The model predicts summer season irrigation requirements acceptably well (R^2=0.73). Differences between modelled and observed soil EC values for most years are within 10%, except in 2008 with 20% and 2007 with 50%. These results show that the model will be helpful in supporting decision-makers to plan appropriately for the establishment of future SRWCs and thereby to optimize their investments and the end uses of the obtained SRWC biomass.

S6 – P10

Key words: POPFULL, biomass harvesting, harvest efficiency, harvest losses, manual harvest, Stemster

Comparative study of different harvesting techniques of a short rotation woody crop culture (POPFULL)

Stefan Vanbeveren, *G. Berhongaray, S. Njakou Djomo & R. Ceulemans*

[1]University of Antwerp, Department of Biology, Research Group of Plant and Vegetation Ecology, B-2610 Wilrijk, Belgium

The large amount of above-ground biomass production of poplar (*Populus*) makes this species most suitable as a short rotation woody crop (SRWC) for the production of biomass for bioenergy. SRWC plantations are generally managed as common agricultural crops, with the main difference being the harvest. Because poplars have only recently been implemented as crops, the experience and knowledge of harvesting techniques are still lacking.

We evaluated different harvesting techniques and machines at the second harvest of the POPFULL plantation (February 2014). This high-density (8000 trees per ha) plantation was established in April 2010, coppiced for the first time after two years (in February 2012) and harvested again after the second two-year rotation. Manual harvest was compared to the Nordic Biomass Stemster MK III. This is a side-operated tractor-pulled stem harvester that consists of a tractor and an inseparable trailer. For each harvesting technique several parameters were quantified: (i) time efficiency, quantified as the number of scheduled vs. productive machine hours; (ii) harvesting efficiency, quantified as the harvested area per unit of time, as the harvested fresh/dry weight per unit of time, as the harvested fresh/dry weight per unit area, and in terms of harvest losses; (iii) economic efficiency; (iv) genotypic variation in different harvesting parameters; and (v) impact on soil compaction. The results are compared with those of the first harvest (Berhongaray, El Kasmioui & Ceulemans, 2013, Biomass & Bioenergy 58).

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S6 – P11

Key words: bioenergy, land use change, GHG emissions, energy ratio, eddy covariance life cycle assessment, POPFULL

Land use, energy and greenhouse gas performances of a poplar short rotation coppice system (POPFULL) for the production of renewable energy

Sylvestre Njakou Djomo, *T. Zenone, T. De Groote, G. Berhongaray, S. Vanbeveren, M.S. Verlinden, L.S. Broeckx, O. El Kasmioui & Reinhart Ceulemans*

University of Antwerp, Department of Biology, Research Group of Plant and Vegetation Ecology, B-2610 Wilrijk, Belgium.

Bioelectricitiy from short rotation coppice (SRC) has the potential to replace electricity from non-renewable sources and mitigate climate change. But the efficacy with which SRC can mitigate climate change depends on how the crop is produced and used, as well as on the emissions from land use changes. Quantifying the full energy and greenhouse gas (GHG) balances of SRC-based bioelectricity is necessary for validating the renewable character and the mitigation potential of these cultures. We established an operational low-input SRC plantation of 18.4 ha on a former cropland in East Flanders, Belgium. We monitored fluxes of different GHGs (CO_2, N_2O, CH_4) between the plantation and the atmosphere during two two-year rotations. We also sampled soil organic carbon before planting and after the second rotation. Flux and soil organic carbon data were combined with on-farm estimates of fossil fuels used as farm inputs and to operate farm machinery, to calculate the land requirement, the net energy yield, and the GHG benefits of the plantation. Bioelectricity from the plantation yielded 6.4 times more energy than required to produce it over two rotation cycles of two years each. The SRC-based bioelectricity emitted about 130.2 gCO_{2e} kWh_e^{-1} and used 0.4 m^2 kWh_e^{-1} of land. Direct land use change (65%) and harvesting (19%) were the principal contributors to the total GHG emissions of bioelectricity. Compared to electricity from the EU non-renewable grid mix, bioelectricity reduced GHG emissions by 78%. While further studies are required, bioenergy from SRC can have significant impacts, and should without any doubt be an important aspect in a multidimensional approach to increase energy security and to mitigate climate change.

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S6 – P12

Key words: poplar short rotation forestry, bioenergy, GHG balance, eddy covariance

Fluxes of greenhouse gases (CO_2, CH_4 and N_2O) above a short rotation coppice plantation after conversion from agricultural land (POPFULL)

Terenzio Zenone & *Reinhart Ceulemans*

University of Antwerp, Department of Biology, Research Group of Plant and Vegetation Ecology, B-2610 Wilrijk, Belgium

Energy production from biomass currently has a high political priority, as shown by the European Union target of a 20% share of renewable energy by 2020. The cultivation of Short Rotation Forestry (SRF) for heat and power generation can make a substantial contribution to meet this target. In this study we report the greenhouse gas fluxes (GHG) of CO2, CH4, and N2O – measured using the eddy covariance methodology – in a poplar SRF plantation for bioenergy production during the first two 2-year rotations. The plantation is located in Lochristi (Flanders, Belgium) and has been converted from previous agricultural land. During the first six months after the establishment of the plantation (June – December 2010) there were substantial CO2, CH4, and N2O emissions, a total of 5.36 Mg CO2eq ha−1. Nitrous oxide loss mostly occurred during a week-long peak emission after an unusually heavy rainfall. This week-long N2O emission represented 52% of the entire N2O loss in 2010. So, accurately capturing these emission events is critical for correct estimates of the GHG balance of bioenergy plantations. The initial establishment (June December 2010) of the plantation resulted in a net CO2 loss of 2.76 ± 0.16 Mg CO2eq ha−1 into the atmosphere. In the second year (2011) there was a substantial net CO2 uptake of −3.51 Mg CO2eq ha−1, while the N2O and CH4 emissions were 0.42 and 0.49 Mg CO2eq ha−1, respectively. After the first harvest in February 2012, the third year (2012), resulted in a net CO2 loss of 5.7 Mg CO2eq ha−1, 0.96 Mg CO2eq ha−1 of N2O and 1.09 Mg CO2eq ha−1 of CH4. Thanks to a longer period of net C uptake during the fourth year (2013; i.e. the second year of the second rotation) there was a net CO2 uptake of – 9.34 Mg CO2eq ha−1 while the N2O and CH4 emissions were 0.54 and 0.82 Mg CO2eq ha−1 , respectively. Over the two 2-year rotation cycles the overall GHG balance (sum of CO2 N2O and CH4) was 2.5 Mg CO2eq ha−1 indicating a net release of GHG from the plantation into the atmosphere. This study underlines the importance of the "non-CO2 GHG" on the overall balance as well as the impact of the harvest on the CO2 uptake rate.

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S6 – P13

Key words: clone-site interaction, yield estimation, spacing effects, rotation length, growth simulator

Joint Research Project ProLoc – Implementing a model for clone-site interaction of poplar and willow in short rotation coppice on a supraregional scale

Christoph Stiehm[1], Martin Hofmann[1], Alwin Janssen[1], Winfried Kurth[2], Jürgen Nagel[3]

[1]Northwest German Forest Research Institute, Department Forest Genetic Resources, Prof.-Oelkers-Str. 6, D-34346 Hann. Münden
[2]Georg-August University of Göttingen, Department Ecoinformatics, Biometrics and Forest Growth, Göttingen [3]Northwest German Forest Research Station, Department Forest Growth, Göttingen, Germany

Until now the extensibility of results from SRC field trials in Germany, attempting to identify yield influencing site conditions, has been limited to the respective trial properties. The Joint Research Project ProLoc aims to close this gap by thoroughly assessing the site/yield interaction on a supraregional scale for environmental conditions typically found in Germany. Under the central administration of the Northwest German Forest Research Station, 23 partner institutions carry out the local supervision of 27 trial sites in a 3 year rotation cycle, 13 trial sites in a 10 year rotation cycle and 3 trial sites with varying spacing. According to the standards in agricultural breeding, all trials rely on a randomized uniform design containing 5 commonly utilized clones (3 poplar and 2 willow clones) in 4 replications. On a yearly basis incremental parameters like diameter and height are being measured along with the survival rate and vitality indicating properties for each test unit. Furthermore the biomass production in the form of dry mass is assessed with each harvest. The large number of sites makes it possible to analyze a huge variety of different site conditions for an identification of the main growth influencing parameters as an input for proper yield estimation for each clone.Results from the second rotation cycle will be displayed along with a comparison with the conclusions from the first rotation cycle. The applicability of different physical/chemical soil properties and climate conditions for yield prediction will be discussed, as well as the impact of different clustering variables on the results. Employing the prior findings as an input, the implementation of a yield predictor in form of a growth-simulator will be discussed, by modifying an existing simulator to conditions in coppice plantations. An outlook on future aims will be given, like the down-scaling from the stand-level growth-simulator to a structural model for coppiced poplar.

Funding support: Federal Ministry of Food, Agriculture and Consumer Protection through the Agency for Renewable Resources

S6 – P14

Key words:
hybrid poplar,
biomass
production,
short rotation
coppice

Hybrid poplar growth under two year coppice biomass production system in Chile

Alejandro Fraga[1,2], *Carlos Gantz*[1], *Mario Olmedo*[1]

[1]Greenwood Resources Chile S.A., alejandro.fraga@gwrglobal.com
[2]Department of Soil Sciences, Universidad de Concepcion, Chile

A study was carried out to follow and assess the performance of three hybrid poplar clones (*Populus trichocarpa* × *populous deltoides*) under a two year coppice system to produce biomass for local renewable energy projects. The trial was established on October 2007 on the Santa Julia farm, located five km south west from Los Angeles, (37º30'14'' S, 72º22'31'' W). The main aim was to assess the growth of Greenwood Resources Inc. - hybrid poplar clones under an intense silviculture regime and a high stocking rate of 5,550 trees per hectare and very short coppice rotation of two years. The trial is being measured yearly and the results after three successive coppice rotations are presented. The trees were measured after every growing season and plots were manually harvested during the dormant period (May to August) following each complete coppice cycle and. The results are very promising in terms of annual growth (3 to 4 m in height) but the coppice rotation should be lengthened along with a reduction in planting density to achieve a higher mean annual increment across successive coppice cycles.

S6 – P15

Biomass production of poplar SRC in Southern Italy

Key words:
poplar clones,
SRC, biomass
production

Gianni Facciotto[1], *Alejandra Navarro*[2], *Marcello Mastrorilli*[2], *Sara Bergante*[1], *Giuseppe Nervo*[1]

[1]Consiglio per la Ricerca e la sperimentazione in Agricoltura (CRA)- Unità di Ricerca per le Produzioni Legnose fuori Foresta (PLF), St. Frassineto Po 35 – 15033 Casale Monferrato (AL) [2]Consiglio per la Ricerca e la sperimentazione in Agricoltura (CRA)- Unità di ricerca per i sistemi colturali degli ambienti caldo-aridi (Bari) (SCA), Via Celso Ulpiani, 5 – Bari (BA), Italy

To extend SRC in Southern Italy and to verify the production of poplar clones in these environments, characterized by low water availability, three trials were established: two in Apulia and one in Sicily comparing five clones. Unrooted cuttings of three genotypes of *Populus ×canadensis* (‘Neva’, ‘Luisa Avanzo’ and ‘Bellini’) and two of *P. deltoides* (‘Dvina’ and ‘Lena’) were planted in the Spring of 2010 with different densities. Two trials have a high planting densitiy (about 5.000 trees per hectare) and biennial harvesting cycle; the third have a density of 1667 trees per hectare and a four years harvesting cycle. Data collection concerned: direct observation during growing season, dendrometric recording and weights of the inner part of each plot at the end of the harvesting cycle. The productivity of the best poplar clones (‘Neva’ in Apulia and ‘Dvina’ in Sicily) is under 5 ODtha^{-1}year^{-1}. When focusing on above ground biomass distribution, in Apulia stem biomass accounted for 75 % of total biomass for ‘Neva’, 70% for ‘Luisa Avanzo’ and ‘Dvina’, 65% for ‘Bellini’ and finally 62% for ‘Lena’. Raw material quality of bark and branches is of lower quality than stem wood, then ‘Neva’ and ‘Dvina’ would be used as SRC species for its quality too. ‘Lena’ and ‘Bellini’, are especially not recommended as SRC species in semi-arid climatic conditions, due to the low values of rooting, sprouting and biomass production. The overall sensitivity of poplar to drought represents a severe limit to the future development of its cultivation in Southern Italy. Considering the present economical situation and the climate change undergone in this period, selection criteria of commercial poplar clones need to be reoriented in order to take into account resistance to abiotic stresses in general, and to drought in particular in addition to a maximum biomass production.

Funding support: FAESI project, Italian Ministry of Agricultural, Food and Forestry Policies.

S6 – P16

Key words:
poplar, willow,
biomass,
production,
marginal soil

Poplar and willow SRC productivity in the Calabrian Apennine (Italy)

Sara Bergante, Laura Rosso, Gianni Facciotto

Consiglio per la Ricerca e la sperimentazione in Agricoltura (CRA)-
Unità di Ricerca per le Produzioni Legnose fuori Foresta (PLF). St.
Frassineto Po 35, 15033 Casale Monferrato (AL), Italy

Currently, about 7000 ha of SRC, mainly with poplar, are cultivated in Italy, of which around 80 % in the North Italy. Due to extreme poplar plasticity, and with the interest on SRC cultivation on marginal soils, these plantations may represent a valid alternative or additional income also in South Italy areas, that have social and economic problems due to work lack and cultivation difficulties related to the conformation of the territory. In Spring 2009, the CRA-PLF of Casale Monferrato, in collaboration with a private nursery ‘Allasia Plant Magna Grecia’ decide to test 13 poplar (*Populus* spp.) and 3 willow (*Salix* spp.) clones in the South Italy Apennine environment with sub-mediterranean climate. Following the SRC model, the trial as a density of 8333 trees·ha^{-1}. The randomized complete bloc with 3 replications was utilized as experimental design and the single plot contain 45 trees. The trial cover an area of 0.4 ha. A minimum cultivation input was applied: irrigations were not necessary because of the abundant rains (1000 mm per year); no pest and diseases were detected. After five years the poplar survival reach a mean value of 70%, the best clone reach 96 %. Also willows show similar results, and best clone reach 84% of survival. The poplar biomass production, during five years, reach a mean of 5.6 Mg·ha^{-1}·y^{-1}. The willow genotypes reach a mean of 6.1 Mg·ha^{-1}·y^{-1}. While in North Italy these genotype show more variable productions (from 6 to 15 Mg·ha^{-1}·y^{-1}) according to water availability. Dry matter content is higher in willows (around 42 %) than in poplar (34 to 38 %). The basal density values seems to be strongly influenced by genotype and vary from 0.31 g/cm^3 to 0.38 g/cm^3. Considering the genotypes availability, and possible cultural model adaptations, the SRC in some areas of South Italy can give a very promising results.

Funding support: FAESI project, Ministry of Agricultural, Food and Forestry Policies.

S6 – P17

Key words:
poplar, SRC,
growth, water
availability,
production

Influence of water availability on growth and production of poplar SRC in Po Valley (Italy)

Sara Bergante, Gianni Facciotto

Consiglio per la Ricerca e la sperimentazione in Agricoltura (CRA)-Unità di Ricerca per le Produzioni Legnose fuori Foresta (PLF). St. Frassineto Po 35, 15033 Casale Monferrato (AL), Italy

In Italy, the Po Valley environment is particularly suitable for poplar cultivations, due to soil characteristics and water availability. In West area of Po Valley the rainfall regime ensure 700-800 mm of rain per year but in the last decade, during the vegetative season (April-October), and particularly during July-August the rains distributions are not sufficient to support productivity in commercial poplar stands. Different irrigation techniques are utilized when possible: flooding, sprinkling, drip system. But the importance of water conservation, as well as the high irrigation costs, force researchers to select drought-resistant varieties and to develop new sustainable farming methods. In this paper we report the results of some irrigation trials conducted in different sites of North Italy. Two trials were established respectively in 2002 and 2009 near Casale Monferrato: one with 14 different poplar clones and one with *Populus ×canadensis* clone “Imola” to evaluate irrigation, and in the second, also N fertilization effects. In this trial four treatments were compared: only irrigation, only fertilization, irrigation plus fertilization and a control not treated. Growth, aboveground biomass, including leaves, were measured at the end of vegetative season. In a third trial, sited near Villanterio, a *Populus ×canadensis* clone “AF2” was cultivated from 2006 comparing drip irrigation plots against no irrigation plots. In this site trees were irrigated during first and second years; then irrigation has been suspended. Growth and biomass production data of first 6 years are available. In other plantations has been detected the influence of water table. Confirming past experiences we emphasizes once again the close link between poplar and water: the trees irrigated produce more or less the double of non irrigated and It was possible to see a certain drought adaptation of not irrigate trees.

Funding support: SUSCACE Project, Ministry of Agricultural, Food and Forestry Policies

S6 – P18

Key words:
poplar, willow,
SRC,
fertilization,
bioethanol

Influence of fertilization on biomass production of four poplars and willows clones for biofuel purpose

Laura Rosso, Sara Bergante, Lorenzo Vietto, Gianni Facciotto

Consiglio per la Ricerca e Sperimentazione in Agricoltura, Unità di Ricerca per le Produzioni Legnose fuori Foresta (CRA-PLF), Casale Monferrato (AL), Italy

While there are numerous options for the production of electricity and heat from renewable sources, for transport sector, biofuels obtained from vegetal tissues are the only renewable alternative to fossil fuels. Among *Salicaceae* genus, there are genotypes in *Populus alba* and *Salix* spp. able to tolerate difficult growing conditions such as those typical of marginal lands. They can be grown avoiding the competition with food crops. The characteristic making these species interesting as biofuel feedstock is the high percentage of cellulose present in their wood. In addition to genetic features, there are other factors that influence the cellulose content in the tree, including technological characteristics and crop management: fertilization can influence wood composition. In this trial, highly productive white poplars, a native genotypes and an offspring of 'Villafranca' (*P. alba*) clone, are compared with two willow clones, *Salix matsudana* hybrids. They are fertilized with three different doses of nitrogen (0, 30 and 60 kg of N per hectare). The trial is located in the CRA-PLF experimental Farm 'Mezzi', the density is 1.111 trees per hectare A Short Rotation Coppice (SRC) cultural model with 5 years harvesting cycle is adopted. Weeds were mechanically controlled during growing season and the trial was drip irrigated during Summer. In the first and second year the analysis of variance of growth data shows significant main effect for genotypes but not for nitrogen and interaction. Regarding biofuel conversion the first results of raw material compositional analysis obtained by the three different fertilization show that this material possess a very low quantity of ashes that ranges from 1 to 1,5% of dry weight. The samples had also a good percentage of water extractives in which are contained no-structural sugars; it means sugars easily available for conversion into bioethanol. These extractives range from 4 to 8% of dry matters.

Funding support: 'Biosegen project'- Ministry of Agricultural, Food and Forestry Policies of Italy.

S6 – P19

Key words:
short rotation woody crops, coppice management, harvest logistics, feedstock quality

Effect of willow cultivar attributes on chip quality and the performance of a New Holland short-rotation woody crop harvesting system

Mark Eisenbies[1], Chris Foster[2], John Posselius[2], Shun Shi[1], and Tim Volk[1]

[1]Department of Forestry and Natural Resources Management, State University of New York College of Environmental Science and Forestry
[2]CNH America LLC, P.O Box 1895, ms 640, New Holland PA 17557-0903, USA

Demand for bioenergy sourced from woody biomass is projected to increase; however, the expansion and rapid deployment of short rotation woody crop systems in the United States has been constrained by high production costs and sluggish market acceptance due to problems with quality and consistency from first-generation harvesting systems. The objective of this study was to evaluate the effect of crop conditions on the performance of a single-pass, cut and chip harvester based on a standard New Holland FR-9000 series forage harvester with a dedicated 130FB short rotation coppice header, and the quality of chipped material. A time motion analysis was conducted to track the movement of machine and chipped material through the system for 153 separate loads over 10 days on a 54-ha harvest. Harvester performance was regulated by either ground conditions, or standing biomass on 153 loads. Material capacities increased linearly with standing biomass up to 40 Mg_{wet} ha^{-1} and plateaued between 70 and 90 Mg_{wet} hr^{-1}. Moisture contents ranged from 39 to 51% with the majority of samples between 43 and 45%. Loads produced in freezing weather (average temperature over 10 hours preceding load production) had 4% more chips greater than 25.4 mm ($P < 0.0119$). Over 1.5 Mg_{dry} ha^{-1} of potentially harvested material (6-9% of a load) was left on site, of which half was commercially undesirable meristematic pieces. The New Holland harvesting system is a reliable and predictable platform for harvesting material over a wide range of standing biomass; performance was consistent overall in 14 willow cultivars.

Funding support: US Department of Energy Biomass Program; New York State Energy Research and Development Authority; Empire State Development Division of Science and Technology and Innovation Technology Transfer Incentive Program

S6 – P20

Highly productive and climate-adapted poplar clones for the energy and forestry sector in the Baltic Sea Region

Key words: adaptation, Baltic Sea region, establishment, *P. trichocarpa*

Anneli Adler[1], *Almir Karacic*[2] *and Mindaugas Šilininkas*[3]

[1]SweTree Technologies AB, Uppsala, Virdings Allé 2A, SE-75450 Sweden
[2]Department of Crop Production Sciences, Swedish University of Agricultural Sciences, Uppsala, Po Box 7043, SE-75007, Sweden [3]Euromediena UAB, Vilnius, S.Daukanto a., LT-01122 Lithuania

The aim of this project is to develop a platform for the deployment of SnowTiger®, a new elite poplar clone mixture from SweTree Technologies AB, on a large scale in Scandinavia and in the Baltic Sea Region in cooperation with Swedish University of Agricultural Sciences (SLU) and Euromediena UAB in Lithuania. The SnowTiger® consists of a unique material bred at SLU for the climate conditions of northern Europe. The material is suitable for establishment of wood fiber plantations for multiple purposes such as energy, pulp, particle board, veneer and environmental applications. There are large areas of land available for this type of plantations in the Region. However, growing short rotation poplar plantations at high latitudes, in a harsh climate and often on various types of former agricultural fields is connected to difficulties, especially in establishment phase. In this project we address these problems by evaluating different types of planting stock in establishment phase and developing a cost-effective production of suitable planting stock for nurseries. Different types of soils may require the use of different site-specific establishment methods or using different planting stock. Poplars can be established using a number of different types of rooted and unrooted cuttings or pregrown plants. The type of planting stock affects the purchasing costs and the costs of planting, transport, storage and handling of the material. One- or two-years-old 2m long poles can be for example used for establishment on cultural grasslands. Using this type of planting stock can be combined with appropriate cultivation practices to decrease various types of biotic and abiotic stress and damages and to attenuate effects of weed competition. The advantages and disadvantages of using different types of planting stock will be evaluated by establishment of 12 R&D poplar plantations on 12 major types of soils on former cultural pastures and on former arable land in the Baltic Sea Region (Sweden, Lithuania, Estonia). The production system for rooted and unrooted cuttings will be evaluated and compared to the presently used commercial production of plants. Finally, the clones used in SnowTiger® mixture will be evaluated in detail for the quality characteristics and usability for different types of products. Funding support: The Eurostars Programme (http://www.eurostars-eureka.eu); VINNOVA (www.vinnova.se)

S6 – P21

Productivity of poplar and aspen hybrids in Latvia

Key words: *Salicaceae*, height, diameter, yield

Aris Jansons, Silva Zurkova, Martins Zeps, Janis Smilga

Latvian State Forest Research Institute Silava, Rigas 111, Salaspils, LV-2169, Latvia

Populus hybrids are recommended for biomass production in Europe; however, there is insufficient information about their growth in the Baltic States, where notable areas of abandoned agricultural lands are available and forest legislation allows using them also in forest lands. The aim of the study was to characterize the productivity of poplar in comparison to that of common aspen and aspen hybrid.

Material was collected in 12 poplar (*Populus balsamifera* x *P. laurifolia*) stands located in central and western part of Latvia (56-57°N, 22-23°E) which were established on fertile soil; initial spacing: 5000 to 7000 trees ha^{-1}; stand age 54–65 years. Data on growth of hybrid aspen (*Populus tremula* x *P. tremuloides*) were collected in the same region in plantations on former agricultural lands; initial spacing 1100 to 2500 trees ha^{-1}, stand age 12-18 years. National forest inventory plot data were used for comparison.

Mean tree diameter and height in poplar stands varied from 29±1.6 cm to 45±3.9 cm and from 24±0.9 m to 31±0.8 m, respectively. Mean diameter and height of hybrid aspen reached 19.0±0.46 cm and 21.6±0.84 m, respectively. Both mean diameter and height of hybrid poplars and aspen was significantly higher compared to Norway spruce, but the differences were age-dependent. Tree ring analysis showed that annual increment of poplars had notably decreased since age 45 years. The number of fallen and standing dead trees, reaching up to 14–46 % from the number of living trees, indicated intense self-thinning; the dead trees had significantly smaller dimensions. Mean annual volume increment of all poplar stands was 13.8 m^{3}ha^{-1}y^{-1} (in some stands reaching 21.0 m^{3}ha^{-1}y^{-1}, corresponding to 4.6-7 t of dry matter per year); whereas of hybrid aspen plantations: 20.3±3.3 m^{3}ha^{-1}y^{-1}. Thus, the results suggest that poplar could be a highly productive species in climatic conditions of the Baltic States, however, growth curves must be studied further to set an optimal rotation age.

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S6 – P22

Key words: poplar, willow, biomass, productivity, multiple rotations.

Twelve-year productivity of willow and poplar clones in a high density energy plantation in Escanaba, Michigan, USA

Raymond O. Miller, Bradford A. Bender

Michigan State University Forest Biomass Innovation Center

Willow hybrids are adaptable and productive plants for use in short rotation energy cropping systems in northern Europe and in the northeastern portion of North America. Shrub-form willows are planted once at high densities and are repeatedly harvested on 3-year cycles, re-sprouting after each harvest. A long-term test of 12 hybrid willow clones and two hybrid poplar check-clones was established at a density of approximately 18,000 stems per hectare in Escanaba, Michigan, USA in the spring of 2002. This test has now been harvested four times. The most productive of the 12 tested willow clones yielded nearly four times as much biomass as the least productive clone after 12 years. The development and selection of superior hybrids will substantially improve the profitability of energy farming. Total productivity of the top two willow clones averaged 84 dry Mg/ha and was comparable to that of the two poplar check-clones, which averaged 89 dry Mg/ha over the 12-year life of this test. The growth strategy of the two taxa differed however. Poplar mean annual biomass productivity rates initially averaged 8.3 dry Mg/ha•yr but declined to 5.2 dry Mg/ha•yr toward the end of this 12-year period. The mean annual biomass productivity rate of the top two willow clones was initially 3.3 dry Mg/ha•yr but increased to 9.0 dry Mg/ha•yr – eventually exceeding that of poplar. Willow has reached a plateau of annual productivity while poplar has declined. This information is critical when determining the number of times a grower can expect vigorous re-sprouting after a harvest before it becomes necessary to remove the old and replant a new energy plantation. Observations of productivity in this test will continue for at least two more rotations.

Funding support: Partial support for this work came from the USDOE Regional Biomass Feedstock Partnership and Michigan State University AgBioResearch.

S6 – P23

Key words:
native poplar clones, biomass production, Mediterranean climate

Evaluation of *Populus nigra* L. and *P. alba* L. clones for adaptability and biomass production in Sicily

Vietto Lorenzo, Giorgia Carletti, Giuseppe Nervo, Gianni Facciotto

Consiglio per la Ricerca e la sperimentazione in Agricoltura (CRA), Unità di ricerca per le produzioni legnose fuori foresta (PLF), Strada Frassineto Po 35,15033 Casale Monferrato (AL), Italy

With the aim of selecting native genotypes of *Populus nigra* L. and *Populus alba* L. for biomass production, two field trials were established in Sicily (Italy). A large collection of these two native species was previously evaluated at the Research Unit for Intensive Wood Production in Casale Monferrato. First pools of 60 genotypes of *P. nigra* and 60 genotypes of *P. alba* were selected for growth rate and resprouting capability. With the purpose to a better evaluation of these clones in the Mediterranean climate condition two experimental fields were established in Sigonella (sandy-silt soil) and Gela (sandy soil) using 20 clones of *P. nigra* and 27 clones of *P. alba* respectively. A Randomized Complete Block design, with three replications was used in both sites. Whips 100 cm length were planted 80 deep and a planting density of 625 trees per hectare was used (4 m on and between the rows). Weeds were mechanically controlled during the growing season by disk harrowing; emergency watering to trees by drip irrigation during the drought season was assured in both trials. Survival and growth rate was measured each year. A good survival rate was recorded at the end of the first season (95%); the range of the diameter at breast height varied from 3 cm to 5 cm and the height from 4 m to 6 m at the end of second year. On the base of these preliminary results, several clones of both species performed quite well; these materials could be utilized for biomass production also in Mediterranean climate condition, with a harvest cycle of at least 5-6 years.

Funding support: FAESI project, Italian Ministry of Agricultural, Food and Forestry Policies.

S6 – P24

Key words:
hybrid cottonwoods,
growth rates

Exceptional hybrid cottonwood growth in B.C.'s southern interior---A Stettler-Heilman *Populus trichocarpa X Populus deltoides cross: 53-242*

Michael R.Carlson, *Vicky G.Berger*, *Nicholas K.Ukrainetz*

Tree Improvement Branch, Ministry of Forests, Lands and Natural Resource Operations, Vernon, British Columbia, V1B 2C7 Canada

In the early 1970s basic research into the mechanisms of reproductive isolation among species of the Genus Populus resulted in hundreds of interspecific crosses. Reinhard Stettler and Paul Heilman of the University of Washington and Washington State University respectively selected individual clones within certain crosses of *P. trichocarpa and P. deltoides (TxDs)* for growth and vigor. These clones have since been planted far and wide in the U.S Pacific Northwest and in southern British Columbia. On several low elevation sites in the B.C. southern interior some TxD crosses have demonstrated excellent growth and frost tolerance and some also performed well in coastal environments.

After short-term testing of several TxD and other interspecific hybrids in the southern interior a few promising clones were planted in the Kootenay River valley at 49 degrees north latitude and 545 meters elevation on high quality farm land relatively free of competing vegetation. One TxD clone in particular, 53-242, with a Granite Falls, Washington female black cottonwood parent crossed with a central Illinois male eastern cottonwood parent performed exceptionally well. Planted at a 3m x 4m spacing (833 stems per ha) in 1998, 53-242 produced a stem volume mean annual increment (MAI) of 33.3 m^3/ha/yr at age 14 yrs with a survival rate of 80 %. Certain diseases and insect pests were also evaluated and 53-242 performed average or above other clones tested.

We are not aware of tree stem volume production rates greater than those reported here in any tree species planted north of 49 degrees north latitude. Our challenge to the World Poplar Community… show us stem volume production rates greater than 33.3 m^3/ha/yr north of 49 degrees.

S6 – P25

Key words:
planting stock,
hybrid poplar,
plantation

Long-term growth comparison of three hybrid poplar planting stock types in an irrigated plantation system

Jose Zerpa, Grant Beauchamp, Austin Himes, Jake Eaton

GreenWood Resources Inc

A hybrid poplar trial at the Boardman Tree Farm, in Oregon's mid-Columbia basin, USA (45°45'58.08" N, 119°36'51.79" W) was established in 2008 to test the effect of three different planting stock types on the growth of four hybrid poplar varieties (two *P. ×canadensis* and two *P. ×generosa*) over the course of a 12 year rotation. The three planting stock types were 23 cm dormant branch sections called "sticks," one year old intact stem sections approximately 3-4 m long called "whips," and two year intact stem sections approximately 6-8 m long called "poles." A split-plot design was used to establish the experiment, with planting stock as whole-plots and clone as sub-plots. Measurements of total height and breast height diameter (dbh) were collected annually starting at the end of the growing season. The key findings from the first five growing seasons are: 1.) *P. × canadensis* clones are outperforming *P. × generosa* clones by 14% in height and by 8% in dbh. 2.) The average height and diameter after five growing seasons was largest for trees established from poles, followed by trees established as whips and least for trees established from cuttings. For the length of the trial, average growth rates have been greatest for whips, followed by cuttings, and have been the lowest in poles. 3.) There appear to be interactions between variety and planting stock type. Data from the sixth growing season of the trial is being collected, including a quantitative assessment of stemform, and will be included in an oral presentation in the "Management & Application - Putting biology into practice" session of IPS VI.

Funding Support: GreenWood Resources Inc.

S6 – P26

Key words:
willow, osier, poplar, craft, bioenergy, greening, management

Poplar and willow coppices - from greening, fence and crafts till bioenegy - historical changes of management during last century

Dagnija Lazdina[1],[2]

[1]Latvian State Forest Research Institute "Silava", forest regeneration and establishment department [2]COST action FPS 1301 WP1

Willows and osiers were well known as a material for different craft works, fences and apiculture as early flowering tree, poplars is excellent windbreaker, shelter and fast growing greening tree, commonly planted across roads and on borders between properties. Across all Europe different species of willows were widely distributed in forests. Introduced poplars are well adapted and now-days naturally and vegetative distributing species, what have a high yield potentials in short term.

During last century, because of more intensive and economically targeted management of forest stands, willows were neglected as low value wood and timber, now willows are mainly distributed near water reservoir banks, protected areas – wetlands and "poorly managed" forest properties and coppice forests. Species of willows and poplars having high increments at juvenile stage, were domesticated for chip and fire wood production. Some fast growing clones characteristic with considerable nutrient catchment are used as alternative of sewage treatment plants and for re-cultivation of former mining areas. Breeds of decorative species are widely spread in flower gardens and industrial parks.

Presence of willow and poplar species in Europe coppice forest, plantation forest and short rotation coppice plantations, management practised, length of rotation period, and products for marked in countries are different and correlate with legal frame of stands and traditional knowledge. Exchange of experience how to utilize and domesticate of *Salicaceae,* could to help find locally new solutions for utilizing of coppice, to reduce knowledge gaps and to raise the (economic) appreciation of active management.

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S6 – P27

Key words: fertilization, coppice management, stand density index, aspen clones, aspen propagation

Aspen agroforestry: Five years of results in the Central Iowa Corn Belt

Richard B. Hall, William L. Headlee

Natural Resource Ecology and Management, Iowa State University,
Ames, IA 50011, USA

A multidisciplinary team at Iowa State University is developing strategies for diversifying the corn and soybean landscape with alternative sources of cellulose for energy that will also provide important environmental services. The authors have been in charge of the hybrid aspen cropping system that is being tested across five landscape positions from ridge top to the riparian zone. The test plots were planted in 2009 with the original intent of a ten-year saw-log crop followed by coppice biomass harvests for several cycles. That plan has changed to a mid-rotation conversion to coppice with a plan to harvest sprout regrowth on two or three year cycles. The decision was based on farmer input and observed development of the root network that could support an earlier conversion. The initial planting was done with winter triticale in the ally ways between trees, but the growth and yield of both aspen and triticale was less than expected. The impact of adding fertilizer pellets to the aspen planting holes was promising. In spite of two very dry years in 2012-13 and very heavy deer pressure, the aspen has survived and grown well, with the best growth on the riparian zone. We are collecting data on edge effects on productivity since edges would make up a significant portion of narrow contour bands of aspen interspersed in agriculture fields. An effort is underway to determine a stand density index for judging when to harvest the coppice growth before excessive stem exclusion starts. More work is planned on determining the best clones for agroforestry applications, scaling up new selections to support planting programs, and alternative cover crops during the establishment phase.

Funding support: Iowa Leopold Center for Sustainable Agriculture and the College of Agriculture and Life Sciences, Iowa State University, Ames, IA 50011, USA

S6 – P28

Key words: *Populus tremula* x *P. tremuloides*, thinning, stem growth, green crown dynamics

Management options in dense hybrid aspen sucker stands

Lars Rytter

The Forestry Research Institute of Sweden (Skogforsk), Ekebo 2250,
SE-268 90 Svalöv, Sweden

The Nordic countries have adopted a vision of independence of fossil energy by 2050. Biomass from forests will be a major source of renewable energy. This means that productivity has to be enhanced and this can, for example, be reached by using fast-growing tree species. Hybrid aspen has been tested in the region for about 70 years and commercial stands have been established since the 1990s. The knowledge of management effects in the first planted tree generation is reasonably good, while information on forthcoming sucker generations is scarce. A study was conducted to evaluate the effect of early pre-commercial thinning in dense second generation sucker stands. The main hypothesis was that weaker thinning strategies would lead to slower growth of remaining trees. Three pre-commercial strategies were performed in a stand with 77 000 suckers ha^{-1} after the second growing season: 1) no thinning during the first 4 years; 2) corridor thinning after 2 years where 67% of the stems were removed; and 3) cross-corridor cleaning after 2 years where 89% of standing volume was harvested. All plots were subsequently thinned to 1 100 stems ha^{-1} after 4 years. The remaining stems were studied for another 8 years. After the thinning at 4 years there were clear differences among trees of different treatment. The no thinning strategy resulted in slimmer trees and smaller green crowns, while cross-corridor cleaning showed the largest stem dimensions and largest green crowns. During the 8 years of observation the green crowns became of similar size for the different treatments but the difference in stem dimension remained and the mean annual increment of the differently treated plots continued to diverge. The study showed that the strength of early pre-commercial thinning has a large effect on the continuing growth of remaining stems. The importance of thinning strategy, when striving for large fast-growing trees, is highlighted.

Funding support: Swedish Energy Agency, Nordic Energy Research and Skogforsk frame programme

S6 – P29

Key words:
Populus deltoides,
domestication,
genetics,
silviculture,
North India.

Matching genetics and silviculture for domesticating *Populus deltoides* in North India

Ramesh Chand Dhiman

Wimco Seedlings Division (WIMCO Ltd.), Kashipur Road, Bagwala, Rudrapur, USN, U.K., India.

Matching appropriate genetics and silviculture has proved useful in the domestication of *Populus deltoides* in North India. Major share of its culture is taking place on farm land in the Indo-gangetic plains south of Himalayan Range. This region has never been home for any poplar species till *P. deltoides* (Eastern Cottonwood) were introduced for matchwood production around four decades ago. The tree has now well adapted to this exotic environment and is grown in integration with agriculture crops. Selected cultivars developed from low latitude populations of *P. deltoides* were introduced and appropriate silviuclture inputs were developed which together played a significant role in its successful domestication. Many cultivars produced from hybridization with some other high latitude and altitude *Populus* species fail to tolerate extremes of weather conditions those exist in this region. Around two dozen poplar cultivars are routinely grown, which show both endemic and wide adaptability depending on local conditions. Poplar is now a highly productive tree which has been achieved with development and application of appropriate silvicultural inputs. Some of the cultural and tending operations, like juvenile sapling production with late planting of nurseries, synchronization of planting time with appropriate soil working, irrigation and fertilizer schedules, and pruning helps in making poplars highly productive in term of quality and quantity timber production. The tree is claimed to produce timber sometimes exceeding 50 m^3/ha/year in selected plantations and is harvested at short rotation varying from around 4 to 12 years. Current and mean annual increments are culminating at around 3-4 years period. The presentation includes experimental data in support of the role of both genetics and silviculture leading to successful domestication of the species in North India.

S6 – P30

Key words: *Populus*, bio-energy, plantation yields, silviculture, wood costs

Genetic and silvicultural management for *Populus* bio-energy plantations in the eastern United States

Jeff Wright

ArborGen Inc.

Bio-energy forest plantations will supplement woody biomass from other sources such as logging residues. In the southern US, projections are for an increase of up to 25 million “new” tons of woody biomass demand for bio-energy. To supply this woody biomass demand will require purpose grown plantations of various species including hybrid poplar and cottonwood, amongst others. Forest plantation yields can be greater than 30 green tonnes/ha/year (MAI 30) on rotations of 5-12 years. Utilization of this renewable and sustainable biomass resource will be as feedstock “designed” for a large number of bio-energy applications. Much of the emphasis has been on hardwood plantations due to their ability to coppice, continued genetic improvement programs as well as the opportunity to combine fast growth and wood properties in selected clones. In the specific case of *Populus*, there are a large number of commercial planting programs in countries outside the US. Feedstock characteristics are important in bio-energy hardwood plantations. Firstly, the plantation hardwood species has to be adapted to the soil and climate conditions. The hardwood feedstock has to be acceptable in harvesting, field processing and ultimately for conversion to bio-energy. Lastly, the growing (stumpage), harvest, haul and preparation costs have to be favorable compared to other biomass options.

S6 – P31

Key words:
bioenergy,
biomass,
economics,
marginal land,
Populus

Comparing poplars to eucalypts and loblolly pine on marginal lands in the southeastern USA – preliminary results from a project modeling biomass productivity and economic performance

William L. Headlee[1], *Richard B. Hall*[1], *Ronald S. Zalesny Jr.*[2], *Matthew H. Langholtz*[3]

[1]Department of Natural Resource Ecology & Management, Iowa State University, Ames, Iowa, 50011 [2]Institute for Applied Ecosystem Studies, U.S. Forest Service Northern Research Station, Rhinelander, Wisconsin, 54501 [3]Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, Tennessee, 37831

Conversion of marginal lands to short rotation woody crops (SRWC) can provide biomass for sustainable bioenergy and stimulate rural economies, while minimizing impacts on the food supply and preserving environmental quality. Past SRWC research provides valuable insights into the performance of certain species in selected environments. However, studies comparing an array of species side-by-side across a wide range of conditions are lacking. Process-based models, which account for environmental influences on tree growth, can be used to compare species performance across a range of environmental conditions in a quantitative, spatially-explicit manner. To better inform woody crop selection and optimize economic performance on marginal lands in the southeastern USA, the objectives of this project are as follows: (1) adapt the Physiological Principles Predicting Growth (3-PG) model for poplars, eucalypts, and loblolly pine within the region, (2) use existing geographic information system (GIS) layers for soils and climate to generate productivity estimates for marginal lands within the region by climate zone and soil type, and (3) determine the optimum rotation age and economic value of each species, as well as the economically optimum species (i.e. highest economic value), by climate zone and soil type. Preliminary outcomes of this ongoing project will be described, including early results of model fitting and mapping efforts.

Funding support: USDA National Institute of Food and Agriculture (Project IOW05356)

S6 – P32

Hybrid poplars: Can they survive in the lower Mississippi alluvial valley

Randall J. Rousseau, *B.L. Herrin, J. Mack*

College of Forest Resources, Mississippi State University, Box 9681,
Mississippi State, MS 39762, USA

Key words: hybrid poplar, *Septoria musiva*, clonal performance

DN - Hybrid clones resulting from crossing *P. deltoides* x *P. nigra*

TD - Hybrid clones resulting from crossing *P. trichocarpa* x *P. deltoids*

DT - Hybrid clones resulting from crossing *P. deltoides* x *P. trichocarpa*

DM - Hybrid clones resulting from crossing *P. deltoides* x *P. maximowiczii*

Hybrid poplars have proven their worth in the Pacific Northwest and the Midwestern United States as well as at other geographic areas around the world. However, the use of hybrid poplars in the lower Mississippi Alluvial Valley (LMAV) has been non-existent. Although limited testing of hybrid poplars has been done in western Kentucky, southeast Missouri, and southern Illinois on both alluvial and upland sites, the results were consistent in that all of the hybrid poplar clones eventually exhibited *Septoria musiva* stem cankers and no clone survived longer than 10 years. In fact, a limited number of TD and TDD taxa survived for only three years when planted on Mississippi River alluvial sites.

Under the Sun Grant Populus Program, there was an opportunity to test a variety of hybrid poplars including DN, TD, DT, and DM taxa and eastern cottonwood clones on alluvial sites within the LMAV and an upland site some 100 miles east of the Mississippi River. A screening study was established in 2010 on both alluvial and upland sites, in addition an additional test site was planted in 2011 on the same upland site as the 2010 test site. Measurements of these tests included total height, diameter, crown ratings, and incidence of stem cankers. Age-three growth data from the alluvial site indicated that in general the eastern cottonwood taxa performed better than the hybrid poplar taxa tested. Differences among hybrid poplar taxa and eastern cottonwood showed that the hybrid poplar taxa exhibited a considerable amount of ramets per clone showing stem cankers. Although these two tests are only three and four- years-old, the amount of stem canker exhibited was rather high. While the results of this study was very similar to previous information it is limited to the clones tested and additional testing of newly developed hybrid poplar taxa clones may prove resistant to this disease.

Funding Support: USDA Sun Grant

S6 – P33

Key words:
black willow,
Salix nigra,
biomass, clonal
variability

Evaluating black willow as a viable biomass crop for the Lower Mississippi Alluvial Valley

Randall J. Rousseau[1], *T.D. Leininger*[2], *E.S. Gardner*[2], *J. Mack*[1], *B.L. Herrin*[1]

[1]College of Forest Resources, Mississippi State University, Box 9681, Mississippi State, MS 39762 USA [2]Center for Bottomland Hardwoods Research, Southern Research Station, USDA Forest Service, P.O. Box 227, Stoneville, MS 38776 USA

In 2009, Mississippi State University and the US Forest Service Center for Bottomland Hardwood Research began developing genetically superior black willow (*Salix nigra* Marshall) clones for use on poorly drained former agricultural sites in the Lower Mississippi Alluvial Valley (LMAV). The overall process included genotype selection from designated geographic areas, establishment of cutting orchards to produce unrooted cuttings needed for testing, establishment of screening trials, early-age selection, establishment of a clonal refinement tests, and selection of superior genotypes.

To date, we have selected the best performing clones from our initial clonal screening trials of 113 clones. These better performing clones were moved into highly repetitive clone tests. Data from four screening trial test sites indicated that geographic origin was significant for all traits measured, but accounted for a lower amount of the total genetic variation compared to individual genotypes. Two clone tests have been established on a single test site in 2012 and two test sites in 2013. Two additional test sites will be established in 2014. Performance by geographic source and clones within geographic sources will be compared across the screening trials.

In 2013, 150 clones from five additional geographic areas were added to the test population providing a complete sampling of the Mississippi River from south of Baton Rouge, LA to Osceola, AR. This material was used to establish a new cutting orchard to produce unrooted cuttings for screening trials beginning in 2014.

Funding Support: US Forest Service

S6 – P34

Energy of the future – growing willow in Kazakhstan

Key words: willow, bioenergy, environment, sustainable development, global warming and climate change

Sara O. Kitaibekova

S. Seifullin Kazakh Agro Technical University

Energy is the natural resource paving the way for the stimulation and development of the lives of human beings. Energy supports the model of societies and, as a multifaceted concept, sparks intriguing questions regarding the universe and humanity itself. Societies' access to energy sources directly determines concrete scenarios in their social, economic and environmental development. Scientific research and technological advances, linked to the optimization of the various energy sources, define the functional approaches of societies and can drive a sustainable energy future. Expo Astana 2017 Future Energy is conceived as an extensive, integral project that addresses the fascinating topic of energy from several perspectives, allowing for it to be contemplated as a determining factor in how societies and everyday life function.

Future Energy is a broad and thought-provoking theme for addressing the challenges of today and anticipating future solutions.

The Future Energy project is supported and organized on the pillars of sustainable development, understood as a process aimed at meeting economic and social needs as well as those relating to cultural diversity and a healthy environment.

Future Energy has the clear objective of exploring strategies, programmes and technologies aimed at sustainable energy development, promoting energy security and efficiency, encouraging the use of renewable energy, and guiding visitors through their essential, active role in the design and execution of a plan for energy efficient production and use. Astana EXPO 2017 will help us look at energy from a common, global viewpoint that takes into account the challenges and concerns facing humanity, safeguarding our health and the environment while boosting economic and social development. This balance depends on many elements and is everyone's responsibility.

S6 – P35

Key words: importance, management, *Populus* plantation, skill

Importance of management skills and proficiencies from the viewpoint of Iranian poplar plantations owners

Amir Mohammad Abdolahian Sohi

Department of Wood Science and Technology, Islamic Azad University, Science & Research Branch, P.O.Box 14515/775, Tehran, Iran

Poplar plantations are widely used for timber production and ecological restoration in Iran, a region that experiences new approach in forest sciences. Poplars (*Populus deltoides*, *Populus nigra*, *Populus alba*, *Populus teremula*) are some of the major components of the forest resource of Iran. The uses and economic importance of these resources increased dramatically in the 2000s. Therefore, more attention in the management of these poplar plantations will be lead to better usage and application of these species and also have a vast improvement in Iranian Forestry. The aim of this descriptive – co relational paper is to determine the "Importance of Management Skills and Proficiencies from the Viewpoint of Iranian Poplar Plantations Owners". The population of 7930 Poplar Plantations Owners in the crop year of 2012-13 was defined. From this population, 225 persons were selected by using Cochran's formula with Stratified sampling. Finally 193 questionnaires were completed and analyzed. The results revealed that the three important required management skills for Poplar Plantations are: Operational Skills, Determination of Goals, and Seeking. Also the results of Friedman Test have shown that there was a significant difference between the importances of Operational Skills in comparison to the others.

S6 – P36

Key words:
bioenergy,
forest management,
volume models,
biomass models

New empirical production models for poplar plantations on farmland in Sweden

Birger Hjelm[1], *Tord Johansson*[2], *Blas Mola*[1], *Ioannis Dimitriou*[1]

[1]Swedish University of Agricultural Sciences, Department of Crop Production Ecology [2]Swedish University of Agricultural Sciences, Department of Energy and Technology

Hybrid poplar plantations in short rotations (≤ 20 years) established on farmland in Sweden have shown promising production figures of up to 300 t d.w. ha^{-1} (ca 25 m^3 ha^{-1} $year^{-1}$) and been in focus as a future biomass feedstock for energy. Taper, biomass- and volume models have been developed for individual poplar trees, biomass models for individual stumps (including roots) and for coppiced poplars. One study focuses on development of mixed effect models for stand volume. The models were developed on data from 55 stands, age 7 – 23 years (stump/coppice biomass models 10 stands) located in south and central Sweden. The models enable improved harvest and thinning estimations and will be a useful tool for management operations.

The developed biomass equation estimates the stem, twigs and leaf fractions. One equation was constructed for stem volume estimation and compared with a number of published equations. The best ranked equations resulted in a bias of 3.8%. Models for estimation of individual stumps and coppice biomass were developed. Biomass production of 1000 excavated stumps could be 45-50 t d.w. ha^{-1}. The stump was 74% on average of the total stump-root biomass. Biomass of 7-year-old coppices on 1000 stumps could be 30-35 t d.w. ha^{-1}. Taper models estimate diameters along stems to corresponding heights. We developed a simple taper equation and compared it with common published taper equations. The best ranked resulted in a bias of 3.7%. Experience tells us that volume, biomass, height etc. differ between stands with the same diameter distribution. A traditional model does not calibrate for this but fits the whole data set, the fixed effect. A mixed model that also calibrates for the random group effect is more complex but gives usually higher accuracy in the estimations and can reduce the bias up to ca. 25%.

Funding support: Swedish Energy Agency, Skogssällskapet Foundation

S6 – P37

Key words:
poplar, SRC,
fertilization,
irrigation, wind
damage

Effect of windstorm on poplar trees grown in SRC with different fertilization and irrigation management

Gianni Facciotto, *Sara Bergante*

Consiglio per la Ricerca e la sperimentazione in Agricoltura (CRA)-
Unità di Ricerca per le Produzioni Legnose fuori Foresta (PLF) St.
Frassineto Po 35 – 15033 Casale Monferrato (AL), Italy

Severe windstorms or thunderstorms with hail, strong winds, and intense rainfall, are the major abiotic risks of the poplar stands of the Po Valley (Italy), they influence the productivity and wood quality. Also the management operations can affect the occurrence of these abiotic hazards. In this work we examined the wind damages among trees of *Populus ×canadensis* clone 'Imola' in a experimental short rotation coppice (SRC) trial, managed with different irrigation and fertilization practices, following a severe windstorm in July 2013. The trial was established in spring 2009, on sandy-loam soil of Po Valley, in the experimental farm of CRA-PLF, Casale Monferrato (AL), Italy. Planting density is 1.111 trees per hectare and harvest cycle is 5 years. A Split-plot design with three replications is utilized. The two level of irrigation (0, 1) were assigned to the plot and two level of nitrogen fertilization (0, 60 kg ha^{-1}) to the sub-plots. Disk-harrowing was carried out two times only in the first year of growing; then only one mechanical weeding per year was done. For every year of growth have been measured survival and growth. Aboveground biomass productions (stem and branches) were estimated via non-destructive measurements methods each year. After windstorm each stem was assessed for damage due to the wind. Seven damage categories were used: undamaged, slight bent of the stem, stem bent <45°, stem bent >45°, uprooted tree, stem broken 0-2.5 m, stem broken 2.5-5 m. During the storm the nearest weather station recorded a peak gust of 45.9 m s^{-1}; this highest wind speed originated from NW. Significant difference in damage occur among the four different management practices. Undamaged trees were 89% in not treated plots, 68% in only fertilized, 4% in only irrigated. In fertilized and irrigated plots 93% of trees were bent, 4% uprooted and the remaining broken.

Funding support: SUSCACEI project, Italian Ministry of Agricultural, Food and Forestry Policies.

S6 – P38

Key words:
hybrid poplar, economics, land rent, discount rate, biomass, 3-PG

Impacts of supplyshed-level differences in productivity and land costs on the economics of hybrid poplar production in Minnesota, USA

William F. Lazarus[1], *William L. Headlee*[2], *Ronald S. Zalesny Jr.*[3]

[1]Department of Applied Economics, University of Minnesota
[2]Department of Natural Resource Ecology and Management, Iowa State University[3]Northern Research Station, USDA Forest Service, USA

The joint effects of poplar biomass productivity and land costs on poplar production economics were compared for 12 Minnesota counties and two genetic groups, using a process-based model (3-PG) to estimate productivity. The counties represent three levels of productivity and a range of land costs (annual rental rates) from $128/ha to $534/ha. An optimal rotation age (ORA) was calculated that minimizes the annualized, discounted per-dry Mg biomass cost for each county, genetic group and land cover, and for two discount rates (5 and 10%). The ORA for the lowest-cost county (Todd) with specialist genotypes and a 5% discount rate is 14 years and the breakeven price at that age is $71 dry Mg-1, while for the highest-cost county (McLeod), the generalist genotype and a 10% discount rate the ORA is 10 years and the breakeven price at that age is $175 dry Mg-1. Planting after a previous poplar stand increased breakeven prices and increased the ORAs by 1 to 2 years relative to planting after a previous annual crop. The discount rate has a large impact on optimal poplar rotation ages and breakeven prices. All other factors being equal, an increase in the discount rate from 5 to 10% is expected to reduce ORAs by 2 to 3 years. High-productivity supplysheds can also be expected to have ORAs that are 2 to 3 years shorter than low-productivity ones.

Funding support: This study was funded by the U.S. Forest Service Northern Research Station, Institute for Applied Ecosystem Studies (RWU-NRS-13).

S6 – P39

Key words:
poplar
plantation,
crown
dimensions,
irrigation, weed
control

Comparing crown dimensions of poplar in plantations with different irrigation treatments

Nafiseh Fanaei[1]*, Vahid Etemad*[1]*, Reza Baghery*[2]

[1]Department of Forestry Natural Resources College, University of Tehran, Shahid Chamran blv, Karaj [2]Institute of Forest and Rangelands, P. O. Box 13185 – 116, Tehran, Iran

In SR forestry systems, fast-growing species are grown for high biomass yield. Highest yields are obtained under intensive management systems, including site preparation, weed control, fertilizer application and irrigation. Of these techniques, irrigation and weed control are the main factors. Consequently, it is essential to assess the effects of irrigation and weed control on the successful management of poplar plantations. Namely, water quantity and availability periods have a large influence on poplar vegetation. Different varieties of poplars have either open crown or closed crown which the rate of their dimensions is affected by multiple factors, including irrigation treatments. Therefore, this experiment was implemented in order to determine and compare crown dimensions of six clones—top clones—in two irrigation regimes (eight 12-day periods). This research was done in a poplar plantation of Alborz province (Iran). First, crown diameter was measured and crown area calculated. Then, we defined whether there are any significant differences between clones of two regimes and each regime There were significant differences between them. The results proved different treatments have a remarkable effect on crown extent.

Funding support: University of Tehran and Institute of Forest and Rangelands

INFORMATIONAL SESSION – Oral Presentations

IS – T1

Key words:
science,
taxonomy,
ecosystems,
operations,
environment,
utilization,
sustainability,
international.

‘Poplars and Willows: Trees for Society and the Environment’ – a new publication

Jim Richardson[1] *and J.G. Isebrands*[2]

[1]Poplar Council of Canada, 1876 Saunderson Drive, Ottawa, Ontario, Canada K1G 2C5, [2]Environmental Forestry Consultants LLC, New London, Wisconsin, 54
961 USA

The accumulated global knowledge and information on poplars and willows could fill many volumes. In 1980 the Food and Agriculture Organization (FAO) of the United Nations published a comprehensive volume on poplars and willows. That book is now out-of-date and out of print. As a project of the International Poplar Commission (IPC), a new book entitled ‘Poplars and Willows: Trees for society and the environment’ has recently been produced. It is a co-publication of FAO, under which IPC is a statutory body, and CABI, a UK-based scientific book publisher specializing in agricultural and environmental topics. The book provides a worldwide overview and guide to the basic characteristics, cultivation and use of poplars and willows, as well as issues, problems and trends relating to them. Its 13 chapters, covering 650 pages with 170 black and white illustrations and 48 colour plates, were prepared by 67 contributing authors from 15 countries worldwide. Its contents cover most aspects of poplar and willow taxonomy, ecosystems, physiology, genetics and breeding, production, environmental applications, abiotic stresses, diseases, insect pests, products and markets, as well as their importance for rural livelihoods and sustainable development. It goes beyond the scope of previous publications to include more information on willows, as well as thorough and up-to-date treatment of taxonomic and classification issues, more global reporting, and evaluation of applications to provide industrial, environmental, social and economic development benefits. An outstanding feature of the book is its nearly 2500 references, making it an invaluable sourcebook. The latest knowledge as shown in results of current research on poplars and willows is linked to implementation to meet sustainable livelihoods, land-use and development. This new publication supports the idea that poplars and willows, as trees for society and for the environment, can help us meet future development and environmental challenges sustainably and on a sound scientific basis.

IS – T2

Key words:
Pacific Albus,
lumber, veneer,
plywood, FSC

The product and market development story for Pacific Albus® lumber

Lee Jimerson

Collins, Portland, Oregon, 97201 USA

Also poster
S6 – P40

Pacific Albus can trace its beginning to over twenty years ago in a hybrid poplar pulpwood plantation operation in the Pacific Northwest. Seven years ago, GreenWood Tree Farm Fund (GTFF) was formed and transitioned the plantation to the production of Pacific Albus saw logs under the precepts of the Forest Stewardship Council (FSC). Pacific Albus is marketed by Collins as a low density, light colored sawn lumber. As a GTFF partner, Collins designed and built a state-of-art saw mill to bring out the best in the Pacific Albus log. After two years of testing and studying Pacific Albus lumber and prototyping products, the mill started operations in the fall of 2009. The mill currently produces over six million board feet of lumber monthly. Pacific Albus products include, inter alia, upholstered furniture frames, edge-glued furniture panels, drawer sides, pencil stock, wooden ceiling grid. In the 2014 Winter Olympics, two gold medals and one silver medal were won by athletes skiing on snow boards with Pacific Albus cores. Pacific Albus substitutes for, inter alia, alder, Paulownia, aspen, basswood, incense cedar and yellow poplar. It has superior in-service stability and edge-gluing properties as well. Pacific Albus is ideal for applications that require a light weight wood that has a great weight-to-strength ratio (1:1.16). Its bright appearance makes it ideal for light colored indoor finishes which contribute to indoor lighting quality. Being FSC 100% certified, Pacific Albus can contribute to LEED Materials and Resources credits under the U.S. Green Building Council’s LEED program. Pacific Albus has now become a well-accepted product by manufacturers world-wide where it competes favorably on a cost, value and quality basis.

Funding support: Collins

Index of Authors

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