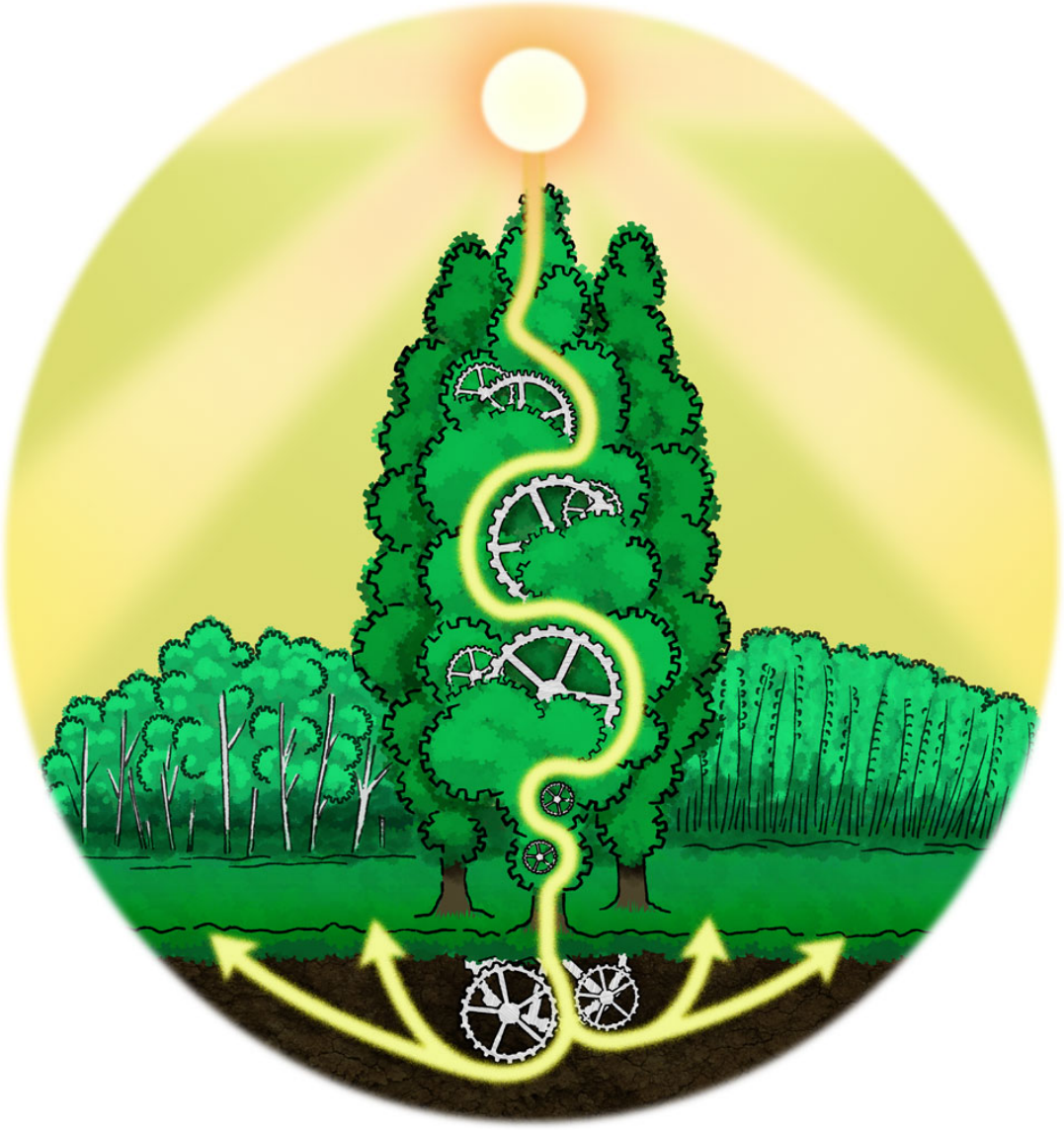
26th New Phytologist Symposium

Bioenergy trees

INRA-Nancy, France



17–19 May 2011

Programme, abstracts and participants

New Phytologist

energy poplar

INRA

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Bioenergy trees

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Organizing committee

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Programme, abstracts and participant list compiled by Jill Brooke.
'Bioenergy trees' illustration by A.P.P.S., Lancaster, U.K.

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Speaker Abstracts

Session 1: Tree genomics
Chair: Brian Ellis

1.1 *Populus* resequencing: towards genome-wide association studies

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Genome wide association studies (GWAS) have been used to identify regions of the genome related to various phenotypes in humans, corn, rice and cattle. Successful application of this approach to bioenergy crops such as *Populus* requires 1) an appropriate mapping population, 2) high-quality phenotypic data and 3) informative genotypic data. With the goal of reducing the recalcitrance of lignocellulosic biomass for economic production of biofuels and understanding basic mechanisms of cell wall formation in Populus we established 4 clonally replicated common gardens experiments each with 1100 native *P. trichocarpa* genotypes collected from along the northwest coast of the U.S. and Canada. A high throughput phenotyping pipeline was developed to measure cell wall chemistry, pretreatment response and enzymatic sugar release. Initially 18 genotypes were resequenced to an average 30X depth in order to design a SNP array to test for statistical association using MMAX and PCA methods of testing among 2500 candidate genes. Genetic structure and linkage disequilibrium (LD) was assessed using SSR and SNP markers. Outlying genotypes were excluded from the analyses and estimates of LD were used to design the bead array. Candidate genes were selected based on QTL intervals, expression profiling within developing xylem and expert opinion. MMAX and PCA results revealed similar significant associations for all measured phenotypes and several SNPs within the candidate gene set explain a relatively high degree of the phenotypic variance. As a result, resequencing has continued in order to conduct GWAS in *Populus*; the complete set of 1100 genotypes will be complete in 2012.

1.2 DNA methylation in the poplar genome

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To describe genome-wide methylation patterns during development in the reference tree and bioenergy species *Populus*, we performed methylated DNA immunoprecipitation followed by Illumina sequencing (MeDIP-seq) with seven *P. trichocarpa* tissues. The tissues included roots, leaves, phloem, xylem, male and female catkins, and dormant buds. A total of 64 Illumina lanes were sequenced, representing 1 to 3 biological replicates for each sampled tissue. Numbers of reads obtained ranged from 16M – 125M per tissue type. Reads aligning to unique positions in the reference genome covered ~30% of genome space. Average sequence depth within covered regions varied by tissue type, ranging from 4 to 12 reads/nucleotide. Numbers of significantly methylated tiled 1Kb genome windows called by RPKM calculations at a 1% false discovery rate varied by tissue type, ranging from approximately 2,000 (xylem) to 40,000 (buds). In all tissues, transposons and other repeat elements were enriched relative to their overall representation in the genome, with LTR-gypsy retroelements being the most highly enriched transposable element type. Gene methylation exhibited a pattern of higher methylation at promoters, middle of coding region, and 3' UTRs relative to 5' and 3' ends of coding regions. Numbers of methylated genes varied by tissue type and gene region considered, and represented 3-5% of the genes in the genome. We have developed a customized genome browser (Gbrowse version 1.69) at which our data can be explored: http://poplar-dev.cgrb.oregonstate.edu/cgi-bin/gbrowse/poplar_v2/.

1.3 Reconstructing the poplar pan-genome

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The analysis of variation in plants has revealed that their genomes are characterised by high levels of structural variation, consisting of both smaller insertion/deletions, mostly due to recent insertions of transposable elements, and of larger insertion/deletion similar to those termed in humans Copy Number Variants (CNVs). These observations indicate that a single genome sequence might not reflect the entire genomic complement of a species, and prompted us to introduce the concept of the plant pan-genome, including core genomic features common to all individuals and a Dispensable Genome (DG) composed of partially shared and/or non shared DNA sequence elements. The very active transposable element systems present in many plant genomes may account for a large fraction of the DG. The mechanisms by which the CNV-like variants are generated and the direction of the mutational events are still unknown. Uncovering the intriguing nature of the DG, i.e. its composition, origin and function, represents a step forward towards an understanding of the processes generating genetic diversity and phenotypic variation. Additionally, since the DG clearly appears to be for the most part the youngest and most dynamic component of the pan genome, it is of great interest to understand whether it is a major contributor to the creation of new genetic variation in plant evolution and more specifically in the breeding process. We will present an analysis of the pan genome in poplar looking both at variation among species as well as within species.

1.4 Breeding by genomic selection: capturing the missing heritability of complex traits in forest trees

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Genomic selection (GS) involves selection decisions based on GEBV (Genome Estimated Breeding Values) estimated as the sum of the effects of genome-wide markers capturing most variation for the target trait(s). GS captures the "missing heritability" of complex traits in forest trees beyond the few effect variants that association genetics typically identifies. GS accuracies should match phenotypic BLUP-based-accuracies even with low-density marker panels (2-3 markers/centiMorgan) in populations with effective sizes Ne≤ 60. Genotypes at ~3,500 DArT markers and de-regressed phenotypes for Height (H), Diameter at Breast Height (DBH) and wood density (WD) were obtained in two Eucalyptus breeding populations (CEN and FIB) with contrasting effective sizes (Ne=11; Ne=55). Realized GEBV accuracies for H and DBH were 0.67 and 0.69 for CEN and 0.62 and 0.54 for FIB; 0.53 for WD in FIB. Not surprising GEBV accuracies were low (~0.18) across populations implying variable genotype-phenotype associations across backgrounds so that population-specific GS models will be necessary. GS-based reduction in breeding time by 50% should provide gains ≥100% in selection efficiencies. With advances in genotyping-by-sequencing methods together with increasing numbers of independent GS studies in forest trees, the perspectives are that GS might soon cause a paradigm shift in forest tree breeding practice.

Session 2: Optimised yield for bioenergy trees
Chair: John Ralph

2.1 Branching in short rotation coppice willow

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Willows (Salix spp.) are among the most advanced biomass crops in temperate regions because of their potential for high yields in short growth cycles, ease of vegetative propagation, broad genetic base and ability to resprout after multiple harvests. Biomass willows are grown in short rotation coppice cycles in which the planted cuttings are cut back after an initial year of growth to promote a coppicing response in the following spring, and plants are subsequently harvested every 2-3 years. Coppicing appears to re-invigorate growth and help maintain biomass yields. The numbers of shoots which grow out from the base (stool) influences the architecture of the canopy and characteristics of the stem (e.g. diameters, composition). However, despite its central importance, little is known about the genetic regulation of coppicing and shoot architecture in willow. This presentation will review our findings from several projects in which these aspects are being investigated. These include investigation of the role of the *more axillary branching (MAX)* genes in willow and BSBEC-BioMASS where canopy and light interception, as well as biomass composition, are being related to stem characteristics. Our results so far are helping to dissect the complex genetic regulation of branching control and are revealing different growth strategies utilised by willows in attaining efficient light interception and high biomass yields.

2.2 Poplar genomics and improvement of poplar as a cellulosic biofuel

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Populus trichocarpa (black cottonwood) and *P. balsamifera* (balsam poplar), are native to North America where large natural populations exist. *P. trichocarpa* has an extensive north-south range along the west coast, while *P. balsamifera* has a northerly range across the continent from Alaska to the North American east coast. Both species are well suited for highly productive bioenergy biomass plantations in north-temperate climates. We are investigating the large reservoirs of natural genotypic and phenotypic in wild populations of these species, with an initial focus on *P. trichocarpa*, to identify allelic variation underlying optimal biofuel and biomass traits that could be used for accelerated domestication. We sampled genetic variation in *P. trichocarpa* by Illumina transcriptome resequencing of 20 individuals from provenances along a latitudinal gradient. This analysis revealed extensive expression and splicing variation in 10,000 xylem-expressed genes. Alignment of transcript sequences to the Nisqually-1 reference was used to identify over 500,000 SNPs. These SNPs were combined with a larger SNP set generated by whole genome resequencing carried out by the US DOE Bioenergy Sciences Center to generate an Illumina Infinium bead array for genotyping 38,000 SNPs in 3,700 candidate genes. In parallel, we carried out extensive phenotyping, which revealed high variation in cell wall, fiber quality, biomass, and physiological traits in collections of 700 accessions grown in common gardens. Population genetic analysis of the SNP data, its use in an association study to identify candidate gene alleles underlying variation in biofuels, biomass, and adaptive traits, and the prospects for marker assisted breeding will be discussed.

2.3 Leaf development for bioenergy yield in poplar: QTL, genes and association genetics

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Leaves are the primary site of carbon capture and fixation. As such, it is well established that leaf area development and biomass productivity are linked, but the use of leaf development as a predictor of biomass yield for bioenergy breeding and improvement programmes is limited, partly because there is little understanding of the genomic and genetic mechanisms underpinning leaf development in bioenergy trees such as poplar. In this paper we will summarise the evidence that links biomass yield to leaf traits and propose that cell production rather than cell expansion is a key characteristic determining leaf size. In an F_2 mapping population we have identified a set of QTL for leaf and cell traits in several environments. Underlying candidate genes were identified from the physical sequence of *P. trichocarpa* and a set of shoot apical meristem genes were identified from the literature, including *ASYMETRIC LEAVES1 (AS1), ASYMETRIC LEAVES2 (AS2), ANGUSTIFOLIA (AN), E2F* and *PHABULOSA (PHAB).* These were utilised in a wide association population study of black poplar, *P. nigra*, where associations between SNPs in candidate genes were found between shoot apical meristem patterning genes and biomass yield. The nature and importance of these associations will be discussed.

2.4 How do poplars deal with nitrogen?

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Poplars are fast-growing tree species, whose cultivation as feedstock for bio-energy production is of increasing interest. In this context the molecular physiology of nitrogen utilization needs to be addressed in the ecological and agronomic context of poplar cultivation. In soils nitrogen is available in different forms such as ammonium, nitrate or amino acids. Depending on habitat, the dominant nitrogen form changes. Poplars can use different N-forms but show different growth responses. To disentangle the role of ammonium and nitrate nutrition for wood formation in poplar, we analyzed N-responsive transcriptional networks and physiological performance. In practical terms, the exploitation of the full growth potential of poplars may require application of nitrogen-containing fertilizers. High nitrogen availability stimulates growth and biomass production, but it also affects wood properties such as cell composition and density. The influence of such treatments on the energy gain will be addressed. Furthermore, excess nitrogen may result in negative effects on mutualistic microorganisms such as mycorrhizas with consequences for plants stress tolerance. Links between ecosystem processes and N physiology will be discussed.

2.5 4-Coumaric acid:coenzyme A ligase 1 (4CL1) can identify and distinguish the *cis*- and *trans*- structures of its substrate 4-Coumaric Acids

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4-coumaric acid:coenzyme A ligase 1(4CL1) is one of the important key enzymes in the lignin biosynthesis pathway and catalyzes the reaction of the formation of phenolic acid: Co-enzyme A esters. 4-coumaric acid is a natural secondary metabolite of plants and one of the substrates of the 4CL enzyme. Both trans- and cis- isomers of 4-coumaric acids are found in the nature and transformed into each other under the illumination conditions. To investigate the properties of trans- and cis- substrates preferability of the 4CL1 enzyme, 4CL1 gene was cloned from the *Populus tomentosa* and prokaryotic heterogonous over-expressed in *E.*coli strain. The over-expressed 4CL1 enzyme was purified by one-step Ni-affinity column and applied to the experiment. Commercially purchased 4-coumaric acids are trans isomer products. Cis-coumaric acid was prepared by photoisomerization from trans-coumaric acid and purified by preparative HPLC on a C18 column. The results showed that the 4CL1 enzyme could distinguish/discriminate the trans- and cis- conformation of the coumaric acid and only catalyzes the trans-coumaric acid into its CoA ester. The products of the enzymatic reaction were on-line detected and characterized with the HPLC-ESI-MS. Both the substrate 4-coumaric acid and the product 4-coumaric acid:CoA ester can be photoisomerized from one state to another. The result of the HPLC chromatograph behavior on a C18 column implied that the 4CL1 enzyme uses the trans-coumaric acid as the substrate and produces a cis-p-coumaric acid:CoA ester. The ESI-MS fragmentation mechanism of the 4-coumaric acid, CoA and 4-coumaric acid:CoA ester is meanwhile presented in this work.
Keywords: trans-coumaric acid, cis-coumaric acid, 4CL1 gene and enzyme, photoisomerization, HPLC-ESI-MSn.

The abbreviations used are: 4CL, 4-coumaric acid:CoA ligase;CA, coumaric acid; HPLC, high performance liquid chromatography; GC, gas chromatography; MS, mass spectrometry; IR, infrared spectra

[*]The contributions of these authors are equal in this work.

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Session 3: Physiological and molecular control of wood formation
Chair: Jerry Tuskan

3.1 Analysis of cell signalling during vascular morphogenesis in Arabidopsis and *Populus*

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Vascular plants have a long-distance transport system consisting of two tissue types, phloem and xylem. The cell lineages of the root vascular cylinder harboring phloem and xylem and the intervening procambial tissue originate from stem cells near the root tip. We and others have taken a combination of genetic and genomic approaches to understand how the specification of vascular cell lineages is determined at a molecular level. We have recently identified AHP6, an inhibitory pseudophosphotransfer protein for cytokinin signaling as a spatially specific regulator facilitating protoxylem specification (Mähönen *et al*. Science 311, 94). Subsequently, we have identified two regulatory interactions that specify the vascular pattern through interaction with cytokinin signalling. We have shown that cytokinin and auxin interact in a spatially specific manner during procambial development. Furthermore, in collaboration with the laboratories of Philip Benfey, Ji-Young Lee and Annelie Carlsbecker, we have also shown that the miR165/6 species act non-cell autonomously to regulate the differential gene dosage of the class III HD-ZIP genes at the central and peripheral position of the xylem axis (Carlsbecker, Lee *et al*. Nature 465, 316). The molecular mechanisms of movement of the various signals between cells are discussed. Furthermore, the role of this spatial regulation of cytokinin in Arabidopsis and *Populus* is discussed.

3.2 Physiological and molecular control of wood formation

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Secondary cell walls in wood are very plastic in their chemotype and structural patterning. This shows on a large potential for molecular breeding towards feedstock genotypes with different and designed properties. However, it also highlights the advantage to characterize not only chemical composition, but also cell wall structure and microchemistry to understand gene function in mutant analysis. I will present recent advances in wood chemotyping using Py-GC/MS and how this is applied in large-scale phenotyping of a collection of aspen transgenic trees. I will also present the use of FT-IR microspectroscopy technique for cell specific chemotyping and chemical imaging. In both cases multivariate tools have been instrumental for data analysis. Finally the role of wood expressed fructokinase and sucrose synthase in wood cell wall biosynthesis will be discussed. Both are key enzymes in primary sugar metabolism, leading to the supply of wood polymer precursors and energy for cell wall biosynthesis. Downregulation of these enzymes primarily result in a relative decrease in the proportion of cellulose and hemicellulosa, but also in overall wood biomass and cell wall structure. Both enzymes seem to be critical for carbon supply to wood cell walls and result in similar chemotypes.

3.3 Lignin biosynthesis pathways

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Global warming, environmental disasters, and increasing oil prices have catalyzed a worldwide trend to use plant biomass as a renewable source for liquid biofuels and bio-based materials. Plant biomass can be processed into bio-ethanol by enzymatic depolymerization of the cell wall polysaccharides into simple sugars, followed by fermentation. However, the presence of lignin in the cell wall is an important recalcitrance factor. One approach to overcome this hurdle is to engineer lignin amount or alter its composition to make lignin more susceptible to chemical degradation. Down-regulation of cinnamoyl-CoA reductase (CCR) in poplar results in reduced lignin content. Field trials have been initiated under short rotation coppice culture in Belgium and France to evaluate their potential as raw material for bioethanol production. The latest results from these trials will be presented. Furthermore, we have used a systems biology approach in Arabidopsis to study how the lignin biosynthetic pathway is regulated and how it integrates into the wider plant metabolism. The results clearly demonstrate that perturbations of lignin biosynthesis have wide-ranging consequences on various metabolic processes. These metabolic reactions of the plant can be used to identify novel biosynthetic routes and the genes involved in them.

3.4 Variability in poplar cell wall traits: opportunities for improvement in bioenergy production

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Photosynthetic carbon capture by terrestrial plants represents a major sink for atmospheric CO_2, ultimately terminating in the synthesis of a secondary plant cell wall – a complex matrix of polysaccharides intricately linked to lignin. The production and coordinated deposition of this lignocellulosic composite confers both protective and structural properties to the plant cell. These same inherent properties also represent a major obstacle for its effective use as a lignocellulosic substrate in biofuels production. This paper will discuss genomic strategies to overcome these limitations, and the inherent natural phenotypic diversity in a range-wide collection of >500 *Populus trichocarpa* that could form the basis for the selection of desired genotypes for future bioenergy applications. The phenotypic variability (including wood chemistry, wood ultrastructure, growth parameters and physiological tree attributes), will also be employed to establish associations between alleles of genes that may control lignocellulosic cell wall attributes and lignocellulosic biofuels traits. This information could ultimately be used to facilitate breeding and selection strategies for the optimization of future lignocellulosic feedstocks dedicated to bioenergy production.

3.5 TREEFORJOULES, a Plant KBBE project to improve eucalypt and poplar wood properties for bioenergy

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TREEFORJOULES is a Plant KBBE project starting in April 2011 and gathering 13 research groups from public and private organisations from France, Germany, Portugal, and Spain. The overall goal is to identify the major factors underpinning the physicochemical properties of cell walls, the recalcitrance of which remains a key scientific challenge for establishing highly efficient, sustainably produced, second-generation biofuels. This knowledge will be invaluable for breeding fast-growing elite trees such as poplar and eucalypts for improved down-stream processing and efficient degradation. TREEFORJOULES aims are to:

- Identify and characterize the regulatory candidate genes (*i.e.* transcription factors and miRNAs) that control wood properties relevant to bioenergy through integration of existing and new transcriptomic resources, delineation of the transcriptional interactome, functional characterization of candidate genes (CGs) in transgenic wood sectors, assessment of environmental and seasonal impacts on CGs expression and correlation with biomass production of high-performing genotypes.
- Develop high-throughput phenotyping methods for key wood and cell-wall chemical constituents, assess their impact on saccharification, bioethanol and bio-oil production, and develop and apply micro methods for phenotyping of transgenic wood tissues.
- Delineate and characterise genomic regions in eucalypts and poplar that control wood properties valuable for efficient cellulosic bioenergy production through comparative analyses at both the structural (comparative genetic and physical mapping) and functional (comparative QTL mapping) levels.

3.6 Altering lignin biosynthesis for improved biomass processing

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Lignin remains one of the most significant barriers to the efficient utilization of cellulosic substrates, in processes ranging from ruminant digestibility to industrial pulping, and in the current focus on biofuels production. Up- and down-regulating genes for enzymes in the monolignol biosynthetic pathway can produce at times striking alterations in lignin composition and structure that may positively or negatively impact a given processes. A few approaches hold considerable promise for reducing the severity and energy demands of various processes. At the same time, we are gaining some insight into what features are required by ideal lignin monomers and are beginning explorations into possible lignin monomer replacements. And now that monomer substitution in the lignification process is well authenticated in various transgenic plants, it is opportune to begin explorations into actually designing lignins to improve the ease with which they can be removed from the cell wall. Here we highlight the logic behind one approach, our idea of utilizing monolignol conjugates to introduce readily (industrially) cleavable bonds into the backbone of the polymer. Already, model-cell wall studies have shown dramatic improvements in processing efficiency, reducing the temperature required for pulping, for example. Successfully engineering plants to incorporate such monomer conjugates, or other monomer replacement strategies, therefore has the potential to vastly reduce the energy demands of processing.

3.7 Novel modes of tubulin regulation in *Populus*

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Cortical microtubules are composed of alpha- (TUA) and beta-tubulin (TUB) heterodimers whose polymerization and depolymerization drive the microtubule dynamics thought to underlie cellulose microfibril deposition. The *Populus TUA* and *TUB* families exhibit several characteristics not reported in other species. The *TUB* family is disproportionately expanded relative to the *TUA* family. The C-termini of the encoded proteins are unusually hypervariable, which may reflect as yet undiscovered post-translational regulation of microtubule dynamics. For these reasons, we are analyzing tubulin function in transgenic *Populus* with perturbed ratios of *TUA* and *TUB* expression, as well as ectopic expression of tubulin post-translational modification (PTM) mimics. Xylem-abundant *TUA* and *TUB* genes or their PTM mimics were co-transformed into *Populus* in various combinations. Transformation efficiency, organogenesis frequency, effect on vascular development and lethality varied depending on the transgene combinations. Only two of eight combinations attempted led to whole-plant regeneration, and interestingly, both involved PTM mimics. The transgenic trees appeared morphologically normal, except for mature leaves which displayed a range of ontogenetic epinasty phenotypes. The transgenic effects will be discussed in the context of tubulin PTMs, and target wood properties in the transgenic plants.

Session 4: **Gene association and breeding for domesticated bioenergy poplar**
Chair: Michele Morgante

4.1 Dissecting the genetic basis of growth in European aspen (*Populus tremula*)

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The initiation of growth and dormancy represents critical ecological and evolutionary trade-offs in perennial plants and and growth as been shown to vary with important phenological traits in many plants. In European aspen (*Populus tremula*) the most important environmental cue regulating the transitions between growth and dormancy are changes in photoperiod. QTL mapping have implicated genes in the photoperiodic pathway in the control of growth and the transition to dormancy. Here we present data from a study on the genetic basis of variation in growth in European aspen (*Populus tremula*) across a latitudinal gradient. We show that growth is intimately linked to seasonal changes in phenology. Despite low levels of genetic differentiation at SNP markers putatively involved with regulating phenology and growth, we found several significant associations between naturally occurring variation in genes from the photoperiod pathway and genetic variation in phenology and growth.

4.2 Accelerating the domestication of bioenergy trees: from genetical genomics to genomic selection

MATIAS KIRST
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Growing worldwide demand of wood products for bioenergy and increasing evidence of climate change creates a pressing need for the development of more productive germplasm that is adapted to existing and novel sources of biotic and abiotic stress. To address these needs we are deploying advanced tree breeding approaches based on genomic selection to rapidly identify individuals with allelic combinations that are optimized for bioenergy production. Genomic selection prediction models developed for a breeding population of loblolly pine show very high (~75%) accuracies for biomass growth and composition traits and gains of over 100% in selection efficiency are achievable. By combining genomic selection with advanced methods of flower production and vegetative propagation, the timeframe for loblolly pine breeding and seedling production can be reduced from decades to only a few years. In parallel, we are dissecting the genetic regulation of bioenergy traits by integrating genomic and genetic information to identify their regulatory genes. In this effort we have targeted carbon partitioning, hydraulic conductivity and biomass growth traits, defining quantitative trait loci and their putative regulators. Here I will discuss our progress in applying genomic selection and genetical genomics to improve bioenergy traits in pines and poplars.

4.3 Breeding of bioenergy-domesticated poplars

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Poplar breeding programs around the world have achieved substantial increases in growth and yield potential through careful combination of intra/interspecific hybridization and clonal selection. Major challenges of future poplar breeding for bioenergy use include continuous genetic gains for recognized economic traits while addressing new breeding goals and optimization of large-scale deployment of the selected genetic variation. To meet these demands for both adaptive and productivity traits, poplar breeding programs require more optimal short and long term management of genetic diversity gathered in breeding populations and deployed according to different cultivation schemes.

Breeding strategies specifically devoted to the development of high performing poplars for bioenergy are facing the following challenges:
(1) Definition of more adapted ideotypes for different cultivation systems and new environments and optimization of multi-trait selection in case of trade-offs and high levels of phenotypic plasticity
(2) Estimation of part of genetic variation and co-variation in cell wall chemistry and wood anatomy to match the range of conversion technologies
(3) Exploration of genetic variation available in the different gene pools and potential interest of inter-specific hybridization for biomass quality which is inescapable to reach maximum biomass yield
(4) Management of targeted and non targeted genetic diversity to control risks associated to clonal forestry and more specifically pest resistances in intensive production systems
(5) Shortening of breeding and evaluation phases to quickly adjust poplar resource to the bioenergy demand.

In the next future, the true challenge for manipulating the complex nature of selection goals linked to bioenergy use will depend on our ability to accurately phenotype poplars in well chosen environments and to connect these phenotypic performances with the overwhelming amount of DNA base information thanks to efficient and user-friendly tools.

Session 5: Saccharification and life-cycle analysis
Chair: Gail Taylor

5.1 Production of fuel ethanol from softwood by simultaneous saccharification and fermentation

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Production of bioethanol from renewable sources is one way to meet the demand for alternatives to fossil fuels. Currently, commercial bioethanol is mainly produced either from sugar-based materials, e.g. sugarcane and sugar beet, or from starch-containing materials such as corn or wheat. However, further expansion of bioethanol production requires the use of lignocellulosic biomass, including forest and agricultural residues, e.g. softwood. This so-called "second-generation" bioethanol, has the advantage of coming from an abundant renewable source, which do not compete with food. The lignocellulosic materials contain cellulose (30-60%) and hemicellulose (15-30%), which need to be hydrolyzed before fermentation to bioethanol. A process based on enzymatic hydrolysis and fermentation is today regarded as the most feasible option in converting the carbohydrates in biomass to ethanol with high yields and low production cost. However, it is usually necessary to perform some form of pretreatment to access these polymers. Treatment of chipped biomass with high-pressure steam, especially with addition of a small amount of sulfuric acid or sulphur dioxide, has been shown to be a successful method of pretreating several lignocellulosic materials prior to enzymatic hydrolysis and fermentation.

A high ethanol concentration (above 4wt%) prior to distillation is crucial for the overall process economics. This could be achieved using high-solids loadings during hydrolysis and fermentation, but the influence of inhibitory substances have to be considered. Cultivating the yeast on the hydrolyzate obtained from pretreatment before fermentation as well as fed-batch addition of substrate in fermentation are two methods to reduce the inhibition problem. However, to produce ethanol from lignocellulose in a cost-efficient way, it is also essential to utilize all components of the material. Ethanol can be produced from the carbohydrates while the remaining soluble organic compounds and solids (lignin) can be utilized to produce co-products such as biogas, electricity and district heating. This way the revenues of the process increases and ethanol production cost is reduced. This presentation will address the latest results obtained at Chemical Engineering, Lund University on production of bioethanol from spruce.

5.2 Life cycle analysis of bioenergy poplar

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Liquid biofuels, especially bioethanol, provide one of the few options for fossil fuels substitution in the short to medium-term. Lignocellulosic resources are receiving special interest as potential feedstocks, driven by concerns over high fuel prices, security of energy supplies, global climate change as well as the search for opportunities for rural economic development. Poplar, as short or very short rotation coppice (SRC or VSRC), is an important potential feedstock for the production of lignocellulosic bioethanol. The EC FP7 EnergyPOPLAR project is in progress to create improved Poplar trees specifically designed for this purpose. To gain an understanding of the potential environmental impact of using Poplar for bioethanol production, using both current and future feedstocks and conversion technologies, a scenario-based Life Cycle Assessment (LCA) was conducted. Twenty-four different scenarios were developed to represent possible combinations of EU locations (North, South, East and West Europe), cultivation methods (SRC or VSRC) and years (2010, 2020 and 2030). The environmental impact of each scenario was compared with conventional gasoline ('petrol').

The LCA outcomes indicate:-

- Significant reductions in greenhouse gas emissions compared with petrol for all poplar bio-ethanol scenarios, this benefit increasing significantly from 2010 to 2030 as improved feedstock and processing comes on-stream.
- Under 2010 conditions, the production and use of bioethanol from Poplar had a greater acidification and eutrophication potential than petrol in all scenarios. Under 2020 and 2030 conditions however, the acidification potential of all scenarios was below the level of that for petrol, as was the eutrophication potential of all SRC scenarios, where fertiliser was not used in the cultivation stage.
- Western Europe, particularly the centre region of France, was the location where Poplar derived bioethanol production exhibited the lowest environmental impact.
- Improvements in biomass yield bring a greater environmental benefit in VSRC scenarios than in SRC scenarios, as the former show higher impact at cultivation stage.
- Although using current conversion technology, an increase in the cellulose content of Poplar would decrease environmental impact, the reverse may be true with efficient pentose fermentation in the future. This is a consequence of the impacts caused by the larger quantities of cellulase enzymes required to break down the additional cellulose (the hemicelluloses are usually hydrolysed during the modelled dilute acid pretreatment phase).
- Research into the structure of Poplar biomass should focus on manipulating the cell wall composition to allow for easier conversion requiring lower inputs of process chemicals and enzymes.

Poplar derived bioethanol has the potential to play a significant role in reducing the greenhouse gas emissions from road transport in the EU. The LCA model is being used to develop ongoing insights into SRC poplar biofuels within the EnergyPOPLAR project and to maximise environmental benefit from implementation of this technology.

5.3 Life cycle analysis of cellulosic ethanol

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Increasingly biofuels have been under intense scrutiny for claims of reduction in fossil fuel consumption, carbon dioxide emissions and benefits to environment. Many studies indicate that some of the issues such as food vs. fuel debate, intensive use of agricultural inputs such as fertilizers and pesticides, low net energy balance, and uncertain environmental impacts are partially addressed by cellulosic ethanol. However such conclusions are not without controversies. It is therefore imperative that potential benefits and tradeoffs of cellulosic ethanol be investigated comprehensively using process modeling, techno-economic analysis and attributional life cycle assessment (LCA).
In this presentation, overall energetic assessment methods will be discussed. Effect of choice of production practices and processing technologies on the energy use and life cycle emissions of greenhouse gases will be addressed. Life cycle assessment of cellulosic ethanol production in conjunction with possible improvements to LCA methodology by incorporating systematic boundary definition methods and water use will be discussed.
Specific case studies for cellulosic ethanol from agricultural residue in Pacific Northwest US will be discussed. Effect of pretreatment process technologies on energy use, techno-economic analysis and LCA for different pretreatment technologies will be discussed to demonstrate the importance of including realistic process data, systematic boundary definitions and incorporating water use in addressing the questions about energetic, economic and environmental sustainability of cellulosic ethanol.
Keywords: Cellulosic ethanol, techno-economic analysis, life cycle assessment, water use, and grass straw.

Poster Abstracts

Listed alphabetically by first author, presenting author is underlined.

1. Identification and characterization of class III peroxidases in *Eucalyptus globules*

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Class III peroxidases are heme-containing proteins present as large multigene families in all land plants but absent in unicellular green algae. In *Arabidopsis* 73 genes are annotated as peroxidase-encoding genes, whereas rice contains 138 members and approximately 200 in maize. Moreover, additional isoenzymes can be produced by post-transcriptional and post-translational modifications increasing the complexity of these proteins. Class III peroxidases are implicated in a broad range of physiological processes such as lignin and suberin formation, auxin metabolism, cell elongation, plant defence reactions, as well as the generation of reactive oxygen species. However, the role *in planta* of most peroxidases remains elusive. *Eucalyptus globulus* is an economically important plant for cellulose industry. Bioinformatic analyses of Brazilian EST-eucalyptus projects returned 83 contigs homologous to characterized class III peroxidases. Using electronic northern analyses candidate genes were selected and their expression assessed by RT-PCR in an induction system based on germinating seedlings exposed to light and sucrose. Peroxidase activity was accessed *in situ* by using TMB and *in vitro* by using guaiacol, syringaldazine and coniferyl alcohol. Spots from guaiacol activity staining electrophoresis gels were used for peptide sequencing by LC-ESI-Q-Tof. The results are discussed in the light of lignin biosynthesis.

2. Biomass yield and vigor of transgenic non-isoprene emitting poplars outdoors in small scale cage-greenhouse cultivation

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Plants emit a variety of biogenic volatile organic compounds with isoprene being the dominant compound. Despite its importance as a significant carbon loss for the plant, the function of isoprene in plants is still under discussion. Different studies provided details that emission of isoprene protects against oxidative stress.
We conducted a long-term outdoor study with transgenic non-isoprene emitting *Populus* x *canescens* plants where isoprene synthase gene expression was knocked-down by RNAi technology. We found that repression of isoprene emission in poplar has no negative impact on growth and biomass yield under temperate climate. Model calculations revealed that the annual release of isoprene from poplar amounted to 2.2% of the total gross primary production. In comparison to non-isoprene emitting plants, isoprene emitters showed 6.9% reduced net growth. It remains to be determined whether the reduced loss of carbon by isoprene is responsible for the increased growth of the non-emitting plants. Additionally, non-isoprene emitting poplars exhibited different sensitivity to pests/pathogens.
In summary, isoprene emission has functions in poplar stress tolerance, amounts to a significant proportion of carbon budget and influences pest/pathogen interactions. These diverse roles need to be more addressed in future with respect to increasing importance of poplar biomass plantations.

3. Effects of nitrogen fertilization on Eucalyptus wood properties

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Eucalyptus species are the most widely planted hardwood trees in the world, representing more than 3,7 Millions ha in Brazil. Their fast growth rates and wide adaptability could allow sustainable and cost-efficient production of lignocellulosic bioenergy. The main limitation is wood recalcitrance to degradation which is linked to cell wall's structure and composition. Lignins, for example, impair the accessibility of cellulose during bioethanol fermentation. The application of nitrogen fertilizers is one strategy to increase growth rates and productivity, but little is known about the effects on wood quality. In poplar, it was recently reported that N fertilization increases aerial biomass, while in wood, an increase in cellulose coupled with a decrease in lignin were observed. Here, we present preliminary results of the effects of nitrogen fertilization on Eucalyptus wood properties and gene expression. We set up an experimental system in which rooted cuttings of *Eucalyptus urophylla* x *grandis* were fertilized during 30 days with three different amounts of N (limiting; adequate; luxuriant). The effects of N fertilization were studied at the phenotypic level including histochemical analysis for lignin and/or cellulose and at the whole transcriptome level using Illumina mRNA-Seq technology and *de novo* assembly. Finally, the saccharification potential was evaluated.

4. MicroEGo- Identification of miRNA in *E. globulus* xylem tissues formed upon gravitropic stimulation

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The understanding of the molecular mechanisms underlying cell wall biosynthesis is of great importance not only for the prospects of future production of pulp and paper, but also for the production of bio-fuels and bio-materials. Many of the genes involved in wood formation have been catalogued amassing a huge potential of knowledge, however the mechanisms in which they act, interact and regulate to determine this complex process of development are still far from being elucidated.

The microEGo project (FCT grant PTDC/AGR-GPL/098179/2008) aims identifying and characterizing *E. globulus* miRNAs and their target genes, involved in the regulation mechanisms of wood formation, using as a model the tension wood forming tissues.

NGS was used to sequence SmallRNA libraries generated from differentiating xylem (DX) samples collected on the upper side (tension wood), lower side (opposite wood), from *E. globulus* bent trees, and also in non-bent trees (control wood). Sequencing data were analyzed to identify and quantify putative miRNA. Additionally, miRNA and putative target genes identification was seeked by establishing sequence homologies against miRNA publicly available databases. Additionally, genome wide bioinformatics tools were also used to identify *in silico* miRNA gene and putative target genes.

These new genomic resource for *Eucalyptus* provides us with new insights into the nature of the molecular machinery involved in tension wood formation and most importantly with the identification of players involved in the variability of wood characteristics.

5. BSBEC-BioMASS – Selecting traits to optimise biomass yield of SRC willow

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Second generation biofuels produced from perennial biomass crops could help reduce dependence on fossil fuels. BSBEC-BioMass is the Perennial Bioenergy Crops Programme of the BBSRC Sustainable Bioenergy Centre (BSBEC) which focuses on improving SRC willow and Miscanthus. Here only the SRC willow research is reported. Work stream 1 of BSBEC-BioMASS aims to increase biomass yield in SRC willow through the identification and manipulation of key processes involved in dry matter production and partitioning. Three routes are being investigated: First, extending canopy duration by looking at the timing of bud flush and senescence; second, maximising carbon fixation via altering crop architecture; and third, selecting for an optimal allocation of above ground (harvested) and below ground (reserve) carbon. BSBEC-BioMASS utilises existing crop resources alongside a dedicated trial planted in 2009 containing four genotypes of willow (and Miscanthus). The trial is designed to provide intensive destructive and non-destructive measurements. Results from the first year show that the genotypes are already displaying differences in architecture, biomass allocation, bud flush and timing of senescence. In addition, profiling of the forms and locations of stored carbon over the growing season in photosynthetic and non- photosynthetic parts is currently underway. In the long-term, key traits associated with biomass yield will be identified for detailed genetic analysis using existing mapping populations.

6. Profiling and localization of phenolics in Arabidopsis lignin mutants

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Lignin, the second most abundant biopolymer on earth, delivers strength and hydrophobic properties to the cell wall allowing the plant to grow upwardly and to transport water, photoassimilates and nutrients. Nevertheless, this aromatic polymer obstructs agro-industrial processes such as the production of pulp and paper from wood, the digestibility of fodder crops and the production of bio-ethanol from lignocellulosic material. A more easily degradable lignin structure, e.g. by introducing easily cleavable units, would alleviate these problems. This could be done by rerouting metabolites that are interesting candidate lignin monomers, to the cell wall. In a first step, this involves the search for interesting phenolics and their subcellular localization. In our study, we profiled the phenolics in a set of Arabidopsis lignin mutants using reversed phase ultrahigh performance liquid chromatography coupled to Fourier transform ion cyclotron resonance mass spectrometry (UHPLC-FT-ICR-MS). Additionally, because most phenolics are transported to the apoplast or the vacuole, we are in the process of profiling the protoplasts and the vacuoles isolated from these mutant lines. The data shows how blocking lignin biosynthesis at alternative steps leads to the accumulation of distinct sets of metabolites in the mutants.

7. Plant microbe interfaces: defining and understanding the relationship between *Populus* and its microbiome

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Plant–microbe interactions can benefit plant health and biomass production by affecting nutrient uptake, influencing hormone signaling, effecting water and element cycling in the rhizosphere, or conferring resistance to pathogens. Studying the integral plant–microbe system in native, perennial plant environments, such as *Populus* and its associated microbial community, provides an excellent opportunity for discovering plant–microbial system functions relevant to bioenergy and carbon-cycle research and the understanding of ecosystem processes. Bacteria and fungi can be found within *Populus* tissues and closely associated with the roots in the rhizosphere. In an effort to comprehensively define *Populus'* microbiome, root and rhizosphere samples from *P. deltoides* growing within upland and lowland sites near the Caney Fork River in central Tennessee, USA were collected and analyzed by 454 pyrosequencing. Endophytic bacterial diversity was found to be highly variable, and on average was tenfold lower than the rhizosphere, suggesting root tissues provide a distinct environment that supports relatively few species. Both fungal and bacterial rhizosphere samples showed distinct phylogenetic composition patterns compared to the more variable endophyte samples. The *Populus* host environment may have a stronger influence than the surrounding soil environment in shaping the microbial community. Additional details related to these phylogenetic analyses will be presented.

8. High-level soluble prokaryotic expression of *Pt4CL1* gene and its immunofluorescent localization in *Nicotiana tobacum*

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4-coumarate coenzyme A ligase (4CL), one of the key enzymes of lignin biosynthesis in vascular plants, plays an important role in cell wall formation. In order to investigate the enzymatic properties of 4CL1 protein of *Populus tomentosa* and its localization, high-level prokaryotic expression of *4CL1 gene of Populus tomentosa* (*Pt4CL1*) was performed. The biologically active Pt4CL1, expressed mainly as soluble protein, was achieved with 0.6 mmol·L^{-1} IPTG induction when the expression temperature was declined from 37°C to 28°C. The 6×His tag enables one-step purification to acquire SDS-PAGE electrophoresis purity of Pt4CL1 protein by affinity chromatography via Agarose coupled with Ni^{2+}-NTA. The optimal substrate for Pt4CL1 was 4-coumarate. However, the recombinant Pt4CL1 has no activity to sinapic acid. SDS-PAGE purity Pto4CL1 protein was used as antigen to achieve the polyclonal antibody. Western blotting showed that the polyclonal antibody has high specificity to Pto4CL1. The fluorescence immunolocalization indicated that the 4CL1 protein was expressed specifically in the xylem of *Nicotiana tobacum*. It provided a basis for further application of xylem-specific expression promoter in transgene to decrease the contents of lignin in biomass so that it can be readily conversed to bioethanol.

9. Ethylene signaling via Ethylene Response Factors (ERFs) modifies wood development in hybrid aspen

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The phytohormone ethylene (ET) has the potential to regulate secondary growth of plants. We demonstrated previously that application of exogenous ET or its *in planta* precursor 1-aminocyclopropane-1-carboxylic acid (ACC) as well as endogenous ET accumulation during leaning stimulate xylem growth in stems of hybrid aspen (*Populus tremula x Populus tremuloides*) and requires functional ethylene signaling[1]. Ethylene Response Factors (ERFs) act downstream of ET perception and activate transcription of ET-responsive target genes. We analyze here whether ERFs are regulators of wood development in hybrid aspen.
We identified 169 *ERF* genes in *Populus* and studied their responsiveness to ethylene, ACC and tension wood formation in hybrid aspen stems using qPCR. Twenty-six ERFs were expressed in stem tissues and inducible by at least two of the three treatments. Twenty of these ERFs were overexpressed in cambium/xylem in transgenic hybrid aspen but caused only mild alterations of height and radial growth in a greenhouse trial, except for one ERF candidate. A Fourier-Transformed Infra Red spectroscopy and Pyrolysis GC-MS based screening of the ERF-overexpressors revealed changes in xylem cell wall composition (lignin abundance and structure (S:G ratio), glycosidic linkages, cellulose abundance) in xylem tissue. This suggests that ERFs have the ability to modify cell wall composition in wood forming tissues.

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10. Water balance and water-use efficiency of a poplar bio-energy plantation

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Here we present the framework and the first results related to the assessment of the water balance and water-use efficiency of a poplar SRC system dedicated to the production of bioenergy. The bio-energy plantation was established on 7-10 April 2010 on a former farmland in Flanders (Belgium) and consists of a 18.4-ha high density plantation of 12 different poplar (*Populus*) hybrids and species maintained over a 2 + 2 years rotation scheme. Measurements of water use and carbon uptake are being undertaken at different spatial (leaf, individual tree, ecosystem) and temporal (instantaneous, daily, seasonal) scales, using a combination of measurement techniques: leaf gas exchange measurements, use of stable isotopes, radial and height growth kinetics, sap flow monitoring, and eddy covariance. The data will (*i*) provide a comprehensive understanding of the physiological and the environmental controls that control water use and carbon uptake at each study scale, and (*ii*) quantify the water balance of the whole plantation. Information collected will also provide the necessary data set to update, calibrate and validate process-based models to simulate the potential of poplar SRC systems to mitigate CO_2 emissions in the not-too-distant future under conditions of global change.

11. Mapping QTLs for leaf traits and plant growth under heat and water stress in *Salix*

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Hybrids of willow (*Salix* spp.) are today grown as biomass crops for energy in several cool-temperate regions of Europe. These trees have a great growth potential also under warmer climates, but here they may suffer from drought and heat. The breeding of willows for biomass plantations in Southern Europe involves the challenge of selecting clones that resist periods of water shortage under high temperature, and it would benefit from the use of easily detectable genetic markers linked to genes involved in drought and heat tolerance. This study examines the effects of drought and heat on leaf traits and plant growth in 440 progenies from an experimental cross of *Salix viminalis* L. and *Salix schwerinii* E. Wolf grown in pots under controlled conditions. We mapped quantitative trait loci (QTLs) for biomass production and partitioning, leaf abscission and leaf temperature under stress by using a dense genetic linkage map developed for the same population and aligned to the *Populus trichocarpa* physical map. Flanking SNP markers anchored to the physical sequence of poplar were used to determine the genomic regions corresponding to the QTLs identified in *Salix*. *In silico* search of these regions was performed to find putative candidate genes for these traits.

12. *De novo* assembly in *Populus nigra*: sequence and polymorphism map

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In the framework of a joint resequencing effort undertaken by the EU projects Evoltree, Noveltree, and EnergyPoplar, we obtained the *P. nigra* genome sequence (86X coverage) using Illumina technology and a *de novo* assembly approach. We developed our assembly pipeline in four phases: assemble Illumina reads into contigs using two softwares, CLC and ABySS, validate the assemblies exploiting the rNA software, characterise the contig content in terms of repetitive elements and peptides, scaffold the contigs obtained. The scaffolding was achieved using the SSPACE software and integrated with the *consensus* obtained from the reference assembly between *P. trichocarpa* genome and *P. nigra* reads.
We also resequenced two individuals at 20X coverage and fifty additional *P. nigra* genotypes representing the European latitudinal range at lower coverage (~2X). We aligned their reads to the assembled sequence in order to detect SNPs to produce a whole-genome map of markers. The *P. nigra* genome sequence provides a valuable resource for improved breeding programs applying genomic selection and for population genetics and genomics.

13. TREEFORJOULES, a Plant KBBE project to improve eucalypt and poplar wood properties for bioenergy

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TREEFORJOULES is a Plant KBBE project starting in April 2011 and gathering 13 research groups from public and private organisations from France, Germany, Portugal, and Spain. The overall goal is to identify the major factors underpinning the physicochemical properties of cell walls, the recalcitrance of which remains a key scientific challenge for establishing highly efficient, sustainably produced, second-generation biofuels. This knowledge will be invaluable for breeding fast-growing elite trees such as poplar and eucalypts for improved down-stream processing and efficient degradation. TREEFORJOULES aims are to:

- Identify and characterize the regulatory candidate genes (*i.e.* transcription factors and miRNAs) that control wood properties relevant to bioenergy through integration of existing and new transcriptomic resources, delineation of the transcriptional interactome, functional characterization of candidate genes (CGs) in transgenic wood sectors, assessment of environmental and seasonal impacts on CGs expression and correlation with biomass production of high-performing genotypes.
- Develop high-throughput phenotyping methods for key wood and cell-wall chemical constituents, assess their impact on saccharification, bioethanol and bio-oil production, and develop and apply micro methods for phenotyping of transgenic wood tissues.
- Delineate and characterise genomic regions in eucalypts and poplar that control wood properties valuable for efficient cellulosic bioenergy production through comparative analyses at both the structural (comparative genetic and physical mapping) and functional (comparative QTL mapping) levels.

14. Understanding the effect of abiotic and biotic stresses and below-ground microbial diversity on sustainable woody biomass on marginal land

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A well recognized benefit of biomass energy from forest trees is the ability to cultivate on marginal lands not suitable for food or other agricultural crops. Long studied for woody plant biomass, the tree *Populus* can be sustainably harvested without replanting in coppice style cultivation. Despite a long history of use in the paper and pulp industry, details of *Populus* biomass accumulation for biofuel or ethanol in field settings are minimal, especially on marginal lands where incidents of biotic and abiotic stress are common. Pre-harvest treatments with the ethylene blocking agent 1-methylcyclopropene (1-MCP) may reduce stress and total lignin content of biomass tissues in *Populus.* We have set up a two-factor completely randomized design consisting of planting space, 1-MCP treatment, and presence or absence of the nitrogen fixing legume, Black Locust (*Robinia pseudoacacia*). In 2009 and 2010, both field measurements and a genomics based gene expression strategy, including above and below ground biological diversity associated with these trees, were taken to assess the growth of these treatments on the accumulation of woody biomass. The goal of this study is to understand the system biology of *Populus* plantations on woody biomass and to determine methods farmers can maximize biomass yields for ethanol production.

15. Sucrose transporter genes in *Populus*: An investigation of their importance as regulators of biomass and carbon partitioning in trees

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Sucrose export from source organs, and subsequent transport to sinks in wood-forming stems and elsewhere depends on the activity of sucrose transporters (SUT). There are no comprehensive reports on SUT function in temperate tree species valued for their lignocellulosic biomass. To begin to address this gap, the SUT gene family was characterized and functionally analyzed in transgenic *P. tremula x alba*. The *Populus* SUT family features the three major groups characteristic of other dicots. Group-1 *PtaSUT3* transcripts localize to leaf vascular traces and stem developing xylem; Group-4 *PtaSUT4* to leaf spongy mesophyll, stem developing xylem, cambium and phloem; Group-2 *PtaSUT5/6* to all leaf cells, stem developing xylem and phloem fibers. The SUT4 ortholog of *Populus* differs from that of other model plants in encoding a vacuolar transporter that is highly expressed in both source leaves and sink xylem. SUT4-RNAi transgenic plants demonstrated a shift of biomass allocation from stem to leaf in both nitrogen (N)-replete and N-limited plants. In those plants, sucrose exhibited a complex pattern of hyper-accumulation in exporting leaves and vascular tissues of the stem, and decreased accrual in the shoot tip and sink leaves. RNAi silencing of SUT4 reduced water uptake from root tissues during drought simulation.

16. Apoplastic H_2O_2 generation mechanisms during extracellular lignin formation in Norway spruce cell culture; effect of H_2O_2 removal on phenolic metabolism

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Apoplastic hydrogen peroxide (H_2O_2) is required for extracellular lignin production in Norway spruce (*Picea abies*) tissue culture as removal of H_2O_2 with potassium iodide (KI) repressed extracellular lignin synthesis. This suggests that peroxidases activate monolignols for lignin polymerisation. At least two mechanisms for H_2O_2 formation were present in spruce apoplast: the one having characteristics of a haem-containing enzyme, and the other of that of a flavin-containing enzyme (Kärkönen *et al*. 2009).

Purified spruce plasma membranes contained several enzymes able to generate superoxide that can dismutate to H_2O_2. Naphthoquinones juglone and menadione strongly stimulated superoxide production. Full-length gene for spruce respiratory burst oxidase homologue (*Parboh1*, NADPH oxidase) was cloned. It had a stable expression during lignin formation and showed a two-fold induction after elicitation (Kärkönen *et al*. 2009).

Phenolic dimers accumulated in both cells and in the culture medium when lignin biosynthesis was inhibited. Cells also started to divide which is in contrast to lignin-forming conditions where cells died soon after lignin formation. Interestingly, removal of KI after a 3-week-treatment restored extracellular lignin formation. The inducible cell culture system enables us to study regulation behind lignin and dilignol formation. The data obtained can be utilised when designing applications e.g. for bioenergy use.

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Kärkönen A, Warinowski T, Teeri TH, Simola LK, Fry SC (2009) Planta 230: 553-567

17. Towards genetic transformation of *Populus* sp. for improving their growth

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Three *Populus* species – aspen *P. tremula* L., black poplar *P. nigra* L. and hybrid poplar *P. x canadensis* Moench were introduced into *in vitro* culture. *P. nigra* and *P. x canadensis* as fast growing clones were provided by the Ukrainian Research Institute of Forestry and Forest Melioration. Thidiazurone and zeatin are used for plant regeneration after the *Agrobacterium* transformation. Explants are transformed with the genetic construct harboured *bar* as the selective gene and *cyp11A1,* gene encoding cytochrome P450scc, protein involved in the biosynthesis of steroid hormones of mammals - important regulators of vital functions. Transfer of *cyp11A1* gene into tobacco genome led to reduced period of vegetative development and increased productivity (Spivak *et al*., 2009). Increased productivity will be very useful trait for poplar as biofuel tree. First steps of this study were to determine the selective concentration of phosphinothricin as well as to create effective protocol for transformation. The gradual increasing of phosphinothricin concentrations through experiments (0,5–2–5 mg/l) demonstrated the better selection. Experiments for obtaining transgenic poplar plants are in progress.

18. Identification of Quantitative Trait Loci and targeting of genes affecting ectomycorrhizal symbiosis in the Poplar

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We have analyzed a *Populus deltoides* × *P. trichocarpa* F_1 pedigree (Family 54B, INRA-Orléans, France) for quantitative trait loci (QTLs) affecting ectomycorrhizal development and for microarray characterization of gene networks involved in this symbiosis. A 300 genotype progeny set was evaluated for its ability to form ectomycorrhiza with *L. bicolor*. The percentage of mycorrhizal root tips was determined on the root systems of all 300 progeny and their two parents. QTL analysis identified four significant QTLs, one on the *P. deltoides* and three on the *P. trichocarpa* unsaturated genetic maps (Jorge *et al*. 2005). These QTLs were aligned to the *P. trichocarpa* genome and each contained several megabases and encompass numerous genes. Expression analysis from a NimbleGen whole-genome microarray was used to narrow the candidate gene list. About 3.4% of the *Populus* gene models were differentially expressed in mycorrhiza of the two parents. Recently we genotyped 300 mapping progeny on a 6K Illumina Poplar SNP array to improve the genetic maps and increase accuracy of gene targeting. Finally, these results suggest that there is a shared molecular communication network between these two organisms and that modification of metabolic pathways may be occurring before, during and after colonization.

19. Functional study of transcription factors involved in the differentiation of G-fibers in poplar tension wood

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Wood is a renewable resource, used as raw material and energy source. In poplar, tilted stems produce, on their upper face, tension wood (TW) characterized by peculiar fibers called G fibers. Tension wood formation is controlled by a number of transcription factors (TF). The goal of this work is to characterize the function of selected TF, in order to decipher the gene regulatory networks underlying TW formation. In a first step, based on data from the literature, we chose about 40 TFs that may play a role in TW formation. Specific primers were then designed and used in Q-PCR to measure TF expression in TW, compared to opposite wood. According to their importance in the regulatory network, 1 to 3 TF will be further analyzed using different complementary approaches: (1) identifying their *in vivo* DNA target(s) by developing ChIP-SEQ experiments (Chromatin Immunoprecipitation followed by high-throughput sequencing), (2) identifying their partner proteins using TAP-tag (Tandem Affinity Purification) methodology, (3) studying their role in TW formation by modifying in planta TF expression using genetic engineering in poplar.

20. Epigenomics in poplar: the methylome of non-condensed chromatin

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Poplars are among the fastest growing trees under temperate latitudes with strong genotypic variability and plasticity facing environmental changes such as a water deficit. Epigenetic variations have been recently reported *between P.euramericana* hybrids and in response to a water deficit (Gourcilleau *et al*., 2010). In addition, a positive correlation was established between traits related to biomass production and global DNA methylation of shoot apex's cells in well-watered condition. Our objective is now to identify methylated genomic loci that could be linked to biomass production (ANR Bioenergy project 'Sylvabiom'). In this context, we have first focused on preferentially *DNase*I-digested chromatin (non-condensed) of *P. trichocarpa* (clone 101-74) shoot apex. Antibodies raised against 5-methylcytosine were used to immunoprecipitate methylated sequences in the non-condensed chromatin fraction. The immunoprecipated fraction was then sequenced using Illumina/Solexa technology. Finally, methylated sequences were mapped on poplar's linkages groups. Our methylated fraction only covered 2% of the poplar's genome but contains sequences corresponding to 74% of poplar's gene models (over 40 000). This confirms the relevance of working on the non-condensed chromatin fraction for methylome analysis. Bioinformatics analyses of methylome will be presented.

21. A SNP-based assay for the identification of commercial willow genotypes

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The use of willows (*Salix sp.*) is becoming increasingly popular in Europe and North America, both for the production of biomass and for environmental applications (phytoremediation). Selection and crossing have been used to obtain different genotypes with specific characters. Currently, about 35 genotypes are being used in short-rotation coppices. Identification of the different *Salix* species and clones using morphological traits is difficult and may lead to misidentifications. Hence, the objective of this project is to discriminate between the different commercial *Salix* genotypes using DNA fingerprinting. Our approach uses sequences of two nuclear and two chloroplastic regions in order to identify single-nucleotide polymorphisms (SNPs) between the genotypes, along ploidy levels. Analyses of 35 commercial willows and comparisons with native species have allowed us to specifically characterize the genotypes at the molecular level. We have developed a molecular approach to identify hybrids and species of *Salix*. We discuss important aspects of this method for the definite identification of commercial willow genotypes.

22. Functional variability of lignification genes in *Eucalyptus urophylla*

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Lignin quantity and composition are major components of wood quality in eucalyptus breeding programs. Optimizing these traits can have enormous economical impact for charcoal and pulp production. However, little is known about the genetic determinism of these traits. Our objective is to establish efficient early selection criteria to identify ideotypes for these traits using gene based markers. To reach this goal, we first estimated genetic parameters for lignin quantity and composition using a factorial design comprising 16 founders and 328 progenies of *E. urophylla*. We found high heritability for both Klason Lignin (h^2=0.85) and Syringyl to Guaiacyl ratio (S/G: h^2=0.62). Then, nucleotide diversity was described for 11 genes involved in the lignification process including structural and regulatory genes. High levels of nucleotide diversity and rapid decay of linkage disequilibrium were observed in a sample of 16 trees. Finally, association mapping was carried out in the 328 progenies using a mixed linear model. A total of 4 SNP (3 in *CCR* and one in *ROP1*) were significantly associated with S/G ratio explaining between 1 and 1.8% of the trait variation.

23. Development of transgenic poplars with multiple transgenes for improved saccharification potential

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Saccharification is the conversion of cellulose into sugars, which itself can be converted into ethanol by fermentation. Lignins are cell wall polymers conferring mechanical strength to the cell wall, but are also barriers to saccharification. Inhibition or surexpression of genes encoding enzymes involved in lignin biosynthesis pathway (CAD, CCR or F5H) can modified quantity or quality of lignins (Vanholme et al., 2008). However, transgenic trees with a reduced lignin level often present lower growth performance. To compensate for this side-effect, we produced transgenic poplars combining lignin genes with genes beneficial for biomass production. To obtain multi-transgenic trees we used two different strategies: re-transformation of transgenic lines improved for one trait and already characterized or cotransformation. Cotransformation allows combinatorial modifications. Four transgenes are used simultaneously: tree genes affecting lignin biosynthesis (CAD and CCR genes, under the control of 35S promoter, and F5H gene, controlled by 4CL or CesA3 promoters) and GA20ox1 gene, under the control of 35S promoter, know to improve plant growth (Eriksson et al., 2000). The transformations events could contain until this four differents transgenes. Results will be presented and discussed. This work is supported by the FP7 European Project ENERGYPOPLAR.

24. Genome-wide haplotype reconstruction from next generation sequencing data of a hybrid poplar pedigree

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In the framework of the EU funded project EnergyPoplar and with the support of Evoltree and Noveltree EU funded projects, we set out with the aim of determining genome-wide haplotype structure in poplar, and of correlating it with heterotic behavior. We obtained Next Generation Sequencing (NGS) data from a factorial design involving 2 *Populus nigra* parents, 2 *P. deltoides* parents and 12 F1 hybrids (average coverage 12x, total coverage 200x). We identified 2,330,301 putative SNPs. Of them, 245,000 SNPs were considered informative for family-based analysis, but we randomly selected a set of 95,000 to save computational resources. Of them, 56,639 passed family-based quality controls and were used for haplotype reconstruction with the package *MERLIN*. Preliminary results, to be experimentally validated, revealed on average 25.365 recombinations per offspring, leading to an estimated genetic length of the poplar genome of 2,536.5 cM. Obtaining an accurate genome-wide haplotype reconstruction in hybrid poplars will enable to correlate haplotype blocks with heterotic behavior. For the first time, we used NGS data to generate SNP markers and to reconstruct poplar haplotype structure. Our results suggest that NGS is a powerful tool to perform cost-effective family-based studies.

25. QTL analysis of wood formation in *Arabidopsis*

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Understanding wood formation is a critical requirement for the successful establishment of second generation biofuels through genetic engineering. Knowledge gained in the *Arabidopsis* plant model system can be translated to bioenergy crops being either grasses such as elephant grass (*Miscanthus sp.*) or trees like poplars and willows. For this study a QTL approach was employed in an effort to identify the genetic determinants behind several wood–related traits. After genotyping and phenotyping recombinant inbred lines, we identified several QTLs, from which the one explaining variation in xylem to phloem ratio was selected for further study. To narrow the QTL region, STAIR lines were used, which were phenotyped and used to fine-map the QTL with different markers. This allowed us to confine the QTL for xylem to phloem ratio in *Arabidopsis* hypocotyls to a 14,2 cM region of chromosome 2. We then generated recombinants in the region of interest which will be used in an attempt to identify the gene(s) responsible for part of the variation in this trait. In parallel, we performed an association analysis for the same characteristic. Our study may ultimately lead to the discovery of new genes involved in wood formation.

26. N-responsive transcriptional networks in wood formation

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Fast growing trees, such as poplars are increasingly used as a feedstock for wood production and biofuel generation. It is therefore critical to understand how nutrient availability affects growth and wood formation of poplar. In this study, we report on the effects of nitrogen fertilization on growth and wood formation in *Populus trichocarpa*. N fertilization stimulated poplar growth rates, influenced physiological and wood anatomical traits of poplar. Gene transcriptional profiles were compared between control and N treatment plants. Networks underlying N-driven secondary growth were tentatively identified by bio-informatic analyses. Based on this information, a working model for wood development was hypothesised and evaluated in Arabidopsis.

27. Genome-wide structural variation in poplar

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In the framework of the EU funded project EnergyPoplar and with the support of Evoltree and Noveltree EU funded projects, we set out with the aim of determining genome-wide structural variation in poplar, and of correlating structural variants (SVs) with heterotic behavior.
We performed next generation sequencing of 16 plants obtained from a factorial design composed by two *Populus nigra* males, two *P. deltoid*es females and 12 hybrids offspring (*P. nigra* × *P. deltoides*), three for each of the possible crosses. Average coverage was 20x in the parents and 10x in the offspring, for a cumulative coverage of about 200x. We used methods based on depth of coverage (DOC) and paired-end mapping (PEM) signatures to identify 1020 genomic regions with a significant copy number variation between the two species; 99 of them were confirmed as deletions by PEM signatures and by experimental validations. We used a custom algorithm for the identification of large insertions and we identified 1598 putative insertions in *P. nigra* or *P. deltoides* with respect to the *P. trichocarpa* reference sequence. We will perform annotation and genotype-phenotype association of identified SV, to provide a list of candidate SVs involved in hybrid vigor in poplar.

28. Wood formation is altered in poplar under ozone and/or elevated carbon dioxide

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The industrial development led to an increase in the concentration of atmospheric carbon dioxide but also resulted in an increase of tropospheric ozone concentrations. Ozone has been suggested to cause the greatest amount of damage to vegetation as compared to other gaseous pollutants. On the opposite, elevated carbon dioxide is usually observed to enhance tree photosynthesis and growth.
We investigated the effects of elevated carbon dioxide and/or ozone on wood formation in young poplars. Trees were bent in order to induce tension wood formation on the upper side of the stem and opposite wood on the lower side. Under ozone, lignin and cellulose biosynthesis decreased probably due to lower availability in carbon skeletons. Cellulose was more affected than lignin resulting in a reduction of cellulose to lignin ratio in wood. Wood anatomy and density was also modified by ozone. Tension wood was generally more altered than opposite wood. All these results showed a strong impact of ozone on wood formation and wood quality and suggested a coordinated regulation of lignin and cellulose synthesis. Elevated carbon dioxide had a low impact on wood formation but the combination with ozone resulted in an attenuation of ozone effects.

29. Shoot Development In Short Rotation Coppice Willow.

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Central to the suitability of willows for biomass production is the vigorous re- growth that occurs following the removal of apical dominance by coppicing. We are beginning to decipher the regulation of this process with respect to bud and shoot behaviour. A dedicated field trial was established in April 2008 comprising six genotypes contrasting in shoot number, stem diameter, and yield. After first coppice a subset of stools was extracted and examined for bud formation, position and number, revealing variation between genotypes in the proportion of buds that sprouted. This process is currently being repeated two years post-coppice, and followed in two mapping populations.

Preliminary QTL analysis placed two *More AXillary branching* (*MAX*) genes within a locus for shoot development in the K8 mapping population. The K8 alleles and alleles from diverse willow species have been cloned and used for transformation rescue of Arabidopsis *max* mutants. Variation in the degree of mutant rescue between allelic variants suggests functional allelic differences that could contribute to differences in coppicing phenotypes. Eleven new mapping populations have been generated with the aim of affirming, and discovering new, bud and shoot regulation QTLs.

This project aims to improve biomass willows in terms of increased yield and the production of biomass products tailored to a range of energy production processes.

30. Changes in protein profile and gene expression during tension wood formation in *Eucalyptus grandis*

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Wood cells are one of the major carbon sinks in the biosphere and constitute the majority of lignocellulosic biomass existing on our planet. Their complex chemical and anatomical composition could represent an alternative source replacing the oil-based industry for a renewable one. The composition of wood is derived from a high variability at the tissue level within a tree. These variations are under developmental and environmental control and can be observed, between normal and tension wood. In this work we induced the formation of reaction wood in young trees of eucalyptus, as an experimental model to follow the changes in gene expression and proteome during wood formation. *Eucalyptus grandis* were bent at a fixed angle and samples from the cambial region taken after 15, 30 and 60 days. Several differences in the expression pattern of genes and proteins involved in structural and secondary cell wall composition changes were observed when compared to normal wood during the induction period. The deposition of G layer was observed after 60 days of tension wood induction. Throughout the induction period, the results suggest an decrease in the flow of carbon for lignin biosynthesis and an increase for cellulose biosynthesis in the tension wood.

31. Fast growing elms as bioenergy trees

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Hybrid elms obtained within breeding programs for DED resistance were usually selected to meet requirements for use as ornamentals. However, it has long been observed that these clones show enhanced growth. Nowadays DED-resistant elms are numerous enough to be considered for short rotation coppice (SRC). Here, growth and stability of performance of 24 DED-resistant elms were studied at three experimental sites in contrasting environments from 2001 to 2009. The study revealed a good growth performance of many clones with mean height increments above one meter per year, and excellent growth of some genotypes. Analysis of variance showed significant effects of clone, site and clone × site interaction, for both height and diameter increments. Stability analysis was performed by using two parametric (CV% and W^2) and two non-parametric (Hühn's $S_i^{(1)}$ and $S_i^{(2)}$) indexes. According to all indexes, two clones showed superior and stable growth. These clones may be suitable for planting in a range of environments. In addition, several other clones had high growth in general or at a particular site. The results support our belief that these elm clones could be successfully used as bioenergy trees, and provide new knowledge for an informed choice of the most suitable genotypes.

32*. Eucalyptus* MYB transcription factors regulating wood formation

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Forest plantations are becoming important sources for sustainable second generation biofuel production, where the whole plant lignocellulosic biomass (mainly composed of secondary walls (SW)) is mobilized. *Eucalyptus* species grow very fast and produce high yields of biomass, representing the main industrial plantations in the world. To improve wood properties related to bioethanol production, we are focusing our efforts towards the identification of regulatory genes controlling the biosynthesis of SW polymers in *Eucalyptus*. We have already characterized two MYB factors (*EgMYB2* and *EgMYB1*) acting respectively as activator and repressor of the SW formation. Here, taking profit of the new release of the *E. grandis* genome, we performed a genome-wide survey of the R2R3-MYB superfamily. The phylogenetic comparison of this family with *Arabidopsis*, rice, poplar and grapevine showed a marked expansion of some clusters putatively involved in wood-related processes, pointing to a diversification of some specific functions in trees. The spatiotemporal expression patterns of members of these clusters are currently being studied and tools to characterize landmark genes (such as a yeast-two-hybrid library from xylem to identify protein partners) have been constructed. Altogether, this should allow getting more insights in the regulation of wood formation by this family of transcription factors.

33. Target genes for improved bioethanol production from wood identified from genetical genomic analysis of saccharification in *Populus*

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The success of lignocellulosic bioethanol largely depends on an improved ability to access glucose, which in its cellulosic form is tightly locked into the cell wall matrix. We have used a Poplar F2 mapping population (*Populus Trichocarpa x Populus Deltoides*) to study the genetics underpinning this issue. We have conducted compositional and saccharification assays on these trees using multiple techniques over multiple coppice cycles. Results show a positive correlation between saccharification potential with Glucose and Galactose and a negative correlation with Xylose. No correlation was seen between saccharification and Lignin content and quality. The data was used to generate saccharification quantitative trait loci (QTL), mapping to 8 linkage groups accounting for 41.9% of the variation. Using Microarrays, 218 genes were identified as differentially expressed between high and low saccharifying genotypes. Of these, 13 were annotated for cell wall functions and 28 for transcriptional control, with 9 of the cell wall genes confirmed by quantitative PCR (qPCR). Furthermore, 2 cell wall genes and 3 potential transcription factors were also present in the QTL. The compositional traits identified for improved saccharification coupled with new genes of interest provides important information in the push to improve Poplar as a viable source of bioethanol.

34. Ecophysiology and productivity of transgenic decreased-lignin *Populus* for use in short-rotation bioenergy cropping systems

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Development of a wood-based liquid fuels industry holds promise of an abundant, sustainable, low-cost energy supply. Yet, current cellulosic feedstocks, such as wood from forest trees, have several barriers to cost-effective conversion to liquid fuels. Among them, the nature of lignin limits enzyme accessibility for cellulose saccharification. Bench-scale studies have shown that transgenic modification of *Populus trichocarpa* for decreased lignin enhances biomass production and saccharification efficiency. Twelve lines of transgenic *P. trichocarpa* trees modified for decreased lignin (22 to 11 %) and altered S/G ratios were planted at field sites in the coastal plain, piedmont, and mountain regions of North Carolina and monitored for growth and physiology for two growing seasons. Growth was greatest at the cooler mountain site, consistent with growth requirements of *Populus*. Five transgenic lines displayed growth similar to the control. Our results show that decreases in lignin of up to eight percent are possible without compromising productivity, which has large implications for the economics of cellulosic liquid fuels production. Wide variation in the performance of individual transgenic lines indicates that more research is needed to produce and identify the genotypes with the most potential to benefit the cellulosic biofuels industry.

35. Exploring the monolignol transport mechanisms in Norway spruce

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While the biosynthesis of monolignols is relatively well known, the transport of monolignols into the apoplastic space is known to a far lesser extent. Our aim is to understand the cell and molecular biology of the transport mechanisms. There are three possibilities: Golgi-vesicles, ABC-type plasma membrane proteins or other channel proteins, or diffusion based on the hydrophobic-hydrophilic properties of the monolignols. We are now studying the ABC-transporters on the plasma membrane using ^{14}C-labelled phenylalanine and transporter inhibitors in a Norway spruce (*Picea abies*) tissue culture line. The results indicate that the ABC-transport inhibitors vanadate and reversin do not inhibit the transport into the tissue culture medium. We have done also an extensive database search on genes related to transport phenomena and their expression levels in different tissues of conifers. As we have now indications that this transport may not be ABC-transporter driven, we have extended the study on other transporters on the plasma membrane. We have not excluded the possibility of diffusion through the plasma membrane due to the hydrophobic interactions with the monolignols or their glucosides. For this purpose we have collected differentiating xylem material of a Norway spruce clone to be used in the membrane transporter study on the protein level.

36. Elucidating the poplar biomass loci QTL at the level of the gene using genetical genomic approaches

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We have identified five biomass QTL in poplar short rotation coppice for bioenergy and named them, the Poplar Biomass Loci (PBL1-5, Rae et al., 2009). These QTL are consistent across two cycles of coppice and 'high' and 'low' biomass extremes form the population were confirmed in a glasshouse experiment. One of these QTL has been investigated in detail using a genetical genomics approach. Firstly, we have completed intensive SNPs genotyping on this particular linkage group and fine-mapped the QTL so that it now accounts for approximately 200 gene models. From the distribution of biomass genotypes we have identified extreme 'high' and 'low' biomass genotypes for further analysis using microarrays. Gene expression using the Affymetrix poplar chip has identified approximately 200 differentially expressed genes between the biomass extremes and from these, eQTL are being mapped from a selected number of differentially expressed genes. Four candidate genes from this transcriptomic study have been taken forward using RNAi in poplar and also through analysis of Arabidopsis homologues. Our research is moving towards the identification of relevant genes and alleles that may be taken forward in breeding programmes and the alignment of these QTL for candidate gene and SNPs discovery in our association population of *Populus nigra* will also be highlighted.

37. Nitrogen supply before bud break strongly impacts spring development of young poplar

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Nitrogen availability widely impacts tree physiological processes. However, research concentrated on endogenous C and N spring remobilization have neglected the hypothesis that nitrogen uptake before bud break may have a significant effect on tree development. We have tested this hypothesis on poplar (*Populus tremula* X *Populus alba*, clone INRA 717-1B4) using one-year whip growing on a nutrient recirculating system in a greenhouse. The following treatments were used: *'control'*, nutrient without nitrate; '*N-Pulse',* nitrate supplied only before bud break; '*N-supply',* nitrate supplied throughout the course of the experiment. Experiments were stopped 15 days after regrowth start. Dynamic curves showed that poplar can take up nitrate before bud break with a high significant effect. Total nitrogen content at the whole plant level for *N-Pulse* and *N-supply* increased by 20 and 70 percent, respectively, as compared to the *Control* treatment. In our conditions, *N-Pulse or N-supply* treatment had no effect on the bud break profile (date, number of bud break and number of leaves per new shoots). However, in these conditions, leaf area increased by 1.26 and 1.44 fold for the *N-Pulse or N-supply* treatments, respectively, as compared to the *Control* treatment. The results suggest that nitrogen supply in spring before bud break can change plant development and quality of the regrowth.

38. Optimization of wood production in bioenergy plantations

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Intensive plantations of trees for bioenergy (short rotation coppice, SRC) are often synonymous with soil depletion. To maintain productivity in the long term while reducing inputs (water, fertilizer), an optimized matching between (1) the characteristics of plant material (genera, species and genotypes, mixed or not) particularly in terms of efficiencies of resource use (WUE, NUE), (2) cultural practices (spraying, densities, pruning, etc.) and (3) the soil and climate conditions is to find. The approach taken to meet this objective is to study the effects of the three categories of above factors on productivity and its determinants through the study of cycles of carbon, nitrogen and water in a network of plantations spread throughout the north of France.
Under the framework of three running ambitious projects, about 20 willow and poplar plantations are extensively monitored in northern France with the objective to test the impact of various cultural practices and pedoclimatic contexts on the links between biomass production and resource use efficiencies. The impact of (1) planting densities, (2) clonal mixing, (3) mixture with nitrogen fixing species (*Robinia*), (4) sludge spreading, (5) first year coppicing, (6) harvest period during the year are tested, (7) cultural antecedent, (8) fertilization, are currently studied.

39. Genomic plasticity in response to drought in a natural population of *Populus nigra:* stomatal patterning gene expression differs between northern and southern European trees.

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The overall aim of this research is to improve the water use efficiency and adaptation to drought in bioenergy poplar and willow trees. A natural population of *Populus nigra* has been collected as part of the projects POPYOMICS, EVOLTREE and ENERGYPOPLAR which now numbers more than 1000 unique genotypes selected from river systems from as far south as Spain and north to The Netherlands.
When grown in well-watered conditions, in the field in northern Europe, we showed that the carbon isotope signature of wood and leaf material differs significantly across the population. Leaf traits also differed significantly with larger, cordate leaves from the northern latitude of origin and smaller rhomboid leaves from the southern latitudes of origin.
A drought experiment was also conducted in the greenhouse in the UK focusing on 6 genotypes showing extreme variation in leaf size and $\Delta^{13}C$, from Spain and from Italy in particular. Physiological traits and gene expression using microarrays and real-time PCR revealed differences in response to drought between genotypes such that 2900 genes were differentially expressed in response to drought in the Italian genotypes whilst only 382 genes were differentially expressed in response to drought in the Spanish trees. These findings highlighted several GO categories that differed most between treatments and this included genes identified for stomatal patterning. The significance of these findings for genomic adaptations to drought in poplar is considered.

40. Modifying Gibberellin Biosynthesis and Signaling to Improve Growth and Morphology of Poplar: Experience from Studies of Eight Transgenes in Greenhouse and Field Environments

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Our laboratory has identified eight constructs that can significantly increase growth rate in greenhouse grown poplars. These genes might therefore be used, in conjunction with conventional breeding, to produce fast growing genotypes for biofuel crop systems. Our studies included five constructs in which the *GA 20-oxidase* gene was driven by different promoters, two constructs that had the *SPINDLY* gene from *Arabidopsis* and *Hordeum* driven by the 35S promoter, and one construct that had the poplar *PHOR-I* gene under the control of its own promoter and terminator. For each construct, we analyzed six to 38 events and two to twenty ramets per event; 2,066 trees were studied in the greenhouse and 556 trees were studied in the field. For two constructs (poplar *GA20ox7* and *PHOR-I* genes, driven by their own promoter and terminator), the transgenes were also shown to cause substantial increases (73-133%) in the levels of bioactive gibberellins. Although we often found dramatic improvements in growth rate of transgenics in the greenhouse, significant growth improvements were not observed under field conditions. Our results underline the critical importance of field trials even at early stages of transgenic biotechnology research.

41. Studies of auxin responses mediators in wood formation in *Eucalyptus*

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Auxin is key regulator of cambium activity and wood formation. Auxin/Indole-3-Acetic Acid (Aux/IAA) and Auxin Response Factor (ARF) transcription factors are well-known auxin response mediators which have been mainly characterized in model plants such as *Arabidopsis*. In contrast, they remain largely uncharacterized in tree species; especially their role on cambium activity and xylogenesis is still poorly known. Thanks to recent sequencing of the *Eucalyptus grandis* genome (http:://www.phytozome.net/eucalyptus), we identified 23 *Aux/IAA* and 17 *ARF* in *Eucalyptus* genome and performed comparative phylogenetic analysis. This study revealed that several *Aux/IAA* and *ARF* subgroups have differentially expanded or contracted among the three dicotyledonous plants studied (*Arabidopsis*, *Populus* and *Eucalyptus*). Expression analysis and EST database surveys are currently underway to explore the transcript levels of each member in the different organs and tissues of *Eucalyptus* at key developmental stages as well as in response to hormonal treatments and to environmental stresses. This gene-family analysis report will be useful in conducting further functional genomics studies to understand the role of auxin signaling in cambium activity and xylem differentiation with a special focus on the regulation of wood cell wall formation taking into account the ultimate goal to deliver tailored sustainable tree lignocellulosic biomass for bioenergy.

42. Molecular features of secondary vascular tissue regeneration after bark girdling in *Populus tomentosa*

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Regeneration is a common strategy for plants to repair their damaged bodies after attack from other organisms or physical assaults. However, how differentiating cells acquire regenerative competence and rebuild the pattern of new tissues remains largely unknown. By using a combination of anatomical observation and microarray analysis, we reported the morphological and molecular features of secondary vascular tissue regeneration after bark girdling in *Populus*. After bark girdling, new phloem and cambium regenerate from differentiating xylem cells and rebuild secondary vascular tissue pattern within one month. Transcriptome profiling analysis indicates that differentiating xylem cells may acquire regenerative competence through epigenetic regulation and cell division, moreover, xylem developmental programs are blocked whereas phloem or cambium programs are activated, resulting in reestablishment of phloem and cambium progressively. Phytohormones also play important roles in regulation of vascular tissue regeneration. Based on our data, we propose a model to illustrate the molecular dynamics during secondary vascular tissue regeneration after bark girdling, which is significant to understand the pattern formation of the secondary vascular tissues and also would shed light on the mechanisms of tissue regeneration in plants.

Participants

** S=speaker abstract; P=poster abstract*

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