

Comparative mapping analysis and QTLs associated to adaptive traits across European Populus native species

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Fifth International Poplar Symposium

Poplars and willows: from research models to multipurpose trees for a bio-based society

20 – 25 September 2010

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Book of Abstracts

Foreword

In the context of the long history of domestication that attends our major food crops, the breeding and selection of forest trees has really just begun. And, unlike food crops, the lengthier generation interval of trees coupled with the complexity of the transition between the juvenile and mature phases of development ensures that their domestication will be a challenging undertaking. To accelerate this process and to elucidate those mechanisms unique to woody plants such as dormancy and secondary wood formation, a "model" tree is clearly needed. Thankfully we have such a model in *Populus* and *Salix*! These two genera are accepted as sound biological models for all commercial trees because of: 1) A relatively small genome, about 550 million base pairs, which is similar to rice, only fourfold larger than *Arabidopsis*, and one fortieth the size of the *Pinus* genome, 2) A worldwide distribution of multiple species, 3) Impressive juvenile growth rates, 4) Facile clonal propagation, 5) The availability of genetic maps and structured pedigrees, 6) Publicly accessible molecular markers, gene sequences, and bacterial artificial chromosome (BAC) libraries, and 7) High-throughput plant transformation and regeneration capabilities among others attributes. But perhaps as noteworthy as their status as model species is the standing that *Populus* and *Salix* have achieved as trees of considerable commercial importance in multiple markets and environmental services.

In the four years since the *Populus* and *Salix* community last met in Nanjing, China at IPS-IV in 2006, a wealth of information has been generated in a variety of disciplines including production physiology, molecular genetics, applied breeding and selection, pathology, ecology, etc. This body of knowledge represents an indispensable resource from which new domestication tools will surely emerge. All of us look forward to the day when such tools are routinely put to work in increasing the adaptability, yield, and carbon sequestration potential of poplar and willows as both bioenergy and timber crops in a variety of cultivation systems.

The International Union of Forest Research Organizations' (IUFRO) Poplar and Willow Genetics' Working Party (2.08.04), the National Italian Research Organisations (CRA, CNR, University of Tuscia, CFS, CNP) and the International Poplar Commission of the UN Food and Agriculture Organization (FAO-IPC) have jointly organized the Fifth International Poplar Symposium (IPS-V), in Orvieto, Italy for the week of 19 September, 2010. In planning IPS-V, our intent has been to stage an open forum where the world's scientific community comes to present its latest research findings and discuss ways in which such knowledge can be implemented in service of humankind. The theme of the symposium, "*Poplars and willows: from research models to multipurpose trees for a bio-based society*", was chosen in view of the increasing imperative that the world's communities face in developing low-carbon, bio-based economies and livelihoods. This challenge encompasses not only the traditional needs for sustainable wood production, but perhaps more importantly, the mitigation of today's environmental extremes of climate change and associated increases in soil and air temperatures, drought, frequency of pest attacks, etc. We wish you an enjoyable and productive time in Orvieto, looking forward to working with you, also in the future, within the Poplar and Willow community.



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Brian J. Stanton, IUFRO WP 2.08.04,
GreenWood Resources, Portland, Oregon, USA

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Plenary Sessions

Forest biotechnology and society: What have we accomplished and where are we going?

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The last two decades have seen great progress and even greater promise for biotechnological approaches to genetic modification of forest trees. A number of studies and commercial experiences have shown the delivery of important traits in the field, including herbicide tolerance, pest resistance, modified wood properties, enhanced nitrogen metabolism, and male-sterility. These studies have also shown that, after initial screening of transgenic events for gene/trait expression, stability in gene expression and limited somaclonal variation is the rule. The development of extensive genomic sequence information for many species, including a draft reference sequence in poplar and one soon to be published for *Eucalyptus*, has provided numerous new tools for continued study, especially for cis/intragenic approaches. Thus, the biological potential for new advances in great. However, a number of serious challenges have developed and are severely constraining further development. These include: 1) Transformation systems that are inadequate in efficiency and generality to address the diversity of tree species and genotypes; this is unfortunate as the application of new advances in developmental genetics could greatly reduce these problems. 2) Regulatory constraints on research that make it extremely costly and risky to conduct field tests; this is a serious problem as most the value and pleiotropy of new biotechnologies cannot be fully appreciated without field study.[1] 3) Regulatory and marketplace obstacles to commercial applications that have limited commercial investment, reduced the attraction of the best students to the field, and slowed scientific progress. Unfortunately, these regulatory constraints reflect deep social, cultural, and ethical issues that cannot be easily addressed by scientific research or dialogue.

If transgenic forest biotechnology is to fulfill its potential to promote forest health and productivity over the long term, it is essential that scientists, scientific institutions, the private sector, and concerned NGOs (non-governmental organizations) work to reduce the regulatory and market stranglehold that is suffocating the field in many parts of the world, including in the USA, Japan, and Europe. This requires engaging on the foundation issues that have prevented meaningful progress.

Keywords: forest biotechnology, genetic transformation, ethical issues

[1] Strauss, S.H., H. Tan, W. Boerjan, and R. Sedjo. (2009) Strangled at birth? Forest Biotech and the Convention on Biological Diversity. *Nature Biotechnology* 27:519-527.

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The population genomics of adaptation to photoperiod 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 latitudinal clines in important phenological traits are common in many plants. In European aspen (*Populus tremula*) the most important environmental cue regulating dormancy initiation is a shortening of the photoperiod. QTL mapping have implicated genes in the photoperiodic pathway in the control of growth cessation. Here we present data from a study on the genetic basis of variation in phenology in European aspen (*Populus tremula*) across a latitudinal gradient. We show that genetic differentiation at neutral markers is low despite strong differentiation in phenology traits, demonstrating local adaptation to the photoperiodic regime. Patterns of nucleotide polymorphism at several genes from in the photoperiodic pathway suggest the action of natural selection along the environmental gradient. We also identified about 100 SNPs from these genes we used to test for associations with naturally occurring variation in bud phenology and senescence.

Keywords: bud phenology, senescence, photoperiod, genetic control

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Populus genome networks and the regulation of growth and development

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The identification of specific genetic elements that regulate plant growth and development is one of the main challenges of quantitative genetics and breeding. When the ensemble of these elements and their interactions are identified, then the complex network of factors that defines the phenotypic properties of an organism can be unveiled. To begin dissecting these genome networks in *Populus* we have integrated genetic and gene expression data, with phenotypic traits measured in a hybrid population of *P. deltoides* and *P. trichocarpa*. Initially, we uncovered the genetic regulation of *Populus* genes expressed across plant compartments, based on expression QTL mapping. Analysis of individual genes indicated that their major regulatory loci are largely distinct in mature leaves, roots and differentiating xylem, demonstrating that control of transcription is highly plastic and variable within an organism. Quantitative analysis of gene expression in the mapping population lead to the development of transcriptional networks supported by functional annotation, that identify their putative regulators and mechanisms of control. Integration of phenotypic information has allowed the identification of putative candidate genes for the regulation of traits such as adventitious root formation, leaf shape and wood composition. While this analysis began to uncover genes and networks that contribute to *P. deltoides* and *P. trichocarpa* interspecific diversity, it does not reveal the more subtle intraspecific sources of variation that impact growth and development within a species. To address this limitation we have begun to define the first layer of genomic information, the DNA sequence, of over 500 *P. deltoides* individuals by resequencing over twenty thousand expressed genes.

Keywords: genome networks, gene expression, QTL

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How can poplar breeding benefit from applied genomics?

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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 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 limit economical and environmental risks associated to clonal forestry. 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.

Potential benefits and present limits of gene mapping technologies, gene expression studies, expanded genome sequences and modern bioinformatics and statistical tools will be analysed in the light of specific situation of poplar genetics and breeding.
The following main challenges will be highlighted:

(1) the increase of selection accuracy for complex breeding goals
(2) the construction of elite genotypes with durable pests and disease resistances
(3) the improvement of inter-specific hybridization: gene complementation; specific combining ability and marker-based distance, management of non-additive effects
(4) the integration of phenotypic plasticity as breeding objective
(5) the development of a merit which integrates target and non targeted genetic diversity
(6) the more combined management of alleles and genotypes in long term breeding populations
(7) the adjustments of selection intensity, phenotyping and genotyping costs and multi-stage marker-assisted selection.
(8) the perspectives of genomic selection in an highly heterozygous species

Even if considerable progress has been made to unravel the genetic make-up of important adaptive and economical traits in poplar, joint efforts from researchers in genomics and poplar breeders are needed for a successful implementation of all 'ics' technologies in practical breeding. In the next future, the true challenge for manipulating the complex nature of selection goals in poplar will depend on our ability to accurately phenotype trees 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.

Keywords: poplar breeding, genomics, gene expression, genome sequencing, molecular markers, association genetics, nucleotide polymorphism, QTL, gene mapping, marker-assisted selection

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Implications of engineering amino acid metabolism in Poplar by ectopic expression of *Glutamine Synthetase*

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Glutamine synthetase (GS) plays the central role in the assimilation of ammonium into amino acids and reduced nitrogen compounds in plants. In poplar, the GS gene family consists of 8 members, 6 coding for the cytosolic GS1, and 2 coding for the plastid form, GS2. Furthermore, our analysis suggests that the GS gene family in poplar is organized in 4 groups of duplicated and differentially expressed genes, *GS1.1*, *GS1.2*, *GS1.3* and *GS2* that display tissue-specific and developmentally-specific patterns of expression.

We have engineered hybrid poplar (*Populus tremula* X *P. alba,* INRA 717-1-B4) to express ectopically the heterologous pine *GS1a*. These transgenics are characterized by enhanced GS activity, increased levels of free cellular glutamine, increased growth in greenhouse and field trials, increased nitrogen use efficiency, enhanced resistance to drought, resistance to phosphoinothricin herbicides, enhanced auxin synthesis, and altered fiber and wood chemistry. Such alterations in the phenotype of GS poplars induced by a single transgene prompt us to profile transcriptomic changes associated with GS overexpression. Microarray analysis has been conducted for sink and source leaves of control and GS poplar subjected to drought stress manipulation. Expression of nearly 7,000 probes was found to differ significantly between control and GS poplar in at least one of the six pairwise comparisons (pre-stress, drought and recovery for both sink and source leaves. Of these, 1,811 poplar probes exhibited 2-fold or greater differences in expression. Gene Ontology (GO) enrichment analysis revealed numerous categories that were significantly over-represented among the genes down-regulated in GS plants under normal (pre-stress) growth condition. Our analyses indicating coordinated expression of networks of genes in GS poplars involved in amino acid metabolism, photorespiration, nitrate uptake and assimilation, and flavenoid metabolism will be discussed.

Keywords: glutamine synthetase, metabolic engineering, microarray analysis

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Update on the transgenic poplar plantations and research in China

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The transgenic poplar plantation has increased to 450 hm^2 this year since the two Bt transgenic poplars were commercialized in 2001 in China. The transgenic poplar plantations have effectively inhibited the fast-spread of the target insects and significantly reduce the times for insecticide application to poplar plantation. The availability of commercial plantation has made it possible to empirically assess the gene flow through pollen and seeds, and the impact of Bt poplar on insect community when intercropping with Bt cotton. The transgenic *Populus nigra* was also used in hybridization with non-transgenic *P. deltoides* as an insect-resistant source for breeding new hybrid clones. Up to now, there are 3 transgenic poplar lines approved for environmental release, and 5 under small scale field trials. Marker free system and multiple-gene construction techniques are developed for safe and efficient transformation of poplar, and diverse traits like freezing tolerance, flowering control and wood modification for better pulping and efficient saccharification are under progress.

Keywords: genetic transformation, insect resistance, gene flow

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Willows beyond bioenergy: *Salix* for ecological engineering and as a specialty crop in low input agricultural systems

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The economic importance of willows is currently increasing not only for biofuel production, but is also emerging in a wide array of environmental applications to restore damaged ecosystems. Species of *Salix* characterized by particular physiological characteristics and ecological resilience are predisposed for use in conservation and environmental projects in many climatic zones with adverse microsite conditions and diverse agricultural, industrial and municipal settings. The current focus is on the multiple land use concept and a combination of traditional plantings with the production of other goods or services on the same tract of land.
The application of multifunctional plantings is under investigation for rural development in the United States, where producers are showing considerable interest in growing alternative crops. Emphasis of this research is on multi-use plantings with the incorporation of horticultural enterprises and new specialty crops that offer multiple benefits for the farm family, including financial returns, effective runoff management, and the involvement of children. Willow species for mid-winter through early spring harvest can be a unique production niche for farmers offering opportunities to supplement income during the otherwise dormant season. Production of brightly colored willow whips for construction of living structures and mini-villages for children is another opportunity for financial returns during off-season time. The project is meant to include junior members of the family into product development and production cycles in order to provide a steady supply of interested, skilled and trained farmers. The willow plantings can be integrated as living snow fences and windbreaks, or riparian forest buffers in order to assist with nutrient management. Willow plantations are sustainable for many years from initial planting, and require only basic input predicated on organic principles. Current research centers on the development of effective production practices; design of new products; study of consumer preference and marketing; development of educational programs.

Keywords: willows, environmental applictions, multifunctional plantings, multiple land-use, educational programs

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An overview of Italian experiences on Poplar and Willows domestication and cultivation

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A brief history of poplar and willows domestication in Italy is presented; hybridization in Italy dates back to the XVII century when cottonwood (*Populus deltoides*) trees of American origin were introduced from France and naturally hybridized with local populations of black poplar (*P. nigra*). Modern Italian poplar breeding began in Villafranca, Piemonte in 1929 and then developed with the establishment of the Poplar Research Institute at Casale Monferrato in the Po River Valley. *P.* ×*canadensis* 'I 214,' perhaps the most widely planted *Populus* clone worldwide, was developed at the Institute under Giovanni Jacometti .
Present day activities on domestication of *Populus* and *Salix* are centered on: (1) conservation and evaluation of genetic resources, (2) controlled hybridization and varietal development for improved growth rate, wood quality, and soil adaptability, (3) breeding for resistance to pathogen and insect pests, and (4) the development of biotechnology and marker-aided selection for varietal development.

Keywords: Poplar domestication, hybridization, crop systems, conservation of genetic resources, international cooperation

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Greenhouse gas aspects and Life Cycle Assessment of bio-energy systems

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The talk will address relevant issues for the cultivation of biomass energy crops as regard to greenhouse gas (GHG) balance of biomass and bioenergy systems.
Special attention will be given to the development and implementation of Life Cycle Assessment using willows and poplar for bioenergy production.

Keywords: bioenergy crops, willows, greenhouse gas budgets, life cycle assessment

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Domesticating poplar using genetic engineering to improve their utility as a bioenergy crop

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The process of domestication converts a wild plant, whose morphology and physiology have become adapted via natural selection for survival and reproduction in its native environment, into a cultivated plant that has been drastically altered by artificial selection to yield products useful to humans. Under modern agricultural systems, domesticated plants are significantly more productive than their wild ancestors.

Domestication of modern agricultural crops through traditional means of plant breeding and agronomic modifications has resulted in advances in plant growth, pest resistance, and adaptation to cultivated environments. Although increasing the resources available to domesticate trees as biomass feedstocks using conventional approaches also would be successful over the very long term, their perennial nature, lengthy generation interval, and large size would severely constrain progress.

Recent increases in energy costs, a growing investment in bio-based products, and documented rises in atmospheric CO_2 have led to heightened interest in producing biofuels from bioenergy crops to replace fossil fuels, and to reduce the effects of climate change. Hybrid poplars are being investigated for their utility and cost-effectiveness as a renewable source of biomass feedstock. However, in most cases, the available clones and cultivars represent at most only a few generations of genetic improvement over their progenitors, whereas hundreds of generations have been needed to domesticate modern agricultural crops.

Accelerating the domestication procedures for biomass-feedstock plants has the potential to increase yields, improve feedstock uniformity, tailor the physical and chemical characteristics of the feedstock to their intended uses, reduce the cost of supplying bio-refineries with feedstock, and improve conversion efficiencies. Leveraging modern molecular genetics techniques, which do not require time-consuming sexual crosses that combine rare, desirable traits into a single cultivar by conventional breeding, should allow us to hasten the development of biomass species, such as those within the genus *Populus*.

An overview will be presented of the various domestication-related projects underway in my lab, including water-use efficiency, salt tolerance, rooting ability, and lignin modification. Special emphasis will be given to recent results with *Corngrass1* (*Cg1*), a gene that has the potential to solve many biofuel-related needs, and to expedite the commercialization of genetically engineered trees.

Keywords: tree domestication, water use efficiency, salt tolerance, biofuels, *Cg1* gene

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Volatile isoprenoids: central molecules in defending poplars and willows against stress factors

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Plants have many ammunitions to defend themselves against stressors. At leaf level, non-volatile isoprenoids (e.g. carotenoids) are known to be powerful antioxidants, but the role of volatile isoprenoids (isoprene, monoterpenes and sesquiterpenes) has not been equally elucidated. Fast growing trees such as poplars and willows invest a large share of the photosynthetically fixed carbon into volatile isoprenoids, predominantly isoprene. Such a metabolic cost must reflect a key benefit for these plants. Here I note that volatile isoprenoids are reactive molecules whose biosynthesis is elicited by a general stress condition, and that volatile compounds commonly exert signaling functions. I will show evidence that isoprene, the primary and most abundant volatile isoprenoid in poplars and willows, a) primes plant response to stress by activating H_2O_2 signaling; b) quenches reactive oxygen species *in vitro* and *in vivo*; c) reacts with NO, indirectly modulating the signaling of programmed cellular death upon stress occurrence; and d) intercalates and strengthens cell membranes. It is concluded that volatile isoprenoids are able to protect the photosynthetic apparatus of fast growing trees against a range of stresses, by mediating, directly or indirectly, the oxidative status of the leaf.

Keywords: volatile isoprenoids, plant signals, stress resistance, photosynthetic apparatus

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Volatile organic compounds in *Populus spp.* defences against abiotic and biotic stresses

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Volatile organic compounds (VOCs) are emitted by plants constitutively manner, but rapid quantitative and qualitative changes take place under abiotic and biotic stresses. Typical for normal *Populus* VOC emissions is the dominance of isoprene, while this isoprenoid is not emitted by many other deciduous tree species. Isoprene has shown repellent effects against some herbivorous insects.When damaged by herbivores *Populus* leaves start to emit inducible mono- and sesquiterpenes and Green leaf volatiles (GLVs) which are six carbon alcohols, aldehydes, and their acetate derivatives. All of these compounds can act as attractants to herbivorous and carnivorous insects. Recently we have shown that increasing night-time temperature results in similar increase of herbivore inducible isoprenoids in *Populus tremula* emissions. Some of the recentstudies suggest that under biotic and abiotic stresses *Populus* VOC emission have a typical shift from highly volatile isoprene emission to emission of less volatile isoprenoids like homoterpenes and sesquiterpenes. So far, the reason for this drastic chance in emission profile under stress is not fully elucidated, although many of the induced compounds can attract natural enemies of herbivores. I will discuss the potential ecological and atmospheric effects of the stress-induced changes in volatile emission profiles and how it is related to plant defence.

Keywords: volatile organic compounds, biotic stresses, herbivorous insects

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Scientific sessions

Session 1: Population genetics, biodiversity evaluation and conservation of genetic resources

Populus nigra as keystone species able to cope with the ongoing climate change

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Populus nigra L. is one important species of the european alluvial forests, that are protected under Habitats directive 92/43/EEC in Europe. This species is often regarded as good indicator of geomorphological and biological quality of this ecosytem and is an active support of riparian biodiversity. This species is threatened by anthropogenic disturbances and gene introgression, justifying a french and european (EUFORGEN) programme on in situ and ex situ conservation of its genetic resources.

In addition, riparian forest ecosystem and trees will face global and local climate modifications this century. *Populus nigra*, that establishes naturally within or along the active channel and that is strictly dependent of the morphodynamics of the river, will be exposed to three new threats : (i) enhancement of the frequency and severity of drought with summer decrease of the water table level, (ii) extreme heat waves, especially in summer (temperature is a key factor for survival and development of seedlings) and (iii) more intense flooding. The basis for the ability of trees to survive, adapt and evolve under changing environmental conditions is genetic diversity and the adaptive capacity to such changes involve three processes : (a) plasticity, i.e. the capacity of existing trees to respond differently to different environmental conditions ; (b) adaptation, which includes adaptive genetic diversity and the occurrence of natural selection in a dynamic system and (c) migration potential through natural dissemination of seeds to more suitable areas.

This presentation will focuss on the two first responses in *Populus nigra*, as the third process is restricted, due to the limited capacity of this pionneer species to migrate in latitude or elevation along the constrained range of the fluvial corridors. Data on genetic diversity and phenotypic variability of adaptative traits in french populations (foliar characteristics, water relations, architecture, floral and foliar phenology…) and from in situ and *ex situ* experiments, testing drought and temperature in different genetic material (cuttings, seedlings) in various conditions (sandy banks, nursery, greenhouses, growth chambers) will be presented. This communication will demonstrate that *Populus nigra* has resources (genetic characteristics) and mechanisms (ecophysiological characteristics) to cope with the ongoing climate change.

This research was mainly supported by regional, national and European funds and realized under the french Populus nigra genetic resource conservation programme.

Keywords : *Populus nigra*, genetics, climate change

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A first look at Aspen (*Populus tremula*) phylogeography across Eurasia

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We have started expanding our investigations of aspen and white poplar (*Populus alba*) phylogeography from Europe into Northern Eurasia. We are investigating variation in chloroplast DNA in order to reconstruct the evolution of the chloroplast DNA molecule, and to make inferences on any shifts of the distribution ranges of the species during the last glaciations, i.e. post-glacial re-colonization. It is interesting to us whether European populations evolved independently, or if populations form the rest of the vast distribution range of *P. tremula* contributed to the present genetic diversity in Europe (via gene flow, including hybridization, or long-distance migration events). Five regions in the chloroplast have been identified as polymorphic in one (or both) species in Europe. While a number of chloroplast variants (haplotypes) are present in both species in Europe, a geographical structure is only evident in *P. alba*, while *P. tremula* shows no evidence of separate evolution of sub-populations (e.g. during the ice ages). With the help of colleagues, we have now obtained samples from the Moscow area, Ekaterinburg, Novosibirsk/Krasnoyarsk, and Kyrgizstan. Preliminary results indicate that at least two of the chloroplast regions are also polymorphic in some of the samples. A comparison of haplotypes and their distributions in Europe and Russia / Central Asia will be presented.

Keywords: Aspen, phylogeography, chloroplast DNA

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Growing large leaves from a small-leaf gene pool: evolutionary trajectories in *Populus nigra L.* (black poplar) in context of a changing climate

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The major river systems of Europe have been heavily modified by anthropogenic factors, causing significant changes to the riparian communities. *Populus nigra L.* (black poplar), a forest tree endemic to the river floodplains of Europe, western Asia and northern Africa, is declining due to habitat loss and hybridization with domestic poplar varieties. Changes in temperature and precipitation predicted for the next century are expected to further impact *P. nigra* populations through changes to flooding patterns.

P. nigra displays significant phenotypic variation across western Europe, with trees from Spain having small leaves and a branching architecture, and trees from the Netherlands south to northern Italy having large leaves and higher biomass. Trees from France are intermediate, having a range of leaf sizes and architectures. Phenotypic differences were summarized using discriminant function analysis, and scores from the first discriminant factor correlated with the minimum monthly precipitation at the site of origin (P=0.006), indicating adaptation to water deficit has contributed to the phenotypic variation observed across the French populations.

Molecular analysis of ten microsatellite loci (SSRs) identified significant allele frequency variation among populations, with differentiation greater than observed in many forest trees ($\Phi PT = 0.121$, P=0.001). Admixture analysis indicates the Spanish samples to be distinct from trees from Italy and Germany, consistent with previous analyses of putative glacial refugia. Trees from France display genetic similarity to both the Spanish and Italian populations, however, indicating admixture contributed to the genetic variation observed in these populations.

Combining morphological, physiological and genomic data from *P. nigra* has revealed the patterns of evolution of these complex, adaptive traits in natural populations. Our data are consistent with the phenotypic divergence of putative glacial refugia located in the Iberian peninsula, the Italian peninsula, and Balkan regions. Trees from France are phenotypically intermediate to the small-leaved and large-leaved morphotypes, but are genetically more similar to the Spanish samples, indicating that these populations likely arose from the Iberian refugia but have been under selection for the large-leaf phenotype of central Europe. If morphological traits reflect adaptation to precipitation patterns, changes in summer rainfall predicted for central Europe may favour the small-leaf morphotype, changing the evolutionary trajectory of this important forest tree. Future work will investigate whole genome scans to assess the genomic and evolutionary consequences of intraspecific admixture in *P. nigra*.

Keywords: leaf size, phenotypic variation, microsatellite loci

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Optimization of Experimental Efforts in Association Genetics Studies with Clonal Material

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Quantitative traits are controlled by complicated polygenic modes. Association mapping aims at dissecting this polygenic background and the identification of gene(s) affecting these complex traits. The successful identification of genotype-phenotype association is dependent on the sample size used and the nature of the studied material. Species with cloning propensity, such as poplar and willows, offer a unique opportunity in the determination of the optimum experimental efforts needed for effective association genetics studies. Issues such as the use of clonal data vs. clonal averages, cloning vs. no-cloning, and fewer replicated clones vs. more genotypes, all have been evaluated using computer simulations. We created various population and familial structure scenarios using the correlated allelic frequencies approach of Fu et al. (2005) and utilized the Unified Mixed Model of Yu et al. (2006) to evaluate these scenarios. Evaluations were based on testing 1000 SNPs harbouring 10 randomly identified QTNs with known allelic effect. Comparisons are based on false discovery rate, ability to identify simulated QTNs, and the magnitude of the positive allelic effects.

Keywords: quantitative traits, association genetics, polygenic modes, experimental data analysis

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Contemporary gene flow between poplars with exotic component and natural populations of native species across Canada

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Populus species and their hybrids are favoured for applications in biofuels and carbon sequestration. As poplars have weak reproductive barriers between species, patterns of realized hybridization within the genus have an immediate practical relevance to the sustainable deployment of new poplar cultivars (likely to be considered as Plant with Novel Traits) for new purposes across Canada. Spontaneous hybridization between PNTs and their wild relatives may pose risks to the genetic integrity of native species, with implications for long-term forest sustainability. We have adopted an integrative approach to risk assessment that teams theoretical modelling and genomic population studies, using exotic genes from introduced species as a proxy for any genomic invasion through introgression. Previous results obtained for Eastern Canada showed that the rate of spontaneous hybridization differs markedly among pollen donor species and among native recipient species *(Populus balsamifera, P. deltoides).* Additionally, small peripheral populations are likely to be more at risk. F1s are fertile, as we observed advanced-generation hybrids (2.4%) across a sympatric zone of two native poplars *(P. balsamifera, P. deltoides)* within which exotic *P. nigra* is found. Based on frequency of F1 formation and the direction of backcrosses, *P. balsamifera* is more likely to be at risk than its congener *P. deltoides.*
Now we are conducting a survey for second and later-generation hybrids that have established within natural stands in central Canada (southern Alberta and Saskatchewan) to compare with our similar results from eastern Canada (Québec). We focus on introgression into populations of native *P. deltoides* and *P. balsamifera* from the exotic *P. nigra* and *P. laurifolia*, as *P. laurifolia* x *P. nigra* hybrids have been introduced and deployed since the 18th century to serve as windbreaks for farms (well documented by the Shelterbelt Center, AAFC-SK). There has been enough time for later-generation hybrids to have become established. We have sampled trees of various ages from 10-15 natural populations from sympatric zones of native poplars and/or exotic poplars, located in central Canada. We employed our newly developed Sequenom iPlex Gold high-throughput genotyping assay (Sequenom, Inc.) of species-specific SNP-markers that includes the exotic component *P. laurifolia.* This method enabled us to rapidly genotype several hundreds of trees using 36 multiplexed SNPs. In southern Saskatchewan, genetic analysis of seed genotypes revealed that 0.1-2.4% were spontaneous hybrids between cultivated exotic poplars and the indigenous *P. balsamifera.* This is a lower rate than that observed (20-72%) for *P. balsamifera* in eastern Canada. Notably, advanced generation hybrids were also detected in three out of the studied populations, suggesting that introgression of *P. nigra* genes into the *P. balsamifera* genome has already occurred. These studies have allowed us to obtain robust estimates of the overall level of introgression across a diversity of environments, enabling us to select localities for ecological modelling and for further work detailing the impact of hybridization on insect communities.

Keywords: spontaneous and realized hybridization, environmental variation
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Genetic diversity in *Populus nigra* (*L.*) populations along rivers in Eastern Austria, Slovenia and Croatia as revealed by microsatellite markers

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The European black poplar (*Populus nigra L.*) is a pioneer tree of alluvial forests (the priority habitate type 91E0*) and one of the most threatened indigenous tree species in Europe. We analysed the genetic diversity of 10 black poplar populations along the main river systems across Slovenia and Croatia (rivers Soca, Sava, Drava and Mura) by using six microsatellite loci (WPMS16, WPMS20, WPMS14, PMGC14, WPMS09, WPMS18) and combined analysis with Austrian data for rivers Mura and Danube with same microsatellites. Eleven common cultivated clones of *Populus* x *canadensis* were included in order to assess the level of introgression of genes of the American Eastern cottonwood, *P. deltoides*, into the gene pool of native black poplar.

The results of this study indicate that the gene pool of remaining *Populus nigra* populations maintains high genetic connectivity across three European countries, even if fragmented today. In natural populations the introgression of genes of *Populus deltoides* was very low. The obtained results are further compared with current status of *Populus nigra* populations in Central Europe and discussed in view of nature habitat conservation and its protection at regional and national level.

Keywords: *Populus nigra L.*, microsatellite markers, genetic diversity

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Meeting river restoration and conservation of native poplars on the Po river: the "Isola Colonia" case study

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Over the last decade the CRA-PLF has promoted several relevant experiences on environmental applications of poplar and willow biodiversity. In co-operation with the Po river Fluvial Park it has carried out several pilot trials on the Po river basin testing plant materials and sustainable planting and cultivation techniques. The present paper presents the preliminary results of the "Isola Colonia" project. The restoration plan was set up in 2006 in the Palazzolo Vercellese district on the upper-mid Po river catchment. An agricultural area of 30 hectares that suffered from continuous changes due to flood occurrence and was heavily infested by noxious weeds and alien species was restored considering genetic, demographic and ecological factors. Over 13.000 poles and seedlings of forest and shrubby species were used to afforest 19 hectares from 2005 to 2008. It is a multipurpose project aiming mainly to convert areas dedicated to conventional crops and intensive poplar cultivation into floodplain forests for recreational purposes and for the conservation of native poplar genetic resources. *Populus nigra* is the most representative tree species of riparian habitats but the populations of black poplar along European rivers have become sufficiently fragmented in many places that there is concern for their genetic viability; considering the suitable site conditions for natural regeneration an artificial *in-situ* gene conservation unit was created to start a dynamic conservation process for this species according to the EUFORGEN strategies. In order to increase the resilience to environmental and climate changes, a large population of over 700 black poplar poles containing a balanced sex ratio and composed of individuals characterized by high genetic diversity was established; together with other units created in the same river stretch, it could be a founder population for new establishments over rather large distances, a source for gene flow (pollen and seeds) into neighbouring scattered stands and, lastly, a seed source for reproductive material to be used for restoration activities. Genetic diversity and possible introgression in the first offsprings will be monitored together with habitat evolution, hydro-geomorphological changes, trees survival and growth performance according to different pedological conditions and water table variations. The project outputs may help riparian ecosystem managers to deal with similar situations.

Keywords: poplar genetic resources, riparian ecosystems, *in-situ* gene conservation

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Genetic variability of carbon isotope discrimination in seedlings from *Populus nigra* L. populations grown under contrasting temperatures

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Climatic events predicted for this century should involve drier and warmer summers, such as those that occurred in 2003 and 2005 in France. The higher temperatures could become a limiting factor for the regeneration of *Populus nigra L.* species. During establishment and development in summer period, seedlings must be able to resist high temperatures (until 57.8°C on sand on a Loire island in July 2009). Therefore, maintaining a high transpiration rate (E) for leaf cooling (and therefore a high stomatal conductance to water vapour (g_s)), may be a prerequisite for seedlings to survive under high temperatures. To test this hypothesis, we used bulk leaf carbon isotope discrimination (Δ) as a time-integrated index of gas exchange activity (Farquhar et al. 1989). The objectives of this study were (1) to explore the genetic variability of Δ among *P. nigra* seedlings from different female trees coming from contrasting sites and (2) the plasticity in response to two contrasting temperatures. Seeds from 16 open-pollinated females originating from the Loire river (France) and from the Paglia river (Italy) were first grown during 7 weeks in two growth chambers at 25°C. At this time (t_0), one chamber remained at 25°C and in the second one, the temperature progressively increased until 43°C until the first symptoms of wilting appeared (t_1). At t_0, an important genetic variability for Δ ($\Delta_{t0\ 25°C}$) was measured ranged from 24.0‰ to 28.0‰ in both chambers, but no provenances differences were detected. At t1, an important genetic variability for Δt1 25°C and Δ_{t1} 43°C was observed ranged from 22.0‰ to 26.0‰ in each chamber and a significant provenance effect was detected. At t_1, Δ_{t1} 43°C values were significantly higher (0.5‰) than Δ_{t1} 25°C. Moreover, a significant correlation was detected between the Δ_{t1} 43°C values and the symptoms of wilting seedlings, seedlings totally healthy exhibiting higher Δ_{t1} 43°C values. We conclude, under the hypothesis that Δ is mainly controlled by g_s, as already reported in poplar species (Monclus et al. 2006), maintaining high E and therefore high Δ may enable seedlings to survive under high temperature.

Keywords: climatic change, transpiration, carbon isotope discrimination

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Session 1: Poster

Response of an Italian black poplar collection versus bark and leaf diseases and woolly aphid

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During the Eighties and the Nineties of the past century, a set of European black poplar (*Populus nigra*) clones, collected from several Italian sites representative of the whole country, was tested to assess the complex of natural populations inhabiting various ecosystems. The evaluation of many characters, ranging from stem shape to technological and productive features, and including susceptibility to the main poplar pathogens and pests, was made on the purpose of selecting genotypes suitable for hybridization with Eastern cottonwood (*P. deltoides*) genotypes to obtain interesting hybrid (*P.* ×*canadensis*) material.
In this context, a lot of information about the response of almost 500 clones versus the main cortical and foliar parasites recurring in northern Italy was collected. It will be useful in river restoration programs in which black poplar will have a key role, and in phytoremediation as well, widely resorting to this species.
As regards *Discosporium populeum*, agent of bark cankers, the surface colonized by the fungus was measured on a sample of each clone and referred to a synthetic scale, according to five ranks (I: heavy attacks with presence of fruiting bodies; II: as I, but without fruiting bodies; III: heavy attacks only in some years; IV: fairly low or low attacks; V: low attacks, comparable to the levels on the tolerant hybrid clone â€˜I-214'). Less than 2% of northern clones was assigned to the worst rank, together with about 16% of central and almost 40% of southern clones. More than 9% of northern black poplars resulted as resistant as â€˜I-214', while none of the southern clones did. Even considering both ranks IV and V as "sufficiently resistant", 17% was represented by northern clones, 6% by central clones while no southern clone could be included in this rank. This situation is in agreement with the poor thermophylic character of *D. populeum*, that is mainly spread in northern Italy so northern genotypes could coevolve with the parasite.
The foliar pathogen *Marssonina populi* infected on the whole 23% of the genotypes tested, among which almost 40% were southern genotypes and only about 13% were of northern origin. On the contrary, the difference between infections by *M. brunnea*, agent of the brown spot disease of hybrid poplars, on northern and southern *P. nigra* genotypes was faintly unfavorable to the former. The levels of infection, however, were always very low, since black poplar could be considered as a non-proper host versus this parasite.
Also the infections of shoots by *Venturia populina*, agent of heavy spring defoliations on hybrid poplars, were limited and showing no difference between southern and northern genotypes, probably because of an equilibrium induced by the long host-pathogen coevolution, that was broken in the first decades of the past century after the introduction of the so-called Canadian hybrids (*P.* ×*canadensis*) into cultivation.
As regards *Melampsora* spp. (mainly *M. larici-populina* and to a lesser extent *M. alli-populina* too), agent of leaf rust, the infection levels were quite higher than those of the aforesaid foliar pathogens and the distribution of the genotypes among the various susceptibility ranks resulted the same irrespective of the geographic origin. Probably, the strong genetic dynamism of rust pathogens did not allow the onset of the same equilibrium between host and parasite observed in the case of *D. populeum*.
Phloeomyzus passerinii, the woolly poplar aphid, was artificially inoculated during the vegetative season *in vivo*, but under controlled conditions of humidity and temperature, on cuttings of the same *P. nigra* genotypes tested for the aforesaid pathogens. A wide variability was observed in the responses; most genotypes resulted highly susceptible and only 10% showed low infestation levels. These relatively resistant genotypes were of southern and/or Mediterranean origin, whereas the most susceptible ones came from northern and/or

continental areas. This wide set of information should be upgraded through new and frequent observations to follow the ever changing populations of pathogens, especially rust agents.

Keywords: *Populus nigra*, pests and diseases, host resistance

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Characterization of the bud-set process in *Populus nigra* L.

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Among forest trees, poplars are important components of riparian ecosystems and are now accepted by the scientific community as ideal model to study perennial plants. They offer several advantages as a model system, including rapid growth, ease of cloning, prolific sexual reproduction, small genomic size and facile transgenesis. Our work has the objective to contribute to the knowledge of the genetic control of bud set in black poplar (*Populus nigra* L.). For this purpose the genetic variability (CV_g) and the broad-sense hereditability (H^2), at individual and genotypic level, have been examined in a full-sib family of black poplar (POP5) and in different European natural populations of the same specie grown in a common garden experiment. The full-sib family was obtained from parents selected from the germplasm collection (DISAFRI – University of Tuscia) from Italian natural populations, diverging for phenology and other adaptive traits. The full-sib family was planted in two sites in Central (Viterbo 42°25'N, 12°05'E) and Northern Italy (Cavallermaggiore 44°42'N, 07°40'E). The european natural populations were sampled in 5 different countries and 820 genotypes, from 15 metapopulations were planted in a common garden study in Northern Italy (Savigliano 44°36'N, 07°37'E). A randomized block design was defined for the establishment of the experimental plantations in each site. Six complete blocks were used with one replicated genotype randomly assigned to each block. The phenological study has been realized on the basis of a protocol designed to monitor the six phenological phases of bud set in black poplar. Data analysis have allowed to decompose the contribution of the different phases to the dynamic of bud set. Four phases characterized the process (phase 2.5, phase 1.5, subprocess 1 and subprocess 2) and two phenological points were useful to compare different sites (50% of individuals in phase 2.5 and 1.5). Preliminary results of the bud set phenology in *P. nigra* natural population will be also presented and compared to the full-sib family data. These results will be discussed in relationship to the photoperiod and temperature trend introducing, in prospect, the utility of this work for genetic improvement and mapping of QTLs associated to bud set.

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Keywords: phenology, bud set, genetic control, natural populations, QTL

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Flowering phenology as an essential parameter for pollen flow modelling in *Populus nigra* L.

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European black poplar (*Populus nigra* L.) can be found in three interconnected ecosystems: (i) natural riparian forests, (ii) cultivated stands through *P.* ×*euramericana* interspecific hybrids, and (iii) ornamental plantings through *P.nigra* cv. 'Italica' (*i.e.*, Lombardy poplar). As a first step to assess the potential impact of cultivated and ornamental poplars on the genetic diversity of wild populations, pollen flow was modelled within a wild *P.nigra* stand located at close vicinity of a Lombardy poplar row.
An inventory of 484 wild *P. nigra* adult trees was realized on this 11.5 ha study site located along the Loire River. Several physical and biological parameters such as flowering phenology, physical distance, and tree size were measured on all trees. Pollen flow was estimated by collecting 1680 seeds from 31 females distributed over the site, and by modelling pollen dispersal using mating model and paternity analysis based on 10 SSR markers.
We detected a high immigration rate (45%), and 4% of the seeds could be attributed to Lombardy poplars. The model that best fitted the data involved an exponential power distribution curve with a fat-tailed dispersal kernel.
Phenological assortative mating and short distance pollen dispersal were identified as the main factors influencing reproductive success and hybridization between Lombardy and wild poplars.

Keywords**:** *Populus nigra* L., pollen flow, mating model, flowering phenology

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Adaptive mechanisms for drought tolerance identified in a European population of black poplar

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Predictions indicate that summer droughts will increase in frequency and intensity over Europe, as a consequence of global climate change. Therefore the study of adaptation to drought for this ecologically and economically significant genus is important.Wild trees of *Populus nigra* were collected in five European countries for an association genetics study, from Spain to The Netherlands, reflecting a wide range of rainfall and temperatures. These genotypes were grown in a common garden in Belgium under well-watered conditions. Carbon isotope discrimination, biomass and leaf size varied with their population of origin. Small leaf size was observed in genotypes from Spain and South France, and considered a possible adaptive trait to drought tolerance, as it prevents water loss. Six genotypes, from contrasting latitudes of origin and displaying different leaf phonologies and carbon isotope discriminations were then selected for a drought experiment in a greenhouse. Carbon and oxygen isotope discrimination, stomatal conductance, biomass, leaf and stomatal traits were measured to identify physiological differences in large- and small-leaf genotypes in response to drought. Leaf samples were also collected 20 days after drought for RNA extraction for microarray analysis and transcriptomics. Direct comparisons between the transcriptome of extreme genotypes in well watered and drought conditions provide insight into the genomic pathways induced during water deficit. These results provide important insights in genetic variation underpinning adaptation to drought across Europe that may be valuable in future conservation and management of trees in the face of climate change.

Keywords: *Populus*, drought adaptation, carbon isotope discrimination

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Trembling aspen genetic diversity and site productivity

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In this study, we benefit from a natural range of site productivity to examine the nature of the relationship at the genotypic level using trembling aspen (*Populus tremuloïdes*), a pioneer tree species in the boreal forest that is clonal and regenerated through root suckering immediately after disturbance. We hypothesized that aspen genotypic diversity will peak at intermediate site productivity with more clones represented by few ramets per clone (high evenness) meeting their minimum resource requirements. Few more competitive clones unevenly represented (low evenness) are expected to dominate rich stands. To test our predictions we investigated how aspen genotypic diversity (richness (R) and evenness (E)) varied across each of nineteen stands whose site indices (dominant aspen tree height at 50 years) range from 6.6 to 29.9 m. As a secondary goal, we sought to determine how allelic diversity changed along the productivity gradient and whether this variable showed the same pattern as for genotypic diversity. Size-frequency distribution of aspen genotype was highly left skewed with 88% of the distinct multilocus genotype represented by ≤ 3 ramets.The proportion of single ramet clones is higher in high productivity site index class with an average R of 0.53 in comparison to low productivity site index (mean R = 0.32). Evenness (E) values indicate that genotypes are more likely to be represented by similar number of ramets in the most productive stands (E=0.90) and intermediate site index classes (E=0.84) than in least productive stand (E=0.68). Our results also indicate an overall excess of heterozygous in higher productivity classes.

Keywords: Aspen, genotype, genetic diversity, evenness, productivity

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Restoration of Populus species in reparin zone of Serbia – formation of genotype collection

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European black poplar (*Populus nigra* L.) and white poplar (*Populus alba* L.) are among dominant tree species in autochthonous biocenoses of riparian zones in Serbia. However, these species become rare and even endangered, especially in case of European black poplar. Their habitats are used for the establishment of plantations of more productive poplar species euramerican (poplar and eastern cottonwood), as well as for other purposes like: agriculture, urbanization, flooding control etc.
The protection of habitats and reforestration by these species are of crucial importance in restoration of autochtonous biocenoses in reparian zones. Beside the preservation of biodiversity, they are important in flooding control, control of undergrownd water level, water quality, etc.
More than 60 genotypes of european black poplar and white poplar from 18 populations throughout the Serbia are colected and propagated by means of micropropagation. The ACM medium (Ahuja, 1983) was used supplemented with BAP, kinetin and silver ions in order to establish tissue culture and micropropagate chosen genotypes. The genotypes are propagated by axilary buds to reduce chances for somaclonal variation. The genotype collection in form of stoolbed established by this material will be the base for the production of planting material for nursery production and further afforestration, presumably in protected areas.

Keywords: European black poplar, White poplar, micropropagation

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The AgCanBaP Balsam Poplar Collection: An example of poplar genetic resource conservation in Canada

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Balsam poplar (*Populus balsamifera* L.) is a natural element of the Canadian sub-boreal and boreal forest. The range of balsam poplar extends from the Atlantic coast of Canada to Alaska attaining the most northerly distribution of any North American tree species (from 42 ^{o}N to 70 ^{o}N). Because of the vast range it was determined that the species was an excellent candidate for genetic and eco-physiological studies related to climate change and adaptation.

The intent was to sample the geographic range of the species (Fig. 1), therefore we concentrated on sampling populations from five north-south transects across the range of balsam poplar in Canada. In each transect 5 to 9 populations were identified and dormant hardwood cuttings collected from 15 randomly selected individual trees per population. From 2005to 2006 a total of 45 balsam poplar provenanceswere assembled, propagated and planted in a live collection at Indian Head Saskatchewan (50^{o}N). The collectionwas given the name AgCanBaP recognizing the commitment of Agriculture and Agri-Food Canada to long term gene conservation. Additional collaborative common gardenswere established at Vancouver BC (49^{o}N) and Fairbanks Alaska (65^{o}N).

The collection and resulting common garden trials have provided opportunities for a variety of studies on the physiological, ecological and evolutionary genetics of balsam poplar. The collection is also providing foundation stock for Canadian poplar breeding programs and *ex situ* conservation of a representative sample of the species. Collaborative research with the AgCanBaP collection is underway at Agriculture and Agri-Food Canada, Indian Head SK; University of British Columbia, Vancouver BC; University of Alaska at Fairbanks;and NRCan-CFS Laurentian Forestry Centre, Quebec, QC. This paper provides an overview of collection strategies employed, summarizes collaborative research activities under way and reports results on provenance growth and leaf morphology variation at the Indian Head common garden.

Keywords: Ex Situ Conservation, Populus, Adaptation, Provenance Trial, Adaptation

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(65^{o}N).

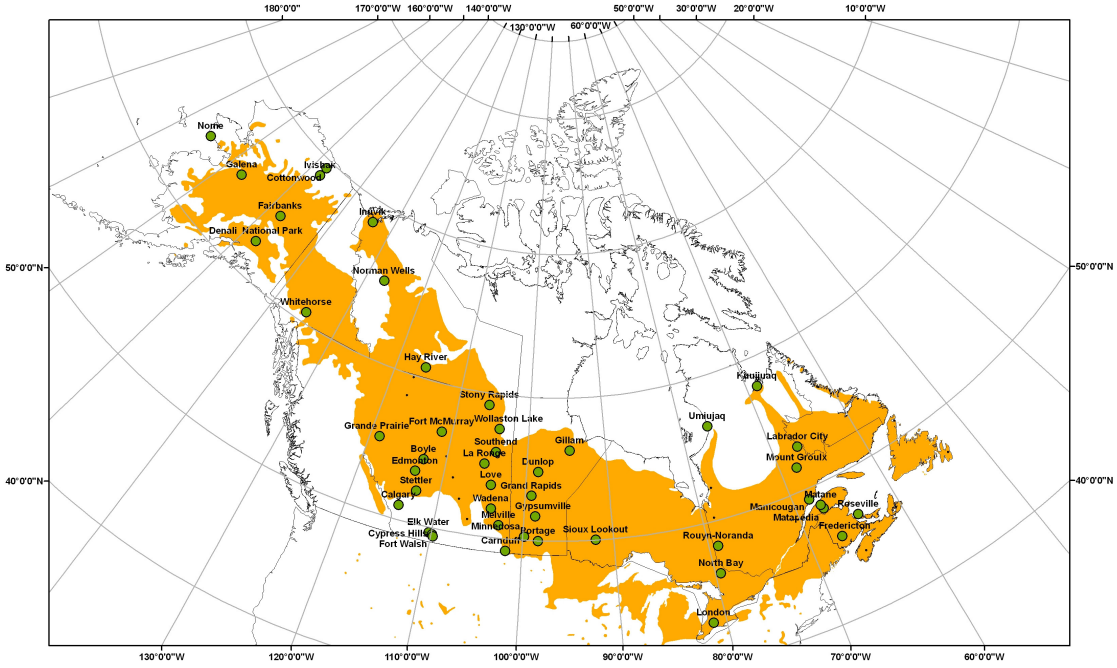


Figure 1: Provenance locations in AgCanBap balsam poplar collection

Tolerance to high temperature and genetic diversity of black poplar families from two environmental contrasting sites

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The European black poplar is an ecological pioneer tree species, adapted to dynamic riparian zones such as river valley floodplains. Our work was focused on studying the consequences of increasing temperature on genetic diversity of two provenances of *Populus nigra* L. coming from two contrasting sites: 1) the Loire river with mild climatic conditions and minimum artificialwater level in summer and 2) the Paglia river with higher water-level fluctuations and semi-arid conditions. We collected seeds, as open pollinated families,from 8 mother trees on each of the two different sites. Seeds were grown for 7 weeks in two growth chambers at 25°C. At this time (t_0), in one chamber temperature was kept at 25°C and in the second one the temperature was progressively increased to 43°C. The number of individuals with symptoms of wilting were recorded at three successive times (t_1, t_2 and t_3). Nine microsatellite markers were used to measure the genetic diversity between the two provenances, among individuals within the same collection site and among individuals within the 16 open pollinated families. At the increasing temperature, differences in temperature tolerance were observed among families in both provenances. Genetic indices as observed and expected heterozygosity (Ho and He), Shannon genetic diversity index (I) and fixation index (F) were calculated. Differenceson genetic diversity and fixation indexwere detected between provenances as well as between families of the same site.

Keywords: Black Poplar, seedlings, temperature, genetic diversity, microsatellite markers

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Hybrid poplar in arid regions: the case for a versatile clone, OP-367

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Recent interest in the production of hybrid poplar in on-farm, planted forests or agroforestry systems is increasing due to the multiple uses of this versatile genus (*Populus* sp.). An array of products include fiber for excelsior and soil conservation materials, peeler logs for pulp, plywood, and building materials, and more recently, as a feedstock for power generation or cellulosic conversion to liquid fuels. Most production of hybrid poplar in the United States has been concentrated in the northeast, southeast, north central, and Pacific Northwest states where precipitation is plentiful. In an attempt to capitalize on increased solar radiation and higher growth rates, limited poplar production in the Pacific Northwest was initially transferred during the 1980's from the mesic coastal zone west of the Cascades to the xeric zones east of the Cascades in the central Columbia River basin of Boardman, Oregon. The success of early drip-irrigated plantations in this arid environment propelled the expansion of the technology to over 14,000 ha currently managed in the area.

Northwest New Mexico has similar environmental conditions as the central Columbia River basin. Following the Boardman model, drip-irrigated hybrid poplar research was started in 2002 at New Mexico State University's Agricultural Science Center in Farmington. Trials were established in 2002, 2003, 2005, and 2007 using OP-367 as a check. This clone was suggested as being suitable because of its vigor and productivity in the Boardman plantations. Both high (4,300 stems ha^{-1}) and low (750 stems ha^{-1}) density trials have been conducted to mimic different production strategies for a range of potential markets. The clone OP-367 has consistently produced greater wood volume and total biomass than other clones in these trials. This hybrid, originally cloned in the 1920's by Oxford Paper Co. in New York State, continues to demonstrate its exceptional productivity, paradoxically in arid regions.

Keywords: hybrid poplar; drip irrigation; arid/semi-arid environments

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Morphogenetic studies on natural European Aspen (*Populus tremula* L.) populations in Giresun-Sebinkarahisar District of Turkey

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In this study a large variation of morphological characters has been identified in sample trees which were selected from six different population of *Populus tremula.* Five trees in each studied population and totaly 30 sample trees were selected in six populations. In selected trees, the length of leaf stem, the width of the leaf, the leaf length, leaf surface spaciousness, the weight of the air-dried leaf, the angle of intersection of the leaf with the branch, the width of bud, the length of bud were measured. Moreover, variations of the germination were also determined in sample trees which are selected from two populations (population D and population E).

According to germination results; it has been determined that the germination of seeds that belongs to sample trees in D and E populations started after 24 hours. It was found germination differences between these two populations. Germination percentage of population D was higher than population E. The percentage of the average germination has changed 34.8 to 92.6 %. Overall percentage of germination was found as 65.88 %. Germination was completed in 5 days and most of the germination was completed in 24 hours. 99 % of germination was carried out in 5 days.

According to all measured morpogenetic charecters, a strong correlation was found among the populations, with $p<0.01$ significance level. Population F was determined as the mostly different population, in terms of morphological characters in studied populations. With 82.2 % success, 6 populations were statistically differentiated.

Keywords: European aspen, populations, morphogenetic characteristics

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Growth Performance of F_1-Hybrids, Backcrossed Hybrids and F_2-Hybrids of *Populus tremula* and *Populus tremuloides*

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F_1-hybrids of European aspen (*Populus tremula*) and quaking aspen (*P. tremuloides*), backcrossings (European aspen × hybrids) and F_2-hybrids ($F_1 \times F_1$) were made successively following an incomplete mating design. Performance of the total of 19 progenies was compared at eight sites among the three hybrid levels with 4, 5, 8 and 2 pure *P. tremula* progenies respectively. Despite of differences between sites, the relative performance among the four groups was constant. Both, the F_1-hybrids and the backcrossed progenies showed a superior growth and survival in comparison to the progenies of pure Eurasian aspen. Among the group of F_2-hybrid progenies the performance was rather homogeneous however, they reached only the level of the pure Eurasian aspen.

Keywords: Species-hybrids, F_1- F_2-generation *Populus tremula*, *P. tremuloides*, backcrossed progenies

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Genetic Analysis of Hybrids from Populus deltoides with Tacamahaca by using SSR Markers

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Poplar is exemplary diploid, meaning it has two copies of gene- one from each parent. To develop desirable genetic traits or elite genotypes from parents, cross breeding is basic way to select new varieties. But traditional selecting program needs long time field trials to possess beneficial traits. Molecular markers may be effective tools to shorten breeding cycles. Simple Sequence Repeats (SSR) markers, or microsatellites, are one of the most advanced marker technologies available in genetic research today. SSR markers linked with polymorphisms (alleles) of *Populus* parents or offspring were used to determine the genomic variation and genetic relations. Differences between the DNA sequences in parents and offspring can be used as markers associated with the genes responsible for desirable traits. The codominant SSR markers are also excellent tools for comparing the allele variation between parents with offspring in order to look for genetic clues and assessing the relation between their polymorphisms and genotypes.

We applied *Populus* SSR primers previously from IPGC SSR resources (*http*://www.ornl.gov/sci/ipgc). In this study six SSRs (PMGC2818, PMGC2392, PMGC2804, PMGC2143, PMGC2541, PMGC562), out of the 20 mapped by Cervera et al (2001) in *Populus deltoides* or *P. trichocarpa* , were selected for their polymorphisms in 6 *Populus* parents(*P. deltoides* clones and natural Tacamahaca species *Populus cathayana*, *P. suaveolens*) and 56 their offspring. One parent originated 1or 2 loci for each SSR. Each offspring out of *Populus deltoides* with Tacamahaca species generated 2 to 4 loci, which was visualized 3 forms−whole, part DNA bands of parents or their regular translocation. Each offspring hybridized between *P. deltoides* clones generated 2 to 3 loci, and most like female DNA bands. These SSRs were not only response to homozygous or heterozygous genes but also revealed breeding genetic rules, which made it possible to analysis offspring genotypes, and compare their relevance with morphological and phonological characteristics at al. in order to help selecting fast-growing and resistant traits early. We also noticed SSRs selected from *P. deltoides* showing different DNA bands in Tacamahaca species, and vice versa, except PMGC2541.These SSR marker is perfect to classify hybrids form species-specific combination or among different forms in same combination, but the offspring from same form in same combination is difficult to separate by using these markers.

Keywords: hybrids, molecular markers, SSR.

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Urban environmental genomics: Elucidating components of asymmetrical gene flow among *Populus* at the Base de Plein-air de Ste-Foy, Québec, Canada

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Detailed environmental genomic studies of hybrid zones in urban habitats may provide insight into components of reproductive isolation and the maintenance of species boundaries between natives and exotics. Previous broad surveys of introgression across an eastern Canadian contact zone of two native poplars (*Populus balsamifera, P. deltoides)* and one European exotic (*P. nigra*) revealed that 2.4% of all sampled trees showed spontaneous admixture, that advanced-generation hybrids were consistently biased towards *P. balsamifera*, and that sites with hybrids were significantly more disturbed than pure stands. One of these perturbed localities, the Base de Plein-air de Ste-Foy, has natural regeneration of *P. balsamifera*, *P. deltoides* and hybrids, adjacent to a city park containing a few mature *P. balsamifera*, and *P. nigra* 'Italica'. Deeper sampling of 100 reproductively mature trees at the site reveals a near equal sex ratio (45 female, 55 male), while genotyping with an interspecific SNP array confirms the presence of a significant number of hybrids of different classes (22%) in addition to pure native species (8% *P. balsamifera*, 70% *P. deltoides*). Despite this striking differential abundance between parental species, hybrids of both F1 and advanced-generation classes are biased toward *P. balsamifera*. Mean reproductive output appears comparable between pure species and hybrids (1000 seed weights, raw number of viable seed), yet germination assays indicate significantly higher mean germination success of *P. balsamifera* and its backcrosses (97.8%, 92.6%) than F1s (77.9%) or *P. deltoides* (54.4%). Potential confounding effects of sampling date on germination success are being examined. A series of inoculation experiments with various species/strains of *Melampsora* is underway under outdoor and indoor conditions to phenotype pure and hybrid classes for the susceptibility of these trees to infection by disease. Preliminary inoculation experiments indicate that the *P. deltoides* genome confers quantitative resistance again the European rust, *Melampsora larici-populina*, a growing concern in North America. Although prezygotic isolation through delayed flowering time of pure *P. deltoides* could potentially contribute to the pattern, it appears as though post-zygotic mechanisms and generalized lower fecundity of *P. deltoides* may also be key factors in the asymmetry. Experimental plantings of open-pollinated progenies from pure *P. balsamifera*, *P. deltoides*, and F1 hybrids in the field and greenhouse will further compare the potential for natural regeneration and recruitment in the different ecological zones of the site. This may help to elucidate the impact of anthropogenic disturbance on patterns of hybridization in urban environments.

Keywords: fitness, introgression, exotic species, disturbed habitat

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Genetic analysis on growth, branch and leaf traits of hybrid clones between *Populus deltoides* and *P. ussuriensis*

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Poplars are considered the fastest growing trees under temperate latitudes that are widely used in the wood industry for the production of paper, plywood, matches, and light packaging materials or for bioenergy. Early selection is an important aspect for the genetic improvement of *populus*, and several beneficial traits including growth, branch and leaf are commonly considered as the pivotal characters on poplar early selection. In this study, a total of 27 clones of poplar hybrids from *Populus deltoides* × *P. ussuriensis* were assessed with *Populus* × *beijingensis* plant as control, and a comprehensive genetic analysis on growth, branch and leaf traits of these clones (four-year old juvenile trees) was carried outã€,For growth traits, the results suggested large variations and significant differences ($p < 0.01$) in H (height), DBA (diameter in basal area) and DBH (diameter at breast-height) among 28 clones. Average H, DBA and DBH of 28 clones at 4 ages were 757 ± 28 cm, 10.8 ± 0.6 cm and 8.3 ± 0.6 cm, respectively. For branch traits, ANOVA analysis showed that F values of number, length, diameter and angle of branch were from 15.02 to 27.75, indicating the extremely significant differences ($p < 0.01$) in branch traits. Meanwhile, striking variations in leaf traits including length, width, area, number of vein, tooth number of edge, radicle angle, tine angle and SLW (specific leaf weight) among the 28 hybrid clones and significant differences ($p < 0.01$) in leaf traits were also found. In addition, correlation analysis revealed significant positive correlation between H, DBA, DBH and branch or leaf traits. As a conclusion, our study provided basic knowledge for the multiple traits-based combination selection in *P. deltoides* × *P. ussuriensis* hybrids.

Keywords: *Populus deltoides*, *P. ussuriensis,* growth traits, branch, leaf, hybrid clones

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Pollen of the wild European black poplar (*Populus nigra* L.) may act as 'mentor pollen' in helping hybrid poplar plantations of *P. x canadensis* spreading their genes

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Hybridization and genetic swamping by related non-indigenous *Populus* taxa is supposed to threaten the European black poplar (*Populus nigra* L.). Cross-compatibility relationship between the European black poplar and the hybrid *Populus* x *canadensis* was studied by a series of hand-pollination cross experiments carried out in the greenhouse. We investigated the relative fertility of hybrid F1 clones of *P.* x *canadensis* and the potential direction of gene flow between both taxa. We tested the effect of pollen interactions on siring success on both *P. nigra* as *P.* x *canadensis* females by using a pollen mixture technique combined with a paternity analysis. We also determined whether a 24 hour delay in arrival time of pollen of *P.* x *canadensis* altered the frequency of hybrid formation and seed set on *P. nigra*.
The potential for gene flow was mainly, although not completely, unidirectional with *P.* x *canadensis* as the female parent. We observed a reduced pollen and seed viability of *P.* x *canadensis* clones compared to clones of the parental wild relative *P. nigra*. Surprisingly, when in combination with *P. nigra* pollen, pollen of F1-hybrids of *P.* x *canadensis* significantly better sired on its own flowers.
The results of this study suggest that hybrid sterility of *P.* x *canadensis* may be overcome in the field by interaction with pollen of the related wild *Populus nigra*. Sequential pollinations of *P. nigra* with a 24 h. time delay between the application of hybrid pollen and pollen of the own species resulted in a slightly higher number of hybrid progeny and a lower seed set. This may indicate that hybridization between *P. nigra* and *P.* x *canadensis* may result in waste of reproductive effort of *P.nigra*.

Keywords: conservation, potential gene flow, mentor pollen

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Are the populations of *Salix daphnoides* in the Western Carpathians (Czech Republic) natural?

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Salix daphnoides Vill. is distributed from southern Europe to Scandinavia. Its natural distribution in Central Europe is confined to mountain regions of the Alps and the Carpathians. The Carpathian Mts. extends to the Czech Republic only in its easternmost part (Beskydy Mts.) and the species is naturally occurring only in this part of the country. From the ecological point of view, we can divide Carpathian populations into three groups: *i*) occurring on river gravel banks; *ii*) Wet *Cirsium* meadows; and *iii*) Ash-alder alluvial forests. Populations from the first biotype are ecologically similar to those ones from the Alps and the Carpathians, are rich in number of individuals and are age-structured. Whereas populations from the second and third biotope are spatially limited and age structure is not well differentiated even in larger populations. We therefore hypothesize that at least some population of the Western Carpathians (Beskydy Mts.) are not natural, but of (older) human origin. *Salix daphnoides* serves as a honey-bearing tree. We therefore address the question whether or not populations on biotopes ii) and iii) might represent spontaneously and clonally reproducing willows for past hundred years. To test this hypothesis, we studied population dynamics, mode of reproduction and genetic diversity of these Czech populations. We characterized populations in the field (age structure, vegetation, spatial structure of populations) and consequently analyzed their genetic diversity using microsatellites (SSR, simple sequence repeats). So far, we found that populations from biotope i) are genetically diverted and the distribution of alleles is significantly in accordance with HW equilibrium. On the contrary, populations from the biotope ii) and iii) are confirmed to be formed by one or two genotypes only suggesting it anthropogenic origin.

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Keywords: *Salix daphnoides*, natural populations, microsatellites

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Fast growing willow family tree clones and hybrids - experience and research in Latvia

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Aspen breeding research in Latvia have been started in early 1950-th. Phenotypic plus trees from stands in best site indexes have been selected. Selections covered all territory of Latvia. First hybrids between American aspen (*Populus tremuloides* Mich.) and common aspen (*Populus tremula* L.) get in 1960-th, but only in 2003 restarted hybrid aspen breeding program. First experimental trials of "energy willows" varieties from Sweden have been established at 2004.

Results of first step are demonstrated based on data from hybrid aspen trials, where tree height and diameter has been measured, stem straightness, branch thickness and angle visually assessed (in 6 grades) and number of spike knots noted. Higher heritability was found in the coefficient of tree height (H^2=0.29 to 0.47) and stem straightness (H^2=0.36 to 0.73), while other traits heritability coefficient values are relatively low. The results show, that selection of clones to maximize both the tree height and stem straightness, can yield a significant selection effect. A high rank correlation of productivity and quality traits was found between 8 and 12 years old trees: tree height (r=0.95), stem diameter (r=0.80), trunk volume (r=0.87), stem straightness (r=0.94), branch thickness (r=0.80), and branch angle (r=0.84). This suggests that superior aspen hybrid clone selection can be done 8 years of age, thus greatly shortening the breeding cycle, and increasing work efficiency.

Evaluation of aspen wood mechanical properties (second selection step) reveal that wood density can be used as a selection criteria, since it has high correlation with bending and compression strength (r= 0.91and r=0.94 respectively).

In vitro proliferation coefficient at least 5 is required for efficient industrial propagation, which is rather high in comparison to average and stipulates high selection intensity in third selection step.

In the register of forest reproductive material 18 hybrid aspen clones have been included. These clones are selected according all mentioned above criteria.

Factors which limiting establishment of willow plantations in Latvia are insufficient knowledge and lack of experience in environmental effect of waste water sludge and wood ash applications, as well suitable species and varieties for local conditions. There have been tested "Swedish" (on experimental trials) and "Latvian" (local farmers) - technologies of establishment and management of plantations. Some promising clones of local species *Salix burjatica* Nasarow and *Salix viminalis* L. are planted on trials and still are in test stage to compare them with breeds and varieties recommended for energy woody crop plantations.

Latest preliminary experimental results show, that the most productive varieties, which can be used in commercial scale in Latvia, are *Tora* and *Torhild*. Recommended rotation cycle in well managed plantations is 3 to 2 years in Latvia. Important is not only fertilizer, but also effect of variety, for example, in the study 3 years old non-fertilized and 2 years old fertilized plantations of *Sven* have nearly the same growing stock 9 - 11 t_{dry} ha^{-1}. Following productivity measures are estimated – varieties *Tora* and *Torhild*, which are recognized within the scope of this study as the most promising in Latvian conditions, in 3 years rotation cycle produces 28 - 31 t_{dry} ha^{-1}.

During this year have been planted some willow varieties from Hungary and Poland and varieties *Klara, Gudrun, Inger* and *Tordis* from Sweden for testing them in Latvia conditions.

Keywords: hybrid aspen, willow, Latvia

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Assessing the dynamics of riparian vegetation through multitemporal segmentation of remotely sensed data and field plot sampling: a case study

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Natural river systems are characterized by highly temporal and spatial dynamics influenced by their hydrology and geomorphology. Riparian vegetation is adapted to exploit the dynamic habitat created by the disturbance regimes and plays an important role in the maintenance of stream and riverbank stability. In addition, riparian zones act as ecological corridors promoting biological diversity and riparian species are recognized as vital to the integrity of river ecosystems. The main objective of this study was to examine the dynamics of riparian vegetation, mainly composed by poplars and willows, along a stretch of Paglia river in central Italy, using multitemporal remotely sensed imagery. Polygons obtained by the multitemporal segmentation applied to aerial orthophotos, acquired in 1989 and 2006, were classified by photointerpretation within a buffer of 200 m along 14,5 km of the river, to quantify land cover changes over the examined period. The study was also supported by the Digital Canopy Model derived from LIDAR data. Field sampling was carried out by random plots in the area under investigation, to assess the change of aboveground carbon stocks of the riparian tree vegetation. The results showed strong developmental dynamics associated with a significant expansion of riparian forests.

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Keywords: poplar and willow natural populations, riparian vegetation, multitemporal remotely sensed imagery

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Session 2: Poplar genomics for breeding and selection

Epigenetic, water deficit and productivity in poplars.

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Several reports on annual plants have already shown the involvement of epigenetic modifiers such as DNA methylation in their adaptation to abiotic stresses. Nevertheless, the genotypic variations of epigenetic modifiers, their possible correlations with morphological traits and the impact of water deficit have not been described for perennial plants. Six genotypes of *Populus deltoides x P. nigra* were subjected or not to a moderate water deficit treatment. Various morphological traits such as the height of the plants, their biomass and the total leaf area were measured to characterize the productivity in both conditions. Levels of DNA methylation, histone acetylation and the activities and isoform accumulation of the corresponding enzymes were measured at the shoot apex, the site of morphogenesis. Genotypic variation was observed for the morphological traits and the epigenetic variables and correlations were established among them. Genotypic variation for DNA methylation was detected in hybrid poplars. A positive correlation was demonstrated between DNA methylation percentage and productivity under well watered conditions. While there was a general decrease of growth for all genotypes in response to a moderate water deficit, genotypic dependant variations of DNA methylation were found suggesting different strategies among hybrids (Gourcilleau *et al*. 2010). In order to confirm these data and to test the long term stability of these epigenetic modifications, a new set of eight hybrids was analysed for global DNA methylation and productivity nine months after being subjected or not to a water deficit. Finally, the relationship established between global DNA methylation in shoot apex and productivity in *Populus deltoides x P. nigra* hybrids was investigated in three populations of *P. nigra*.

Keywords: genomics, epigenetic, water relations

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Whole Genome Resequencing and Population Genomics of *Populus trichocarpa*

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The era of whole-genome resequencing has become a reality with the widespread availability of economical next-generation sequencing technologies. These capabilities have created a plethora of new challenges and opportunities in tree genomics. One of these opportunities is the ability to perform large-scale association studies in which polymorphisms are assessed for their associations with interesting phenotypic traits. We are undertaking a large-scale association study in the black cottonwood tree, *Populus trichocarpa*, which is focused on identifying the determinants of cell wall composition and structure using a candidate-gene approach. The first step in this process is polymorphism discovery. To this end, we have used the Illumina GA2 genetic analyzer to sequence total genomic DNA to a depth of 32X for 16 *P. trichocarpa*, 2 *P. deltoides*, and 1 *P. tremuloides* tree. This dataset has yielded 16.8 million polymorphisms, including more than 11 million that were polymorphic in *P. trichocarpa*. We have also resequenced 10 genes representing 48 Kb in 48 *P. trichocarpa* individuals, including 15 that were shared between the datasets. There was a 93% correspondence in shared genotypes between the datasets, and tolerable rates of type I (no Sanger polymorphism corresponding to an Illumina polymorphism) and type II errors (no corresponding Illumina polymorphism). PCA and structure analysis revealed significant population structure corresponding to a north-south division of the *P. trichocarpa* population, corresponding to divisions in Northern California and near the Columbia River, in addition to a strong division in British Columbia. In keeping with these subdivisions, there was a substantial North-South gradient in allele frequencies for significantly more loci than would be expected by chance. Linkage disequilibrium was low on average, declining to background levels within 1 kb for the vast majority of locus pairs. However, regions of elevated LD and low population recombination rates were common, including extensive regions on each chromosome that may correspond to centromeres. There were also a large number of recombination hotspots across the genome, including loci with several orders of magnitude of range in estimated recombination rates. These data are being used to select SNP that will be assayed using Illumina BeadArrays in 1100 genets in an association population that is replicated at four field sites across the *P. trichocarpa* range. This large-scale dataset should provide fundamental insights into genome structure and evolution in this ecologically- and commercially-important species.

Keywords: genomics, adaptation, linkage disequilibrium

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Next-generation sequencing as source of SNP and structural variants information to improve breeding programs in *Populus nigra*

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Progress in applying new methods to forest tree breeding has understandably been slower because of the difficulty to acquire genomic data in the major commercial species. The situation has improved rapidly through whole genome sequencing in *Populus trichocarpa*, and the construction of large EST databases in poplar. Nevertheless, the genomic tools to develop molecular markers for advanced breeding are still not adequate, in particular for the native European poplar species, *P. nigra*. In the framework of a joint resequencing effort undertaken by the EU funded projects Evoltree, Noveltree and EnergyPoplar, we aim at producing a sequence-marker map made of SNPs, short InDels and copy number variants covering the whole black poplar genome by means of next generation sequencing.

First, by using the Illumina/Solexa technology we resequenced three different European individuals at a coverage > 20X in order to obtain both SNP and structural variants information and a draft sequence for the black poplar genome. Pilot analyses showed the feasibility of using *P. trichocarpa* genome sequence as reference for *P. nigra*, given that 75% of *P. nigra* reads were uniquely mapped on the P. trichocarpa sequence. We will present data on types and frequency of sequence variants at the whole genome level based on the sequences of the three individuals.

Then, to maximize SNP discovery all over the genome, sequence reads obtained from 50 additional European clones, which have been resequenced at 2X coverage, will be aligned to the black poplar draft consensus sequence. Such SNP detection will be supported and confirmed by *P. nigra* sequences of several gene fragments, which were resequenced in different natural European populations by using the traditional Sanger method.

The final SNPs data will be used for high throughput genotyping of an association population made of 1100 poplar clones established in two experimental sites, in order to monitor genetic variation during the selection process and develop Marker-Assisted-Selection and Gene-Assisted-Selection breeding programs.

Keywords: Next-generation sequencing technologies; *Populus* genome; SNP discovery

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Transcriptome resequencing of *Populus trichocarpa* individuals from northwestern North America: applications for SNP discovery, association genetics, and the biology of wood formation.

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Poplars and aspens (*Populus*) are well suited for highly productive plantation forestry in temperate climates. British Columbia is a centre of diversity for *P. trichocarpa*, black cottonwood, which dominates many riparian ecosystems in the province, and has a range that partially overlaps with that of *P. balsamifera*, balsam poplar. Recent advances in sequencing technology enable unprecedented amounts of DNA sequence data from natural tree populations to be collected. This information can be used for a wide variety of purposes, including population genetics, conservation, association genetics, genome annotation, and studies of alternative splicing and gene expression levels. In our work we are using next generation sequencing for a genome wide, range-wide survey of xylem expressed transcripts in black cottonwood (*Populus trichocarpa*). Using the mRNA-SEQ approach, mRNA was extracted from developing secondary xylem of 20 *P. trichocarpa* accessions grown in a common garden, spanning a latitudinal gradient from 59°N to 44°N from British Columbia, Canada to Oregon, USA. Following conversion to cDNA, libraries were constructed and sequenced to high depth using the Illumina Solexa platform and the paired end read approach. For each transcriptome, up to 100M reads, and 4 Gbp of sequence was obtained, and reads were mapped to the *P. trichocarpa* Nis-1 reference genome sequence. Xylem transcriptome data for a *P. deltoides* individual was also obtained. For each accession more than 10,000 genes were sequenced at a depth of coverage of 10X or more, allowing nearly 400,000 SNPs to be identified relative to Nis-1. Because we used non-normalized mRNA libraries, depth of coverage per gene can be used as a proxy for level of expression, revealing potential genotype-specific variation in expression levels of certain genes. Analysis of transcriptome data also revealed extensive alternative splicing, and we have generated dataset of over 3000 novel intron-exon junction borders. As well, up to 6% of transcriptome reads mapped to intergenic regions that could represent transcripts of non-annotated genes, and we have obtained data consistent with the existence of chimeric mRNA species. Population genetic analysis of the SNP data, and its use in understanding *P. trichocarpa* population structure, genetic diversity and as a tool for an association study for candidate gene alleles underlying variation in biofuels, biomass, and adaptive traits will be discussed.

Key words: SNP, transcriptome, genetic variation.

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Branching out from model organisms: insight into the control of shoot development in Short Rotation Coppice willow.

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Short rotation coppice (SRC) willow is grown as a source of renewable energy, providing bioenergy with a near neutral net carbon output. Central to the suitability of willows for biomass production is the vigorous re- growth that occurs following the removal of apical dominance by coppicing. The regulation of this process, with respect to bud and shoot behaviour, is poorly understood at present. By exploiting (i) knowledge from Arabidopsis on branching and bud development; (ii) similarities between the poplar and willow genomes: (iii) segregating willow mapping populations and: (iv) allelic diversity within the UK National Willow Collection (NWC), we are beginning to gain insight into the coppicing response.

Initial QTL analysis in the K8 willow mapping population has placed two of the *More AXillary branching* (*MAX*) genes, previously identified in Arabidopsis, within a locus for shoot development. QTL-associated alleles and diverse *MAX* alleles from the NWC have been cloned and used for mutant rescue of the Arabidopsis *max* mutants. Preliminary results suggest that the degree of mutant rescue varies between allelic variants studied to date, indicating that certain alleles perform their tasks more effectively than others. To enable the discovery of additional components of bud and shoot regulation, a further 11 QTL mapping populations have been generated and are now undergoing phenotypic assessmentments in field trials. These populations comprise around 500 individuals each and are derived from parents that exhibit diverse shoot development phenotypes.

For more detailed phenotypic characterisation of coppice stool development, a dedicated field trial was established in April 2008. This trial comprises four genotypes (A-D) of the K8 mapping population with the following contrasting phenotypes; (A) low shoot number, low stem diameter, low yield; (B) low shoot number, large stem diameter, high yield; (C) high shoot number, low stem diameter, high yield; (D) high shoot number, large stem diameter, high yield. A subset of stools was extracted from the trial in January 2009 after first coppice, and bud formation, position and number were examined. Analysis revealed variation between genotypes in the proportion of buds that sprouted. This process will be repeated in January 2011, two years post-coppice. Measurements of the stools were first taken in August 2008 and will continue to be taken twice yearly, in winter and summer, to follow potential genetic differences in stool development including self-thinning.

To examine the role of hormone response in the physiological response to coppicing, hormone assays developed previously for Arabidopsis were adapted for use on willow shoots. The branching physiology of the K8 parents and the four genotypes of the coppice stool trial are currently being analysed.

This project aims to generate molecular tools for use in the breeding of improved biomass willows, both in terms of increased yield and the production of end products tailored to a range of energy production processes.

Keywords: willow, *Arabidopsis*, branching

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Association genetics of traits controlling lignin and cellulose biosynthesis in Black Cottonwood (*Populus trichocarpa*, Salicaceae) secondary xylem

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Recent interest in poplars as a source of renewable energy, combined with the vast genomic resources available, has enabled further examination of the genetic diversity underlying the lignin and cellulose biosynthetic pathways. In this study, an association genetics approach was used to examine individual genes and alleles at the loci responsible for complex traits controlling lignin and cellulose quality and quantity in black cottonwood (*Populus trichocarpa* Torr. & A. Gray). Forty candidate genes of the lignin and cellulose biosynthetic pathways were resequenced in a panel of 15 unrelated individuals to identify single nucleotide polymorphisms (SNPs). A total of 1,536 SNPs were subsequently genotyped in a clonally replicated population (448 clones). The association population (1,080 trees) was phenotyped using pyrolysis molecular beam mass spectrometry (pyMBMS). Both single marker and haplotype-based association tests were implemented to identify associations for composite traits representing lignin content, syringyl/guaiacyl ratio, and C6 sugars. A general linear model approach, including population structure estimates as covariates, was implemented for each marker-trait pair. This study identified 27 highly significant associations (FDR $Q < 0.10$) unique across 40 candidate genes in three composite traits. Of these, five associations were found to be in the coding region of the candidate genes, with two being nonsynonymous. Haplotype-based associations were performed on 181 amplicons across the 40 genes. For lignin content and C6 sugars, 23 significant haplotypes within 11 genes were discovered. The majority of markers (56%) in all three composite traits were characterized as having additive modes of gene action. These associations provide insight into the genetic components of complex traits involved in the lignin and cellulose biosynthetic pathways in black cottonwood.

Keywords: genomics, association genetics; SNPs; *Populus trichocarpa*; LD; biofuels; wood quality; genotyping

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Quantitative Trait Loci analysis of traits related to biomass production in willow (*Salix* spp.)

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Willow trees (*Salix spp.*) have received increasing attention during the last decades for the purpose of bio-energy production in Europe. Willows are especially suitable for biomass production by short rotation cultivation because of their fast growth and high capacity to resprout after coppicing. However, achieving high biomass yields with relatively low inputs of energy requires development of high-yielding cultivars both suitable for plantation in different environments and efficient in the use of available resources. Selection and breeding of biomass willows would largely benefit from the use of easily detectable genetic markers linked to genes underlying economically important traits. In this study we present the results of quantitative trait loci (QTLs) analysis performed on several traits that are important determinants of biomass production and promising targets for the breeding of biomass willows. We mapped QTLs for growth, resource-economy-related traits such as biomass allocation, water use efficiency, leaf area efficiency, leaf nitrogen content and leaf nitrogen efficiency, and phenology traits such as timing of bud burst, growth cessation, and leaf senescence, in an experimental cross of *Salix viminalis L.* and *Salix schwerinii* E. Wolf . In the QTL analysis we used a dense genetic linkage map that we have constructed for the same population. The map was built using SNP markers located within genes evenly distributed throughout the Populus trichocarpa genome and it is thus aligned to the poplar physical map. We found high degree of synteny and gene order conservation between the genomes of willow and poplar. The use of the map aligned to the poplar genome allowed us to search for annotated genes the regions of the poplar genome corresponding to the QTLs found in *Salix* and to identify candidate genes putatively involved in the control of these traits.

Keywords: willow, genetic map, QTL, molecular markers, growth, phenology, resource-economy traits

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Effect of water deficit on wood formation of poplar clones: physiological and transcriptional analysis in cambial region

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Poplars are among the fastest growing trees in temperate latitudes. Their high productivity is associated with large water requirements. As a consequence water deficit condition is the major limitation to growth and represents the main problem affecting wood productivity. Wood formation is being increasingly studied at cellular and biochemical levels; however, gene expression and regulation during wood formation in response to abiotic stress deserves to be deeply investigated. In this study we used a double approach coupling the physiological parameters to the transcript analysis of defined gene networks, ROS defense and carbohydrates/cellulose metabolism, chosen on the base of a previous microarray analysis at whole genome level.

Young poplar trees (*Populus deltoides*, *Dvina* and *Populus* x *canadensis*, I-214) were grown under reduced soil water availability. The stress intensity was estimated by measuring soil water content, predawn leaf water potential, leaf relative water content, leaf growth, leaf conductance and maximum photosynthesis, maximum daily shrinkage of the stem. Radial growth was recorded by point dendrometers and the effect of water deficit on differentiating xylem and wood was investigated by high resolution stem growth analysis and anatomical investigation. After 16 days of totally withdrawing water, cambial region was sampled by scraping technique. Transcript analysis was carried out on RNA extracted from the cambial region of lyophilized stems by means of relative quantitative RT-qPCR using selected genes of the two metabolic networks.

Our results showed that, under these conditions, poplar plants put into action mechanisms to reduce water loss through stomatal closure which is coupled with a water translocation from the stem to the leaves and primary meristems. After 16 days of withholding irrigation, significant differences were recorded between treatments and genotypes in term of leaf RWC, total leaves number, total leaf area increase and stem length increase. On the basis of our results, the hybrid I-214 has showed a higher susceptibility to water stress than Dvina. RT-qPCR analysis evidenced differences at transcription level between clones and genes of the different metabolic networks.

The relevance of the metabolic changes in cambial region in relation to plant growth during water stress is discussed.

Keywords: water relations, candidate genes, carbohydrates, wood formation

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Session 2: Poster

Leaf proteome analysis of eight *Populus* ×*euramericana* genotypes: genetic variation in drought response and in water-use efficiency involves photosynthesis-related proteins

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Genetic variation of leaf proteome in drought response was investigated among eight *Populus* ×*euramericana* genotypes contrasting for their leaf carbon isotope discrimination (Δ), a surrogate of intrinsic water use efficiency (Wi). Plants were grown in open field on two similar plots. Drought was induced by an 86-day irrigation cessation on one plot while a second plot remained regularly irrigated. Using 2-DE, 863 reproducible spots were detected; about 60% presented at least one significant effect i.e., Treatment, Genotype and/or Genotype by Treatment interaction effect. A significant Genotype by Treatment interaction was evidenced for 62 reliably identified proteins among which, about 65% consisted in chloroplast-associated proteins either involved in the Calvin cycle or in the electron transport chains. The other proteins were involved in oxidative stress, amino acid or protein metabolisms. Correlations between protein abundance and Δ variations were found for 45 reliably identified proteins. The abundance of RuBisCO activase isoforms scaled negatively with Δ regardless of the treatment, suggesting that a large Wi could be due to higher abundance of RuBisCO activase. Under control condition, abundance of enzymes involved in carbon fixation was also negatively correlated with Δ, whereas abundance of enzymes involved in photorespiration or respiration was positively correlated with Δ.

Keywords: water-use-efficiency, water stress, photosynthesis, proteomics

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Comparative mapping analysis and QTLs associated to adaptive traits across European *Populus* native species

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White and black poplars (*Populus alba* L. and *P. nigra* L.) are native to Eurasia and their genetic diversity for their growth potential and stress-adaptive mechanisms is underexploited. A better knowledge of their genome will allow more effective protection and use of critical genetic resources for breeding programs. The main objective of this study was to find quantitative trait loci (QTL) associated with adaptive traits and compare the *P. nigra* and *P. alba* maps among them and with the genome sequence of *P. trichocarpa*. For that, four parental genotypes were selected from contrasting natural Italian populations and crossed to generate two F_1 mapping pedigrees. Highly informative genetic maps were constructed based on amplification fragment length polymorphism (AFLP) and simple sequence repeat (SSR) markers. These maps showed good overall colinearity to each other and a good synteny between the two species for the very high number of common markers. Both species allowed a complete alignment with the 19 haploid chromosomes of the *P. trichocarpa* genome sequence. Comparative analysis among maps of *P. alba* and *P. nigra*, and the *P. trichocarpa* genome sequence revealed a putative region of chromosome translocation between linkage groups II and XIV in *P. nigra*. Such comparative mapping is a crucial step to gain insight into the way in which genome structure and function differ among *Populus* species. Genetic mapping of QTL have also proven its utility in the identification of genomic regions associated with the expression of adaptive traits, such as growth, crown architecture, and phenology. The identification of QTL intervals on the four maps allowed us to perform QTL projection on the *P. trichocarpa* genome sequence, and to identify genome regions including candidate genes for adaptive processes. This approach will greatly facilitate the study of the genomics of *Populus* and how it can be exploited in applied breeding programs. Selection based on molecular markers tightly linked to traits of commercial importance could ultimately prove useful as a powerful adjunct to conventional approaches in *Populus* improvement programs.

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Keywords: comparative mapping, QTL, AFLP, SSR

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Wood production determinants in poplar: where are we?

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Because of its vigorous growth, poplar may be a good candidate for sustainable biomass production to cover renewable energy needs. Due to the wide diversity available within the genus, a screening of the genotypes to be used is necessary. However, selection of suitable genotypes is a time-demanding process and it has to base on relevant traits. For this purpose, the relevance of the traits is mostly estimated through the strength and robustness of their relationships with the target properties, e.g. yield, resistance to biotic and abiotic constraints, efficiency to use water, wood quality, etc.

For bioenergy purposes, a high biomass production remains the main selection objective. However, to avoid concurrence for land use with food agriculture, bioenergy tree plantations are likely to be principally installed on marginal areas, where water and nutrients may be scarce. Traits such as the efficiency with which the trees use resources may be of interest in selection programmes.

Yield is dependent on the individual growth of the trees which is itself defined by numerous determinants: phenology (e.g. length of growing season), canopy and whole tree architecture (e.g. foliar index, ramification, rooting), leaf function (e.g. photosynthetic assimilation, stomatal behaviour, efficiency to use nutrients and water), or biochemistry (e.g. carbon and nitrogen storage and allocation). During the last decade, numerous studies have aimed at disentangling the relationships among these different categories of traits, at leaf, tree and canopy levels, in the genus under various growth conditions and for diverse plant materials in terms of age and parentage. If some traits have proved their relevance as selection criteria, some others showed erratic relationships with yield as well as with resource-use efficiencies.

We propose here a synthesis of the results from 15 studies dealing with productivity determinants. The strength and robustness of the relationships among potential productivity determinants in response to (1) the pedoclimatic context, (2) the age of the plant material, (3) the water availability, (4) the hybrid formula, and (5) the time course, are summarized.

The comparison of these studies shows that many traits can not be considered as relevant selection criteria because genetic variation within poplar clones is too narrow and/or because their relationships with wood production is highly dependent on the growth conditions. Basically, traits can be classified in three categories: (1) traits linked to wood production irrespective of growth conditions and plant material (e.g., leaf area, sylleptic branchiness, bud burst), (2) traits for which the relationships with wood production are dependent on environment and/or plant material (e.g., specific leaf area, nitrogen content, water-use efficiency), and (3) traits showing no link with wood production irrespective of growth conditions and plant material (e.g., chlorophyll content, carbon content).

Keywords: Wood production; Breeding; Genotype x environment interaction

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Phylogenomics of Chloroplast Oxylipin Synthesis Genes using *Populus* as a Model Species

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Plant oxylipins, which include jasmonic acid (JA) and its methyl ester, are signaling hormones critical for regulating plant carbon allocation, growth, and response to biotic and abiotic stresses. JA is synthesized from chloroplast membrane-derived fatty acids via the octadecanoid pathway. We used phylogenomics to characterize three critical gene families involved in JA synthesis: Lipoxygenases (LOXs), CYP74 cytochrome P450s, and allene oxide cyclases (AOCs). JA synthesis is initiated by LOXs, which comprise a large family of fatty acid dioxygenases that have an affinity for linolenic and linoleic acids derived from the chloroplast membrane. The first dedicated step in JA synthesis is presumably regulated by allene oxide synthase (AOS), a member of the large CYP74 family, which converts LOX-derived fatty acid hydroperoxides to allene oxides. Two other CYP74 proteins, divinyl ester synthase (DES) and fatty acid hydroperoxide (HPL), also utilize the hydroperoxide as a substrate to produce a diverse suite of potential oxylipin products. For JA synthesis, AOC then catalyzes the stereospecific cyclization of unstable allene oxide to stable *cis*-12-oxophytodienoic acid (OPDA). OPDA, which presumably has a role in plant growth regulation distinct from JA, is transported out of the chloroplast and subsequently converted to various jasmonates via the remainder of the JA biosynthesis pathway. Within *Populus*, we found 20 LOX genes, 3 DES genes, 2 AOS genes, 1 HPL gene, and 3 AOC genes; which support previous claims of a genetic bottleneck within the oxylipin and JA pathways observed in *Arabidopsis*. Unlike *Arabidopsis*, the diversity of oxylipin genes is greater in *Populus*, suggesting a more intricate role for oxylipins within the biology of *Populus*. The maintenance of a potential bottleneck on such a presumably vital signaling phytohormone is the subject of ongoing work in the genus *Populus*, including the interrogation of other plant reference genomes. We discuss the importance of molecular regulation of stress tolerance and carbon allocation in plants—and forest trees in particular— to providing a framework to enhancing biomass allocation and atmospheric carbon sequestration.

Keywords: phylogenetics, *Populus*, oxylipins, jasmonic acid, biotic and abiotic stress.

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Demographic histories of Salix viminalis and Salix schwerinii – Inferred from multiple nuclear loci

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The increasing interest in bioenergy crops has put the potential of *Salix* biomass production into focus. Breeding *Salix* varieties, adapted to different environments and resistant to pathogens for high and sustainable biomass production is of main concern nowadays. The most commonly used species for commercial breeding of *Salix* is *Salix viminalis* and *Salix schwerinii* and both pure and hybrid variants exist on the market. There is an ongoing program at the Swedish Agricultural University in Uppsala called 'High and sustainable biomass production from willow: Integrating molecular genetics, ecophysiology and plant breeding' with the general aim of implementing marker-based selection with focus on different resistance and tolerance traits and for traits related to biomass production in *Salix*. Within the program, mapping populations and linkage maps have been developed and we are currently performing QTL analyses and fine mapping of selected regions aiming at identifying easily detectable genetic markers linked to genes underlying economically important phenotypic traits in order to increase the efficiency of *Salix* selection and breeding. Markers suitable for marker-based selection must be in linkage disequilibrium (LD) with QTL. Depending on characteristics such as mating system, population size, evolutionary history, recombination rate and breeding strategy, LD varies widely between species and breeding populations.

In the present study we have re-sequenced 30 nuclear loci in 25 *Salix viminalis* and 24 *Salix schwerinii* clones in order to estimate nuclear DNA sequence variation. This data will be used to estimate LD to understand the required density of markers for implementing marker-based selection in commercial breeding of *Salix*. In addition, we will study the degree of divergence between the two species as well as other demographic processes that may have affected their evolution.

Keywords: *Salix*, breeding, genetics

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Identification of rare genetic variants in *Populus nigra* using pooled multiplexed sequencing

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In poplar, cinnamyl alcohol dehydrogenase (*CAD4*) is involved in the biosynthesis of lignin, a major component of plant cell wall which negatively impacts paper pulp processing and biomass fermentation to ethanol.

In the framework of EnergyPoplar project, we set out to screen 1152 poplar trees for mutations in *CAD4*. After Sanger sequencing of 384 samples, we analysed the additional 768 by Illumina next generation sequencing in multiplexed runs, and obtained complete *CAD4* sequences for 12 pools of 64 subjects each.

To measure performance of our method, we also obtained Sanger sequences for two of the 12 pools. Considering Sanger sequencing as the gold standard, next generation pooled sequencing had 100% sensitivity, 99% specificity and AUC=0.99. In addition, we observed strong minor allele frequency correlation between the two sequencing methods (r=0.96). Sensitivity analysis revealed that the best frequency threshold to be used in SNP identification was 0.47%. Using this threshold we identified in the whole sample 51 SNPs; of them 9 were non-synonymous. Our results show that multiplexed pooled next generation sequencing is a fast, reliable and cost-effective screening strategy to identify rare variants in a large sample. We then extended mutation screening to 4 additional genes involved in lignin biosynthesis, and identified in total 31 non-synonymous SNPs; among them two caused a premature stop codon.

Keywords: lignin biosynthesis, gene mutation, SNP

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A model for linkage analysis in a triploid population

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Triploids have been recognized to occur more frequently in natural and experimental populations of many species including poplars than previously thought. Despite their economic and biological importance, linkage analysis for triploids has not well been explored. Herein, we present a statistical model for estimating and testing the linkage between molecular markers in a triploid population. The model is formulated within the maximum likelihood framework and implemented with the EM algorithm. The model is extended to estimate the linkage of genetic loci with dominant markers and multilocus markers. We have also explored linkage analysis in a mixed population composed of diploids and triploids. Simulation studies were performed to test the statistical behavior of the model. The new model for linkage analysis with triploids will provide a powerful tool to study the genetic diversity and origin of a triploid population.

Keywords: triploid, statistical model, maximum likelihood, EM algorithm

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Breeding of hybrid aspen in Finland: performance of individual clones in growth, pathogen resistance and energy production.

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A breeding program with hybrid aspen (*Populus tremula* x *P. tremuloides*) was started in Finland during the mid 1990's. In the beginning individual superior hybrid aspen trees were selected from stands and trials that were left over from an earlier period of hybrid aspen cultivation during the 1950's and 1960's. From these selections the first series of trials including 25 hybrid aspen clones was established in southern Finland in 1998 using one year old plantation material. These trials were measured after 5 and 12 growing seasons. On the best sites after 12 growing seasons the average height growth varied between clones from 14.7 m to 7.4 m with an average diameter growth of resp. 13.4 cm and 4.9 cm. Individual trees reached a height of over 20 m.

In addition to the fiber production concept hybrid aspen is nowadays also considered as an alternative for growing biomass feed stocks. From a number of clones in the measured trials trees were harvested during the winter 2009-2010 to estimate the biomass yield, and to analyze stem and crown components for fuel characteristics.

Ten clones included in these trials were also included in field trials to study the resistance of the clones against leaf and shoot blight (*Venturia macularis*) and canker (*Neofabraea populi*). The results showed variation in resistance against both pathogens. Also correlations between resistance and growth will be analyzed.

The results of these studies will be presented, as well as a short overview of the present status of the breeding program.

Keywords: Aspen, wood production, pathogen resistance, bioenergy

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DNA methylation as a component of poplar phenotypic plasticity toward water availability

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Poplars are among the fastest growing trees in temperate latitudes but their high productivity associated with large water requirements shows strong genotypic variability and plasticity facing a water deficit (Marron *et al*., 2005; Monclus *et al*., 2006; Bonhomme *et al*., 2008). The importance of epigenetic mechanisms in the control of phenotypic plasticity has now been established in plants and recently in *P.euramericana* hybrids submitted or not to a water deficit (Gourcilleau et al., 2010). Thus, we have shown a correlation between productivity traits and global DNA methylation of shoot apex's cells in well-watered condition while a genotype-dependent DNA methylation change was observed in water-deficit condition. Our objective is now to identify target loci of global DNA methylation changes participating to the phenotypic plasticity toward water availability. In this context, we have developed a MeDIP-seq approach (Methyl DNA ImmunoPrecipitation sequencing) focusing on preferentially DNaseI-digested chromatin (non-condensed) of *P. trichocarpa* (clone 101-74) shoot apex. Indeed, the non-condensed fraction of chromatin was expected to be enriched in expressed model genes and to have lower repeated sequences that are preferentially localized in heterochromatic (condensed) regions. Then, 5-methylcytosine antibodies were used to immunoprecipitate methylated sequences in this non-condensed chromatin fraction. Using Illumina high-throughput sequencing (Solexa) technology, methylated sequences were identified with variable coverage density, mapped on chromosomes and analysed for their genomic context. Then, microarray slides based on our methylated sequences have been generated and their analysis (in progress) will identify the variations of DNA methylation and expression of these sequences between shoot apex in well-watered or not conditions.

Keywords: epigenetic mechanisms, phenotypic plasticity, DNA methylation

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Mapping of QTLs for resistance to leaf rust in Salix

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The leaf rust fungus (*Melampsora larici-epitea*) can cause great damage in biomass willow plantations. Production losses of up to 40% have been estimated in susceptible varieties. A high rust resistance is therefore one of the most important breeding goals, and improvements of resistance has also been achieved in the breeding programs during the years. A lasting problem is that the willow leaf rust fungus, like many other rust fungi, has a high capacity to change and overcome resistance of the plant. This is especially true for the kind of resistance that is governed by a single major gene, which is common in many crops. It is therefore preferable to utilize also other types of resistances that are based on several minor genes (i.e. quantitative resistance) and hence more complicated for the fungus to adapt to. The aim of this work is to characterize resistance genes in the current breeding material and develop markers for a variety of resistance genes to be used in marker-assisted breeding.
To identify various resistance genes we are utilizing two linkage maps of *Salix*, one based on a hybrid backcross between *S. viminalis* and *S. schwerinii* (S_1), and the other based on a pure *S. viminalis* family (S_3). By doing controlled infection experiments in growth chambers we were able to characterize various resistance reactions in these families. Measurements were made of latent period, number and size of rust pustules, and necrotic flecking. We have also done assessments of rust resistance in the field on the same individuals. By doing QTL mapping (mapping of quantitative trait loci) a number of genomic regions that are important for rust resistance have been identified in the two populations. In two cases, QTLs were located close to markers for annotated rust resistance genes from the *Populus* genome.

Keywords: disease resistance, genomics

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Revealing the genetic background of poplar breeding material

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The objective of this reserach was to determine the genetic profiles of 15 poplar genotypes in order to evaluate their genetic potentials for breeding programs. The genotypes were of different genetic background (clones, cultivars, hybrids) of two sections (*Aigeiros* and *Tacamahaca*). The AFLP, SSR and RAPD markers polymorphism and its power of discrimination were employed in this study. Six AFLP primer combinations and 16 SSR markers (PTR and WPMS) were used in genotyping while two SSR markers, MmCAT-30 and MlCAG101 and two RAPD markers ($OPG10_{3400}$ and $OPZ19_{1800}$) linked to *Lrd*1 locus were used for screening of tolerance to causal agent of poplar rust *Melampsora sp*. in the studied genotypes. Marker systems applied successfully amplified the genetic profiles of the genotypes of interest. According to cluster and principal coordinate analysis, the differences within genotypes and their belonging to particular poplar section were occurred. Two SSR loci, MmCAT-30 and MlCAG10, indicated the existence of two *Melampsora* species in poplar genotypes. The first is the widespread in Serbia, *M. larici-populina*, while the other according to our preliminary results could be *M. medusae*. Screening 15 poplar genotypes with two RAPD markers it was revealed the existence of locus $OPG10_{3400}$ in three genotypes belonging to *P. deltoides* and *P.*x *euramericana* species while locus $OPZ19_{1800}$ was detected in one *P. deltoides* genotype.

When evaluating polymorphisms within poplar genotypes, SSR, AFLP and RAPD DNA markers applied in this study proved to be a useful tool for genotyping and marker-assisted selection of leaf-rust resistant genotypes. They will be of significant importance for poplar breeding programs.

Keywords: AFLP, SSR, RAPD, genetic characterization, poplar rust, poplar

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A dynamic model for temporal patterns of transcriptome-proteome correlations

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Cells respond to developmental signals by dynamically modulating changes in gene and protein expression. During a process where genes encode proteins, a dynamic relationship takes place between proteome properties and gene expression in complex biochemical networks. In this talk, I will present a dynamic model for identifying the temporal pattern of co-expression of genes and proteins in a time course. This model is formulated within a mixture-model framework in which time-dependent co-expression patterns are approached by biologically meaningful mathematical equations. By estimating and testing the mathematical parameters that specify dynamic changes of gene and protein expression, the model allows the optimal classification of correction patterns between transcriptional and proteomic profiles. The statistical properties of the new model are investigated by simulation studies. As new technologies enable more accurate and cheaper measurement of time-related gene and protein expression profiles, the model will provide a powerful tool for constructing a regulatory genetic network for important biological processes.

Keywords: developmental signals, gene and protein expression

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Populus breeding and hybrid poplar development program in Minnesota, U.S.A.

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An applied *Populus* breeding program located at the Natural Resources Research Institute in Duluth, MN has been producing and testing intra- and inter-specific hybrid poplar germplasm for the past 15 years (1996 – 2010). The primary *Populus* species of interest for breeding and testing in Minnesota include *P. deltoides*, *P. nigra*, *P. maximowiczii* and *P. trichocarpa*. Eastern cottonwood selections from a previous University of Minnesota collection and from other regional *Populus* improvement programs formed the base breeding population. Genetic diversity of the base population has been supplemented with periodic open-pollinated seed collections from native trees along Minnesota river-systems and from natural populations located outside the North Central region of the United States. Seed collections and parent materials have been exchanged with cooperators working in the United States, Canada, Europe and Asia for the purpose of long-term parent population improvement and evaluation under Minnesota climatic conditions. Parent selections have been collected from field trials containing pedigreed populations and many have been archived in dedicated breeding orchards. The operational logistics of each screening phase from the initial nursery progeny plantings through the family-level field trials, advanced clone testing and preliminary yield block evaluations will be discussed. Following a 1- to 2-year nursery screening phase, hybrid poplar pedigrees are established in replicated field trials imbedded in commercial fiber woodlands plantations for critical evaluation throughout the entire rotation period (10-12 years). Growth, disease resistance and ease of establishment are among the selection criteria of new clones for advanced clone and yield testing. General patterns and results of growth gains, disease incidence and clone performance from current Minnesota-based field trials will be presented.

Keywords: *Populus* genetics, hybrid poplar, tree breeding, clone development, population improvement

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Dynamic transcript profile of adventitious rooting in *Populus*

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Adventitious rooting ability is one of the most important target traits for the genetic improvement of economically important plant species. However, despite extensive physiological and anatomical studies of adventitious rooting, the genetic and developmental mechanisms that regulate this biological process are poorly understood. In this study, we used a genome-wide transcriptional analysis to quantify the temporal correlation between transcript changes of genes and the initiation and progression of adventitious roots in a hybrid poplar, Nanlin895, derived from *Populus deltoides* (I-69) and *P. x candensis syn. euramerican* (I-45). At five distinct stages of root growth, we measured the amount of expression for each gene. Using a nonparametric functional clustering approach, a total of 56055 genes were categorized into 11 groups in terms of their expression trajectories. Some groups of genes are expressed consistently over time, although they display different extents of expression, whereas some are expressed, following a logistic curve as expected for root growth. It is interesting to find that the expression of some genes is periodic and exhibits an oscillatory behavior. The results from this study shed light on the genetic control of adventitious rooting ability in poplars.

Keywords: functional clustering, hardwood cutting, transcriptional regulatory network

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Comparative transcriptome profiling of salt stress response in *Populus alba* L.

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Soil salinity is an important limiting factor to tree growth and productivity. *Populus alba*, an economically and environmentally valuable tree, is moderately salt-tolerant. The *P. alba* natural populations are adapted to contrasting environments, thus providing the genetic resources needed to identify key genes for tolerance to abiotic stress, such as salinity. Yet, physiological and molecular mechanisms of responses to salinity stress remain mostly unclear in this species. To elucidate the genetic basis of variation for salt tolerance in *P. alba*, we analyzed the gene expression profiles by using a poplar cDNA microarray. Two contrasting genotypes: 6K3, a salt-sensitive clone, and 14P11, a salt-tolerant clone, originating from North and South Italy, respectively, were challenged with shock salt stress (200mM NaCl). The transcriptome change induced by salinity was analysed in leaves collected from three to 72 hours of stress. Remarkably, the widest changes in number of transcripts were observed at 72 hours after stress initiation, a time that has been further investigated. Approximately 20% of transcripts showed up- or down-regulation at this time point. Down-regulated genes were significantly higher than the up-regulated ones in 6K3 clone, whereas there was no such difference in 14P11 clone. While, most down-regulated transcripts were common to the two genotypes, the up-regulated ones were mostly regulated in a genotype-specific manner. The commonly down-regulated transcripts correspond to 67 genes and are functionally related to a variety of biological processes (Gene Ontology annotation), including photosynthesis, response to stress, carbohydrate metabolic process, and formation of precursor metabolites. The commonly up-regulated transcipts represent 13 genes and are mainly involved in response to stress and transport. The functional annotation of up-regulated transcripts discriminated the molecular response of the two genotypes in terms of molecular function, cellular component, and biological process. Particularly, in 14P11 clone, 21 genes were specifically up-regulated and mainly involved in response to stress, transport, and biosynthetic process, which may contribute to salinity tolerance. But, only 11 genes were specifically up-regulated in 6K3 and were involved in response to stress and nucleoside, nucleotide and nucleic acid metabolic process. The comparative analysis revealed that most genes affected by salt stress behaved in the same manner and were related to the down-regulation of leaf functions, negatively affected by the high salinity. However, the differences in transcriptome between the two contrasting genotypes could address the molecular basis of intra-specific variation in salinity tolerance.

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Keywords: *Populus alba*, salt tolerance, transcriptome

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Discovering genes related to biomass recalcitrance using a combination of high throughput and high resolution plant cell wall characterization methods

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The BioEnergy Science Center (BESC) research program addresses the challenge of converting cellulosic biomass to sugars with an interdisciplinary effort focused on overcoming the recalcitrance of biomass. A key strategy of the BESC is to develop high throughput pipelines that can supply large populations of genetically diverse plant materials as well as measuring important phenotypes such as cell wall chemistry and biomass recalcitrance. Cell wall chemistry traits are determined using high throughput analytical pyrolysis that can screen hundreds of samples per day. A second high throughput system has been constructed that reduces conventional pretreatment and enzymatic hydrolysis methods to microtiter plate-scale with the advantage of being able to quickly screen thousand of samples. These high throughput screens provide an effective means for identify interesting individual samples that can be subjected to more detailed analysis. Results will be shown demonstrating how the cell wall chemistry phenotypes have been used to identify quantitative trait loci in poplar across multiple studies and laboratories. We will also report on the analysis of ~900 poplar samples collected across the Pacific Northwest showing the effect of geographical and environmental factors on cell wall chemistry and recalcitrance.

Keynote: Recalcitrance, High Throughput Analysis

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Exploring the developmental epigenome of *Populus trichocarpa*

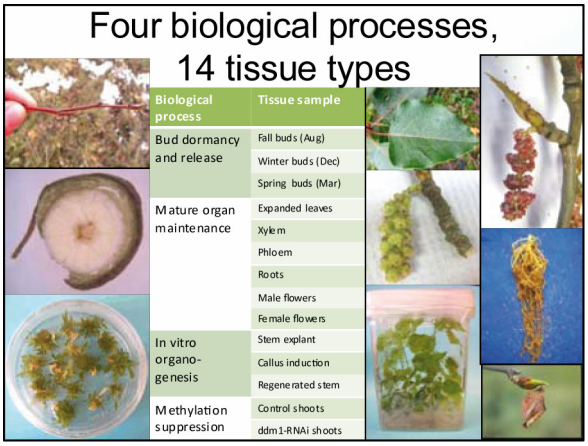
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In addition to its role in suppression of the activity of highly repetitive DNA, DNA methylation has recently been discovered to be commonplace among active genes in plants. Its function in this context, however, is largely unknown. To gain insight into the genic role of DNA methylation in a tree species, we have begun to characterize epigenetic variation during development in poplar. We are using 5- methyl-cytosine DNA immunoprecipitation ("MeDIP") and chromatin immunoprecipitation ("ChIP") followed by high-throughput (Illumina/Solexa) sequencing to study (1) several tissue types (2) stages of the annual dormancy cycle and (3) the effect of *in vitro* regeneration. We will present results that show how methylation varies between 5', coding, and 3' regions of genes in poplar, and that DNA methylation is unusually high in or near to a subset (~1%) of annotated genes in the poplar genome and its relationship to microarray-based gene expression patterns.

Keywords: HT sequencing, methylation, development



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Predicting candidate genes for biomass traits

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The phenotype-genotype problem starts with phenotypic variation and tries to determine which genes are involved. Quantitative trait loci (QTL) are chromosomal regions identified through linkage analysis that assign variation observed in a phenotype to a region on a genetic map. Even well-defined QTL, however, may encompass many potential candidate genes, perhaps hundreds. It is therefore hard to objectively choose underlying candidate(s) that drive the phenotype. We are developing bioinformatics tools to support systematic analysis of QTL regions and prioritise genes for experimental analyses. Prioritisation is generally based on evidence that supports the role of a gene product in the biological process being investigated. The two most important bodies of information providing such evidence are bioinformatics databases and the scientific literature. In this work, we first present a knowledge base (KB) for the Poplar genome created by using comparative genomics, data integration and text mining methods of the freely available Ondex system (www.ondex.org). Poplar proteins in this KB are enriched with functional, phenotype and literature information. Second, we demonstrate our novel web-based tool to query the Poplar KB and analyse a QTL region to identify and prioritise candidate genes for complex phenotypes such us biomass production.

Keywords: QTL, Phenotype-genotype, candidate gene prioritisation

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Functional genomic approaches in poplar

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During the last few decades molecular technologies including genomics, gene technology and proteomics have become an important part for sustainable management of forests and forest tree breeding programs all over the world. Structural genomic approaches like sequencing of the whole genome of an organism are a first step to unravel the molecular basis of life and adaptation of life to the environment. In plants, genome sequencing has been completed in the overall model Arabidopsis thaliana (The Arabidopsis Genome Initiative 2000) and in rice (Rice Genome Sequencing Project), a model cereal. As the third plant species the genome of the black cottonwood (*Populus trichocarpa*) is now available in public domain with the beginning of the year 2004 (International Populus Genome Consortium). The complete sequence for *Eucalyptus* is coming soon.

Sequencing of the genome of an organism provides a tremendous amount of data and information, however, without any idea on gene functions. To understand how a cell works one needs to know the function of almost every gene in its genome. Once whole-genome information is available for an organism, the challenge turns from identifying the parts to understanding their function as well as to improving genome structure. In the short term, the first goal is to assign some element of function to each of the genes in an organism also referred to as 'functional genomics', and to do this with high-throughput, systematic approaches.

Gene technology is a very powerful tool in forest tree breeding programs as well as for functional genomics in order to unraveling gene functions. Tree species under consideration are within the genera *Populus* (poplars), *Picea* (spruces), *Pinus* (pines) and *Eucalyptus* (eucalyptus), and many transgenic lines carrying several gene constructs have been produced mainly under lab conditions so far.

Various reverse and forward genetics strategies are ongoing to determine the functions of genes and regulatory sequences. A significant progress has been made in large scale functional analysis of a number of tree species including conifers, and thousands of ESTs are available from poplar, eucalypts, pine and other tree species. Alternatively, analysis of mutants provides an elegant tool to understand gene functions. However, while many resources are available for high-throughput functional genomics like investigations of proteins ("proteomics") or metabolites ("metabolomics"), in vivo resources in form of tagged mutants are still lacking in forest trees.

In this paper, examples of forest tree gene technology ("transgenesis") comprising approaches on the targeted exchange of genes, transfer of "tags" (T-DNA or transposons) or RNAi strategies as well as analysis of natural mutants will be given.

Keywords: Poplar,tagging, mutant

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Towards marker informed poplar breeding

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Marker informed breeding could be a favorable opportunity to accelerate cultivar selection in poplar. Linking genotype to phenotypic variation of important economical and adaptive traits is the prerequisite. The two strategies chosen are QTL detection in mapping pedigrees and association studies in natural populations. An intra-specific mapping pedigree *Populus nigra* x *P. nigra* of 300 individuals segregating for leaf rust resistance is available. Another large progeny of 1400 individuals derived from a cross *P. deltoides* x *P. trichocarpa* for which two major factors and QTL of rust resistance segregated is already available. QTL for phenology and water use efficiency were also mapped. Large scale phenotyping of wood properties is underway. An association mapping population of 1100 *P. nigra* individuals coming from France, Germany, Netherlands and Spain was evaluated for rust resistance in both controlled and field conditions. Even if most of the genetic variation for rust resistance could be observed at the genotype level, significant differentiation between natural populations was detected. Taking advantages of genomics development in poplar including structural and functional genomics, positional, functional and expressional candidate genes were identified for rust resistance, phenology and wood properties. It became clear that SNP in those genes could be ideal combined to HTP genotyping technologies to identify causative polymorphism for phenotypic variation. We developed collections of SNP useful for both genetic and association mapping. With the help of the genome sequence, unique primer pairs were automatically designed and tested for their uniqueness before PCR amplification and HTP Sanger re-sequencing. Based on the analysis of 850 gene fragments, an average of 1SNP/90bp were found in the three *Populus* species studied. SNP appeared fixed in each species after comparison of SNP position between the three species. Analysis of linkage disequilibrium in *P. nigra* showed a rapid decline of LD with physical distance

Keywords: marker informed breeding, QTL detection, association studies

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Molecular markers for identification of species, hybrids and clones within the genus *Populus*

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Many of the poplar species within a section but also between sections are cross-compatible to each other, thus a high number of interspecies-hybrids occur naturally or have been produced during the last 100 years. Out of the inter-species, hybrid clones have been developed to ensure genetic stability of the plant material used in SRCs. Very often, systematically performed records during production or vegetative propagation of poplar hybrids and/or clones are not available to date. Thus, it is not inevitably clear which *Populus* species or hybrids are behind these clones so that differentiation between them is sometimes difficult. Therefore, we need genetic markers to clearly identify and differentiate the species and hybrids within the genus *Populus* for both identification of existing clones and breeding of new clones.
We tested 290 SSR markers from the *Populus trichocarpa* genome for reproducible amplifying in six further *Populus* species (*P. alba, P. tremula, P. tremuloides, P. nigra, P. deltoides, P. maximowiczii*). Now we have a set of twelve polymorphic SSR-markers for identification of clones within this seven species.
Furthermore we tested 24 published barcoding primer combinations and 14 newly designed primer combinations of the chloroplast genome and 18 primer combinations of the nuclear encoded gene polyphenoloxidase (PPO) for amplification within the seven species and identification of SNPs to differentiate between the species and hybrids. For species identification until now we detected at least one SNP within each of the seven species using the chloroplast primers. In an about 400 bp long fragment of the PPO gene we detected one or more than one SNP for six of the seven species. *P. maximowiczii* can be identified only by exclusion of the SNPs for the other species. With help of combination of chloroplast and nuclear SNP markers we can also detect hybrids.

Keywords: microsatellites, SNPs, molecular markers

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Clonal differences among *Populus alba* selections and hybrids in short-rotation and coppice biomass traits

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A clonal trial of 32 *Populus alba* selections was fall planted with containerized stock in September, 1996 near the Iowa State University campus. Twenty-five of the clones were selected from the progeny of five different crosses, taking the best five growers from each cross in a 4-yr-old progeny trial. The remaining clones were older selections that had been used in other plantings in the North Central Region of the USA. A Libby and Cockerham design was used with individual tree plots spaced 1.7 m between all adjacent trees, 3 replicates per block, and 5 blocks. The original intent was to run the experiment for eight years with at least one intermediate thinning. Due to budget constraints, no thinning was done and a total harvest of surviving trees was not made until March, 2008 at the end of 11 growing seasons.

Initial survival was good for most clones averaging 80% after 7 growing seasons with a range of 33 to 100% and a basal area of 18.2 m^2/ha. Natural thinning over the next four seasons dropped average survival to 64% (range 0 -100%, BA = 32.7 m^2/ha) and accentuated the differences between clones in the sizes attained. Three of the top five clones in biomass production were pure *P. alba* selections, one was a *P. alba* x *P. tremula* hybrid and the other was a *P. alba* x *P. grandidentata* hybrid. Eleven of the new clones out performed the 'Crandon' clone that has generally been considered our best clone in the region. Specific gravity ranged from 0.28 to 0.43. The two best stump sprouters were sibling clones from the *P. alba x P. grandidentata* cross. The vigor of stump sprouting in the first year after harvest varied from 0.4 to 3 MG/ha (average = 1.3) based on projecting the average performance within a clone to full stocking of a stand. One *P. alba* family was produced with a fastigiate male parent (Bolleana cv.); three fastigiate and two normally branched progeny clones were included in this study. The best biomass producer of the five was a fastigiate clone that ranked seventh overall with 87% survival at harvest. However, the other two fastigiate clones were poor biomass producers and one had only 33% survival. The two normally branched clones ranked 14 and 19 overall in biomass production with 60% and 53% survival respectively.

The results show that stands of mixed aspen clones can be grown and do well over rotations that are well matched to the spacing in the plantation. However, carrying the stand past the planned rotation results in substantial mortality for many of the clones. This is not necessarily a problem because the coppice rotations that follow will be made up of the clones that grew the best in the first rotation. Hence, the time necessary for clone/site selection for commercial plantations could be shortened by planting the initial clone test as an intimate mix of promising clones on a variety of sites, growing the plantation until clear size differentiation occurs, thinning out the smaller clones at least a year before harvesting the whole stand, and then converting to coppice rotations. This system admittedly is biased in favor of fast-starting and good root sprouting clones, but that is exactly what we want in developing short rotation biomass stands.

Keywords: clones, yield, basal area

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Wood quality assessment of clones of *Populus deltoides* developed by Forest Research Institute, India through closed and open pollination

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Populus deltoides has firmly established itself as the most preferred plantation species especially in the northern India. Lot of work is going on tree improvement of this species. Forest Research Institute (FRI), India under its tree improvement program has carried out breeding programs and has developed various clones of Populus deltoides through open and closed pollination.

Silviculture Division of FRI raised Seedlings from the seeds of 19 open-pollinated and 24 control pollinated families. Best individuals of each family were cloned after one and a half years of age. The clones were repeatedly tested in nursery. Best 95 clones of open pollination and 74 clones of control pollination have been assembled in a germplasm bank at FRI campus for further multiplication and field evaluation. All these clones were studied for their wood quality with the objective to screen better clones. Specific gravity, Fibre and vessel characteristics were evaluated.

Statistical analysis was carried out for establishing variation in between and within the families and in between clones of the open-pollinated and closed-pollinated families. Multivariate Analysis showed that fibre length (FL), lumen diameter (LD), fibre wall thickness (WT) and vessel length (VL) were significantly different between the families but non-significant differences were found within the family of one year ramets indicating heritability even at juvenile stage.

In closed–pollinated families FL, LD, WT and VL varied significantly in between the 24 families and between the clones, while within a family the variation was non-significant. FD varied non-significantly in between and within the families. However, it significantly varied between the clones. Specific gravity ranged from 0.293 to 0.580. Post hoc tests revealed the cluster of families that differed significantly. Based on Student – Newman – Keuls test (as the number of observations differed in each of the clone) for 24 Families, two clusters were identified. Post hoc tests were also performed to find out subsets of the clones that differed significantly. Based on Student – Newman – Keuls test for 74 clones, eight clusters were identified.

In open–pollinated families FL, FD, LD, WT and VL varied significantly in between the 19 families and between the clones. However, within a family the variation was both significant and non-significant for different families. Specific gravity ranged from 0.335 to 0.485. Post hoc tests revealed 5 clusters of 19 families and 32 clusters of 95 clones that differed significantly.

Keywords: Open pollination, closed pollination, FRI, India

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Selection of willow clones for the production of bioenergy in northern Quebec

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In the Canadian province of Quebec, several small communities of the North are suffering from the closure of paper mills and a traditional forest-based economy. Opportunities exist to convert marginal lands into high yielding bioenergy crops. Amongst the species considered, *Salix* cultivated under Short Rotation Coppiced (SRC) present a high potential. A significant challenge that these communities face in order to establish bioenergy crops is the very short growing season, often accompanied with frequent spring frosts. However, very few performing clones or varieties of willows are currently available for cold climates. Hence, this project aims at identifying the *Salix* genotypes most suited to cold climates and frost resistant.

Twenty (20) *Salix sp.* genotypes were selected on their potential to produce biomass. The genotypes originated from several countries, growth environment and included Canadian native species. Three sites were selected to represent a north-south gradient and the cuttings were planted in a complete randomised bloc design repeated three times at each site. Yield, physiological parameters are being measured during the growth season and biomass production will be evaluated at the end of the cycle.

Preliminary results, after the first year following establishment of the cuttings, showed that most of the clones tested performed well even at the Northern site, including indigenous species. However, genotypes of *S. viminalis* were defoliated by *Empoasca Fabae*, but only at the southern site. A screening test is currently under way to simulate late frosts in a controlled environment. This approach should allow us to rapidly assess the genotypes susceptible to frost and to quantify the damages.

The results obtained in this study should contribute to the revitalisation of small communities by providing a list of willow genotypes suitable for the production of bioenergy in northern regions.

Keywords: Willow, cold, bioenergy, frost, genotypes

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Improving water use efficiency in bioenergy poplar: QTL for stomatal conductance and carbon isotope composition

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Dedicated bioenergy crops including poplar and willow are required as low fossil-fuel input feedstocks for renewable energy in a future drier climate. Capturing natural variation in traits contributing to water use efficiency (WUE) is the first step to developing trees adapted to future drier climates.

Stomatal conductance (g_s), leaf-level WUE and leaf carbon isotope composition ($\delta^{13}C$) were assessed in two *Populus* species, *P. deltoides* and *P. trichocarpa* and their F_2 progeny, grown in three contrasting environments - one site in the UK in different conditions of growth and one in Italy.

P. deltoides had a lower $\delta^{13}C$ compared to *P. trichocarpa*. The g_s of *P. trichocarpa* was less responsive to dehydration and abscisic acid treatment than *P. deltoides*. Quantitative Trait Loci (QTL) were discovered for $\delta^{13}C$ on seven linkage groups (LGs) and 3 QTL on different LGs for g_s. From these QTL and gene expression from microarrays, we have identified four novel candidate gene models underlying QTL hotpots on LG VI and X.

We have begun to unravel the genetic basis of WUE in *Populus*. These data are critical for future breeding and improvement in poplar and willow genotypes with higher WUE.

Keywords: water use efficiency, carbon isotope, QTL

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Genetic imprinting and adaptation to flooding in *Populus alba* and *P. tremula*

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Populus is a riparian genus of tree that is often associated with river floodplains with a general consensus that it may be considered 'flood tolerant'. However, differences in ability to tolerate flood water exist between different species of poplar. *Populus alba* (white Poplar) is often found in areas of Europe prone to flooding whilst *Populus tremula* (European aspen) conversely is more common in areas with greater altitude and therefore is subjected to flooding much more infrequently. These two species however are naturally hybridising in areas of central Europe and could yield the key to finding adaptive traits for flood defence.

The aim of this project is to use *P. alba* and *P. tremula* to find key physiological responses and the genes that control them, aiding the development of flood tolerant crops. This research is utilising physiological monitoring, and the genetics approach is in the process of utilising microarrays and candidate genes. The final portion of this project is to use epigenetic techniques to find the effects of repeated flood stress on the response mechanisms.

Keywords: flooding, adaptation, genomics

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Nitrogen-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 on 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*. Gene transcriptional profiles were compared between the elongation zone and developing xylem under control conditions and N treatment, respectively. Networks underlying N-driven secondary growth were tentatively identified by bio-informatic analyses.

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Keywords: wood formation, biofuel generation, nitrogen fertilization, gene transcriptional profiles

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Sessione 3: Poplar biotechnology for a low-carbon, sustainable society

Phytoremediation of TCE, Explosives, and Pesticides using Poplar

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Chlorinated hydrocarbons, such as trichloroethylene (TCE), are common environmental contaminants of soil, groundwater, and air. TCE, the most prevalent pollutant at SuperFund Sites, is hepatotoxic and a suspected carcinogen. Phytoremediation is the use of plants to restore polluted sites. By overexpressing mammalian CYP2E1 in transgenic poplar plants, we were able to dramatically increase TCE metabolism in lab experiments. In order to identify genes involved in the complete degradation of TCE in poplar, we conducted a microarray analysis, comparing the transgenic poplar to control poplar plants exposed to TCE.

The nitroaromatic explosives, TNT and RDX, are serious environmental pollutants present at phytotoxic levels in military training ranges. We developed transgenic poplar plants that could be used around the perimeters of such areas to prevent the spread into neighboring communties. Transgenic plants containing the bacterial genes *nfsI*, for TNT transformation, and *xplA* and *xplB*, for RDX degradation, were able to rapidly remove these pollutants and overcome the phytotoxic effects.

Poplar and willow take up and degrade the common pesticide, chlorpyrifos. In an effort to improve remediation of pesticides and to provide a low-cost purified extract for on-site application for spills, we developed transgenic poplar and tobacco overexpressing a detoxifying enzyme.

Keywords: Poplars, phytoremediation, chlorinated hydrocarbons, bacterial genes transformation

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Reproductive onset in poplar

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Poplar (*Populus* spp.) has developed a complex reproduction program. It is closely intertwined with vegetative growth and dormancy, and one cycle of flowering is spread over multiple years. Historically, research has focused on physiological and anatomical aspects of this complex trait. With the advent of poplar genome sequence, molecular genetics has been deeply integrated into this area of research. We will present a developmental model showing how genetic, physiological, and environmental factors collectively regulate the onset of reproduction and associated vegetative growth in poplar. In particular, we will focus on the *FT* genes and their signaling pathways. The discussion will include how this knowledge can be used by breeders and biotechnologists to speed breeding and improve tree growth and development.

Keywords: poplar, reproduction, flowering.

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Poplar tolerance to salinity stress is regulated by the metabolome and by summoylation of SP1, a novel stress-associated protein

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Stress-associated metabolites and stress proteins are key regulators of plant adaptation to abiotic stress. Our understanding of the contribution of the metabolome to stress tolerance is just emerging. Here we report recent findings on detailed GC-MS metabolite profiling in a salt sensitive (*Populus tremula*) and tolerant (*Populus euphratica*) poplar (in addition to other species) in response to salt stress and recovery. In addition, the structure and possible role of poplar SP1 and *Arabidopsis* AtSP, two member of a new protein family, are also presented. GC-MS metabolite profiling revealed significant changes in the concentration and organ distribution of about 40 metabolites in response to salt stress in salt-tolerant vs. salt-sensitive plants, in both species mentioned above. The dynamics of metabolite content during the stress and upon recovery from stress was also investigated. We conclude that specific organ accumulation of several mono and oligosaccharides, organic acids, amino acids, osmoprotectants and signaling molecules contribute to plant adaptation to stress. Moreover, we suggest that stress-associated metabolites may in fact drive plant adaptation to abiotic stress. SP1 of the salt-tolerant *P. euphratica*, but not of the sensitive *P. tremula*, was found to be sumoylated upon stress and consequently translocated from the cytosol to the plasmalemma. Several SP1-transgenic *P. tremula* lines show increased tolerance to salinity.

Keywords: salinity and water stress; stress proteins; metabolomics; sumoylation

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Elimination of marker genes and targeted integration of transgenes via the FLP/FRT-recombination system

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Antibiotic or herbicide selection marker genes are needed to control success of the transformation process, however, become useless during later stages of development. Further, the problem of expression variability is more crucial for trees having long vegetative cycles compared to annual plants. To address the issue of expression variability, single-copy transgenes need to be placed precisely into the pre-defined genomic loci already characterized for stable expression.

Site-specific recombination (SSR) systems can be used both to eliminate the antibiotic marker gene and to place a recognition target (reporter gene) within the genome so that the "gene-of-interest" (GOI) can be precisely placed into the target. The aim of this project is to test in a proof-of-concept study the usability of the FLP/FRT-recombination system from yeast in transgenic poplar for two applications: (a) removal of the selection marker gene, and (b) replacement of one gene by a second unrelated one.

Keywords: genomics, antibiotic marker, reporter gene

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The role of SUT4 in regulating carbon allocation, growth and phenylpropanoid homeostasis in *Populus*

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Sucrose transporters (SUT) are classically regarded as integral to the export of sucrose from source leaves in higher plants. In certain tree species they also appear to mediate the export of sucrose from stems (sink organs) to newly expanding leaves in the spring. We have been investigating SUT function in *Populus* where the primary mode of sucrose export from source leaves and its dependence on SUT proteins remain unresolved. In apoplastic phloem loading *Arabidopsis* and Solaneceous species, SUT proteins mediate the active transit of sucrose across membranes. The long distance transport of sucrose in many temperate tree taxa including *Populus* and *Salix*, may feature a significant symplastic component as well. Symplastic transport occurs through plasmodesmatal connections and is thought to be energetically passive. Whether SUT proteins contribute to the regulation of symplastic sucrose transport is not known. We have characterized SUT gene expression in *Populus*, and demonstrated sucrose transport by their encoded proteins in yeast-complementation assays. The most abundantly and ubiquitously expressed SUT gene, SUT4, was selected for functional analysis via RNAi-mediated suppression in transgenic *Populus* (*P. tremula* × *P. alba* clone 717-1B4). To facilitate our functional analysis, GFP tagging and western blotting were used to determine the membrane localization of SUT4 protein Based on detection of SUT4 transcripts in both mesophyll and phloem companion cells in source leaves, SUT4 may function in symplastic as well as apoplastic sucrose export. We have also used in situ mRNA hybridization of stem sections to determine that several SUT genes exhibit overlapping patterns of transcript distribution in the secondary xylem. In addition, xylem expression was relatively strong during summer growth when diameter growth of stems is rapid. The effects of SUT4 down-regulation were investigated in hydroponically maintained plants grown under N-replete and N-limiting nutrition. Allocation of carbon between leaf and stem biomass was altered, as was starch and sucrose metabolism in source leaves. Phenolic glycoside homeostasis was clearly affected, although condensed tannin levels were only slightly altered. Based on plant-wide analysis of carbohydrates, metabolic profiles, and growth, SUT4 appears to be important in leaves for mediating export of sucrose, and in xylem, for mediating the utilization of imported sucrose.

Keywords: carbon allocation, sucrose transporters, SUT gene

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The transcriptional roadmap for delayed autumnal senescence in *Populus* in elevated atmospheric carbon dioxide.

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Extensions to the growing season have been measured over recent decades, at the continental scale using remote sensing and at the plant scale using observations such as bud-burst, bud-set, leaf colour change and leaf drop. While a strong association exists between rising global temperatures and an earlier spring, the association between temperature and the later on-set of autumnal senescence is weak. Here we hypothesise that an increasing atmospheric carbon dioxide concentration [CO_2] may partly account for delayed autumnal senescence and for the first time, through transcriptome analysis, identify gene expression changes associated with this delay.

Using the EUROFACE (www.unitus.it/euroface) facility, a nine hectare experimental plantation of poplar trees growing in central Italy this hypothesis was tested. Experimental plots within the EUROFACE plantation expose trees to Free-Air Carbon dioxide Enrichment (FACE) raising the [CO_2] concentration to 550 µmol mol^{-1} (predicted in 2050 ~ 42% above current levels). Whole canopy remote sensing of Normalised Difference Vegetation Index (NDVI), changes in leaf area index (LAI) and leaf chlorophyll content were all measured to quantify the effect [CO_2] has on the rate of autumnal senescence. It was estimated that an elevated [CO_2] delayed a 50 % canopy leaf loss by ~ 10 days. To generate hypotheses as to how elevated [CO_2] may cause this delay the PICME microarry was used to determining differential gene expression between leaves senescing in either ambient and elevated [CO_2] during this FACE experiment. Leaf anthocyanin and carbohydrate contents were also measured.

Leaf biochemical pathways for secondary metabolism and glycolosis were significantly up-regulated by elevated [CO_2] during senescence, in particular, those related to anthocyanin biosynthesis. Expressed Sequence Tags (ESTs) representing the two most significantly up-regulated transcripts in e[CO_2], *LDOX* (*leucoanthocyanidin dioxgenase*) and *DFR* (*dihydroflavonol reductase*) both down-stream in anthocyanin biosynthesis, gave (e[CO_2] / a[CO_2]) expression ratios of 39.6 and 19.3 respectively.

In line with the growth difference balance hypothesis we propose that elevated [CO_2] stimulates secondary metabolism, namely anthocyanin biosynthsis, during senescence which prolongs leaf longevity.

Keywords: leaf senescence, elevated CO_2, FACE, transcriptome

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Biotechnology for cold tolerance in *Eucalyptus*: could the research in Eucalypts be useful also for Poplars?

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Eucalyptus hybrids are some of the fastest growing and highly productive forest tree species in the world. Some hybrids routinely produce in excess of 20 green tons per acre per year on 5 to 7 year rotations in Brazil when managed for the production of pulpwood. These high yielding fast growing plantations are ideal candidates for biomass plantations since they can be managed on short rotations, are capable of coppice regeneration, have a high ratio of wood to foliage and can be grown at high densities. Unfortunately, these hybrids can not be grown in the southeastern United States except for the southern portions of Florida due to their low tolerance to freezing temperatures.

ArborGen is in the late stages of development of a freeze-tolerant eucalypts variety which harnesses the high productivity of a eucalyptus hybrid while providing freeze tolerance via biotechnology in order to expand the range of this fast growing hybrid to north Florida and southern Georgia, Alabama, Mississippi, Louisiana and southeast Texas. This variety also contains technology designed to prevent pollen production and which may have applications in reducing outcrossing from plantations.

In this talk, we will describe our experiences in developing Freeze Tolerant Eucalyptus and the application of many of the same approaches to the development of Short Rotation Populus (aspen and cottonwood) that contain genes for enhanced growth and productivity.

Keywords: cold tolerance, biotechnology

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Variation in sex expression of *Populus*, and comparative mapping analysis of sex determination across poplar species and hybrids

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The genetic mechanism of sex determination in poplar is still in its infancy. The genus *Populus* is generally considered as dioecious, yet, bisexual trees have been widely reported. Hence, it constitutes a working model for sex determination in flowering plants. As a first evolutionary explanation for dioecious plants, Darwin suggested that it may be advantageous to specialize on one sex rather than two as allocating resources only on one function may provide selective advantages than are comparable hermaphrodites. Recently, another argument suggested that dioecy evolved chiefly to minimize self-fertilization to avoid the deleterious effect of inbreeding depression. Moreover, a body of evidence shows that the creation of the first male or female and nascent sex chromosomes were the result of suppression of genes for female or male function in the monoecious ancestors. The genus *Populus* can shed light on such evolutionary explanations by providing a better understanding of the genetic and molecular mechanisms that control sex determination in dioecious plants. Here, we have embarked in an integrated genomics approach to deciphering the genetic basis of sex determination in *Populus*. Although, genetic mapping studies of chromosomal regions that determine sex development showed the promise of this approach, data on pedigrees are increasingly needed. A *P. x canescens* pedigree obtained in 1998 was carefully monitored for sex phenotype for the last 6 years. This cross revealed that bisexuality in poplar seems to proceed during time and showed segregating ratio of males, females and a significant number of bisexual trees. This allows carrying out repetitive studies to monitor changes over time. Interestingly, the male parent may probably confer the bisexual tree trait. The genetic analysis of sex locus in the *P. x canescens* pedigree will help unravel the discrepancy in the location of the sex locus on linkage group XIX as well as the inconsistencies in the heterogametic sex found in several other species of *Populus*. In addition, gene expression and transgenic studies of floral homeotic genes in *Populus* species provide remarkable insight on the role of these genes in sex determination. These studies of newly evolving sex-determining chromosomes and genes in *Populus* raise many new questions to be answered by further work.

Keywords: dioecy, linkage map, sex inconstancy.

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Sessione 3: Poster

Comparative sequence analysis of two *P. tremuloides* BAC clones harboring sex-linked markers with *P. trichocarpa*

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In the last few years, a number of genetic linkage maps containing a mapped sex trait have been published for the genus *Populus*. On these genetic linkage maps sex was mapped as a genetic marker, constantly located on linkage group XIX but on varying positions and inconsistently on male or female maps. Thus, up to date, sex-linked markers to be used in early selection tests are not available and, moreover the mechanism of sex determination in this genus is still largely unknown.
In our study, a progeny of 126 F1-individuals of an interspecific cross between *Populus tremula* x *P. tremuloides* was used for genetic mapping of linkage group XIX with SSR markers based on the *Populus trichocarpa* genomic sequence. Several completely sex-linked SSR markers were identified and mapped to a central position on the male *P. tremuloides* map of linkage group XIX. Sex-linkage of the SSR markers was validated in other crosses between *P. tremula* and *P. tremuloides*.
To get a first impression of the sequence differences in the genomic regions containing the completely sex-linked SSR markers between *P. trichocarpa* and *P. tremuloides*, we sequenced two BAC clones (BAC96P5a and BAC110J22) of *P. tremuloides* containing mapped SSR markers which show 100% linkage to the sex locus. BAC96P5a includes the genomic region around the SSR marker BP82 and BAC110J22 the region around BP 60 (Pakull et al., manuscript submitted). Extracted DNA of the BAC clones was subjected to 454 sequencing and the generated reads were assembled to contigs automatically using Consed (GATC Biotech AG, Konstanz). For each BAC, the contigs were super-assembled to a scaffold representing the draft BAC sequence.
To check the homology of the generated genomic *P. tremuloides* sequences to the already sequenced *P. trichocarpa* genome (phytozome v2.0; http://www.phytozome.net/poplar) we created a comparative physical map of *P. trichocarpa* chromosome 19 with both BACs, based on the best Blast hit of different BAC regions to this chromosome. The results clearly show that homolog regions exist between the BAC sequences of *P. tremuloides* and the *P. trichocarpa* sequence, however, other parts of the *P. tremuloides* BAC sequences map to complete different regions of *P. trichocarpa* chromosome 19 and, moreover, a few BAC regions show the best hit to other chromosomes of *P. trichocarpa*.
This indicates that irrespective of the high collinearity observed between all the different *Populus* maps and the *P. trichocarpa* genomic sequence so far, translocation rearrangements at least between *P. tremuloides* and *P. trichocarpa* could have occurred in a region of LG XIX showing sex-linkage inheritance in *P. tremuloides*. Mapping and genetic analysis of the two sex-linked markers mentioned above in other crosses than *P. tremula* x *P. tremuloides* will unravel their potential to be used as sex-linked markers in other *Populus* species.

Keywords: *Populus tremuloides, Populus trichocarpa*, floral biology, sex markers

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Indirect organogenesis of *Salix humboldtiana* Willd, a promising species for phytoremediation

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Willows are woody plants belonging to the genus *Salix* L., comprising over 300 species, including trees, shrubs and other than a few inches in height. Willows play an important role in phytoremediation (removal of heavy metals contaminated soil purification) of degraded land, rehabilitation of fragile ecosystems (especially combating desertification) and forest landscape restoration. Willow plants have relatively high rates of transpiration and resistance to seasonal flooding, with roots that extend several feet from the ground which are very advantageous in wetlands, where control of groundwater runoff is essential to reduce pollution. The native willow, *Salix humboldtiana*, has almost been replaced by the willow wicker or willow named "mimbrote". It will be important to have a large number of cultivated plants and vegetatively propagated either to repopulate areas where the native willow is in decline, or to establish strategies for phytoremediation through plantations. Have been reported various biotechnological approaches in *Salix* species. However, there are some difficulties in rooting some species, although most willows are fast growing and easily propagated vegetatively. The aim of this study was to obtain an efficient in vitro propagation protocol of *Salix humboldtiana* (willow native) by indirect organogenesis. Fungicide treatment with sodium hypochlorite was sufficient for the disinfection of immature embryos of willow and made possible the establishment of in vitro culture for this species. The culture media used for growth of callus and shoots from leaves were optimal as well as employees for rooting of shoots. 96% of the explants (leaves) formed callus, 51% of the calluses formed shoots and the remaining 49% is possible to regenerate plants. *Salix humboldtiana* is possible to regenerate by indirect organogenesis. It is recommended the cultivation of leaves from plants established in vitro from immature embryos. This work indicates that variants in the culture medium and the concentration of growth regulators are crucial for obtaining an efficient protocol for regeneration *in vitro*.

Keywords: micropropagation, phytoremediation, sauce criollo

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2n pollen induction in *Populus* × *popularis* by colchicine

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Triploid poplar clones, derived from 2n gametes, in section *Populus* and in section Aigeiros display growing faster over the normal diploids and are widely used in poplar plantation. 2n pollen is a basic material for poplar sexual polyploidization. However, the natural occurrence rate of 2n pollen in poplar is very low, its normally under 5%. Thus, artificially induction of 2n pollen is an essential technique for poplar polyploidy breeding. 2n pollen can be induced by physical factors such as temperature, radial ray, and ultrasonic. High temperature is the widest application factor among them. Poplar and pine male flower buds were treated at prophase I during meiosis with 38-40 ℃ about 2h, gained 2n pollen 94.4% and 84.1%, respectively. 2n pollen also can be induced by chemical agents such as colchicine, oryzalin,and trifluralin. Colchicine was first discovered as an effective cell division inhibitor in 1937 and has been utilized for a long time. Most inductions of 2n pollen in poplar focus on the species in section *Populus* while few in other four sections in poplar genus. Populus × popularis were the hybrid offspring of *P. simonii* × (*P. pyramidalis* + *Salix matsudana* mixed pollen) and cultivated by the Chinese Academy of Forestry. They are widely planted in semiarid area in north China and display high resistance against cold and drought but slow growth. The objective of this paper was to explore the technique of 2n pollen induction and seek for the approach for polyploid cultivation of *P.*× *popularis* in order to enhance its growth for the future biomass production.

Induction of 2n pollen is a required technique for cultivating polyploid via sexual polyploidy. Orthogonal design or Tagichi Design was applied to select the best treatment process of 2n pollen induction in *Populus* × *popularis* from different levels of the meiosis stage of male flower buds, colchicine concentration, inject times, and interval between injections. Flow cytometry and chromosome counting were used to identify the triploids from the offspring of *Populus* × *euramericana*.(Dode) Guinier pollinated with induced pollen of *P.* × *popularis*. The results showed that high 2n pollen rate can be achieved by selecting the flower buds during Diakinesis stage in meiosis, and then injecting 0.6% colchicine 4 times with 2 hours interval. The 2n pollen rate reached 73.95% by this process, and two triploids were obtained, which indicated that it is possible for cultivating triploids via 2n pollen induction by colchicine treatment in poplar. Results and protocol related to 2n pollen induction, polyploid identification and effect of 2n pollen in this study might be applicable in polyploidy breeding in section Aigeiros and Tacamahaca of poplar.

Keywords: 2n pollen, poplar, polyploid breeding, colchicine, orthogonal design

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Characterization of the triploid white poplar PtDrl02 gene promoter in transgenic tobacco and poplar

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The *PtDrl02* gene belongs to the TIR-NBS gene family in triploid white poplar (*Populus tomentosa* × *P. bolleana*) × *P. tomentosa*. Its expression pattern displays tissue-specificity, and the transcript level can be induced by wounding, methyl jasmonate (MeJA), and salicylic acid (SA). Here, we describe the properties of the *PtDrl02* promoter in transgenic tobacco and poplar using β-glucuronidase (GUS) as a reporter. The *PtDrl02* promoter was 986-bp long, and contained a cluster of stress-responsive elements such as W-box, GT-1 motif and ABRE motif. In transgenic tobacco, the *PtDrl02* promoter directed gene expression mainly in the aerial parts of the plants and was confined to the cortex tissues of leaf veins, petioles, stems, and stem piths, showing a typical tissue-specific expression pattern, which was compatible with the expression profile of the *PtDrl02* gene in triploid white poplar. Furthermore, it was found that the promoter activities could be significantly induced by the treatment of wounding, MeJA, SA, ABA (abscisic acid) and NaCl in the stem tissues of transgenic tobacco. In poplar (*P. tomentosa*), *GUS* reporter gene expression retained an aerial-specific expression pattern under the control of the *PtDrl02* promoter, albeit a relative low level of of gene expression in tissues compared to that in tobacco, and that of the native reference *ACTIN* gene. As expected, the *PtDrl02* promoter presented a wounding-, MeJA-, SA-, ABA-, and NaCl-triggered expression pattern (in *GUS*) in transgenic poplar but apparently with a time course manner that, to a certain extent, paralleled to the endogenous pathogenesis-related genes (*PR-1*, *PR-5* and *PR-10*) in Planta. These results indicated a biotechnological potential of the triploid white poplar *PtDrl02* promoter in use for the development of genetic modified plants (e.g. *Populus* spp.) with biotic and/or abiotic resistance, given that the transgene should be precisely controlled.

Keywords: Poplar, TIR-NBS, promoter, resistance, genetically modified plant

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Expression patterns of PtLFY from *Populus tomentosa* and gene silencing by interference structure PtLFY-IR in transgenic tobacco plants

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Chinese white poplar (*Populus tomentosa* Carr.) is an important native tree species in China. In common with most tree species, it possesses a long juvenile phase. To understand the genetic and molecular mechanisms underlying floral development in *P. tomentosa*, *PtLFY*, a *LEAFY* homolog, was isolated from a *P. tomentosa* floral bud cDNA library and its characteristics were analyzed. DNA gel blot analysis suggested that one copy of *PtLFY* is present in both male and female *P. tomentosa* genomes. The expression differences of *PtLFY* in tissues of *P.tomentosa* were detected by RT-PCR, the result indicated that high transcripts of *PtLFY* were showed in root, both male and female floral buds, only faint transcripts were found in stems and vegetative buds, and no transcript was detected in leaves. Furthermore, the *PtLFY* expression patterns were also analyzed during the development of both male and female floral buds in *P. tomentosa* via real-time quantitative RT-PCR. Continuous, stable and high expression of PtLFY transcripts was detected in both male and female floral buds from September 13th to February 25th, but the amount of *PtLFY* transcripts in male floral buds was considerably higher than in female floral buds. Our results also showed that the inverted repeat *PtLFY* fragment (*PtLFY*-IR) effectively blocked flowering of transgenic tobacco plants, indicating that silencing of the tobacco *LFY* homologs *NFL1* and *NFL2* was triggered by interference structure *PtLFY*-IR.

Keywords: Expression patterns, flowering, gene silencing, *LEAFY* homolog, *Populus*, qRT-PCR

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Microclonal propagation and genetic transformation of *Populus sp.* for improving the abiotic stress tolerance

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Due to fast growth rate, easy propagation, broad area of distribution, good applicability for the wood products, paper and biofuel industry *Populus* species become very popular plants for growing in tree plantations. They are also very important trees in fundamental biology as well as in biotechnology. Biotic and abiotic stress tolerance, wood modification, herbicide resistance, fast-growing, phytoremediation are main directions for improving the poplar genome through gene manipulation.

The problem of Chornobyl zone of exclusion is very important for Ukraine due to exclusion the extensive territories from agricultural usage. These polluted sites are not used for food production, but may be used for wood production, particularly for paper and biofuel industry, and poplars are the most appropriate trees for this aim. The purpose of our work is developing the methods for microclonal propagation, direct plant regeneration from explants and genetic transformation of several perspective clones of *Populus* for improving stress-tolerance.

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. Hybrid poplars demonstrate high productivity in plantations, and the fastest growth rate in vitro.

Microclonal propagation on WPM media was carried out. For genetic transformation experiments the methods of direct shoot regeneration from leaf explants were developed. We used TDZ (0,02 – 0,04 mg/l) for induction of direct plant regeneration. Using this hormone we have received up to 5-7 and more regenerants per explant. Some of these regenerants were adapted to greenhouse conditions.

The *Agrobacterium* strain LBA 4404 was used for transformation. The construct pBi2E carries both ds suppressor of proline dehydrogenase (*PDH*) gene from *Arabidopsis* and *nptII* gene as a selectable marker for kanamycin selection. PDH is the first enzyme of proline catabolism, and suppressing its activity increases the level of free proline in cell. Proline is known to defend the cells from abiotic stresses (drought, salinity etc). We used both media with kanamycin and high concentration of NaCl for selection of transformed plants. Now transformation experiments are continuing, candidate plants that escape selective pressure have been obtained and stability of their tolerance to different NaCl concentrations is under study.

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Keywords: *Populus* sp., genetic transformation, stress tolerance

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Fig. 1. *Populus* sp. in vitro culture

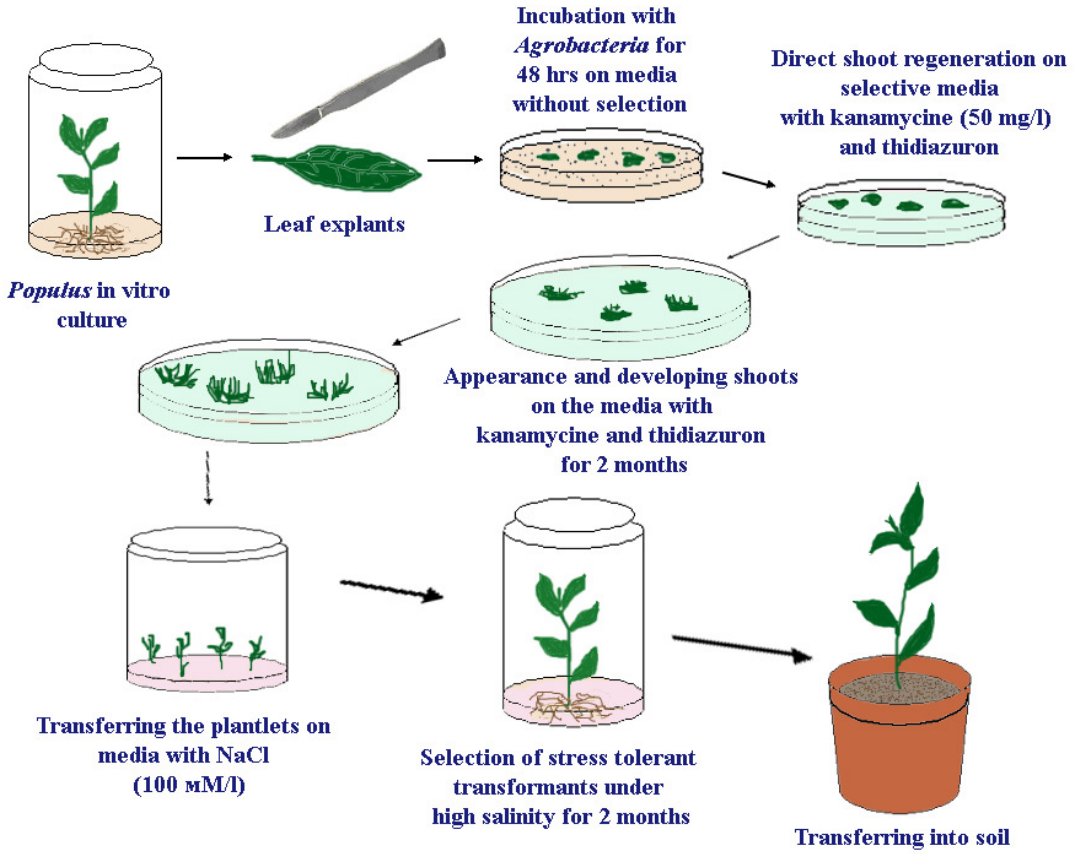


Fig. 2. Genetic transformation of *Populus* and evaluation of transformants under salt stress

Over-expression of SRK2C enhances stress resistance in Poplar

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SRK2C is a member of the SnRK2 gene family within the SnRK2a subfamily and has been annotated as a putative serine/threonine protein kinase. Research in *Arabidopsis* suggested that SRK2C was a positive regulator of drought tolerance in *Arabidopsis* and the drought tolerance coincided with up-regulation of many stress-responsive genes. In this study, stem sections of *Populus tremula* × *P. tremuloides* cv. T89 were used as materials for genetic transformation. The expression vector was constructed by use of Invitrogen Corporation's gateway recombination system and an over-expression vector pH35GS with the homologous gene of SRK2C cloned from the poplar was established. Three over-expression vectors pH35GS with three homologous genes of SRK2C were used for genetic transformation of *Populus tremula* × *P. tremuloides* T89 mediated by the Agrobacterium method. Sixty hyg resistant plants with the pH35GS::PtSRK2C-1 gene were obtained by a selection medium with concentration of 25 mg/l hyg. Forty hyg resistant plants with the pH35GS::PtSRK2C-2 gene and 30 hyg resistant plants with the pH35GS::PtSRK2C-3 gene were also obtained. The resistant plants were determined by PCR and Southern Blotting analysis which showed there were 80%, 73% and 70% positive respectively, and it proved that the three genes were integrated into the genome of *Populus tremula* × *P. tremuloides* T89. The transgenic plants growed normally under PEG and NaCl stress, and its growth was much better than the control plant, indicating that the tolerance of transgenic plants to drought and salt was better than that of the control plant.

Key words: SRK2C, stress resistance, *Populus tremula* × *P. tremuloides* T89, transgenic plant

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Biotechnological *in vitro* manipulation with willows (*Salix* spp.)

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Populations of endangered willow species are extremely limited in number of individuals and in its spatial distribution in high mountains of the Czech Republic. Species are often present as females only at localities. Thus these species propagates only by clonal reproduction at their relict sites. Biotechnological *in vitro* techniques have potential to recover populations of selected species using original genotypes. We have done experiments with micropropagation by nodal segments and leaf buds on common as well as endangered willow species (e.g. *S. alba, S. caprea, S. cinerea, S. hastata, S. herbacea, S. laponum, S. matsudana* and *S. viminalis*). The compositions of media were suit to *in vitro* manipulation with woody plants. MS medium supplemented with low concentration of plant growth regulators (BA, IBA, TDZ, and ZEA) and with glutamine and caseinhydrolysate were tested for segments rooting (MS medium can be replaced by WPM medium). The cultivation of the green young nodal segments of *S. alba* and *S. matsudana* showed the best results up to now. The most positive effect on segment regeneration was recorded on the medium with the combination of TDZ and IBA. For *in vitro* propagation of willows accessions with unisexual flowers (catkins), the anther and ovule culture were tested and optimized. We isolated anthers from male catkins of *S. cinerea, S. caprea* and *S. viminalis*, and ovules from female catkins of *S. herbacea, S. hastata, S. laponum, S. cinerea, S. caprea* and *S. viminalis*. Isolated anthers were cultured on media supported androgenesis (B5 medium with NAA, BAP or MSm medium with 2,4-D and BA, and with high concentration of sucrose). The microcallus of *S. caprea* and calluses of *S. viminalis* was formed. Various media were tested and optimized for *in vitro* willow gynogenesis, due to the unexplored area in this genus. The mixture of different growth regulators (NAA, IAA, IBA, KIN, GA3, BA, TDZ) and other components (caseinhydrolysate, coconut water, glycine) were added to MS, MSm, SH and WPM medium. In this case, there were observed no changes so far. In the end, the callus induction from leaves was tested in *S. cinerea* and *S. viminalis*. Media for callus induction were used (MS media supplemented with NAA, BA, TDZ, 2,4-D). The calluses were observed on one of them (medium MSC, MS medium with NAA, BA).

Keywords: micropropagation, endangered willows

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Session 4: Innovative production systems for low input tree crops

Developing a hybrid aspen coppice system for the Midwest agricultural region of the United States

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While *Populus deltoides* and various selections of *Salix* species and hybrids show considerable promise for short-rotation energy production on good quality floodplain sites, we have begun focusing on hybrid aspens (*P. alba* selections and interspecies hybrids) for use on upland sites that can qualify for conservation reserve status. Yields of up to 224 oven-dry MG/ha at the end of the first 10-yr rotation have been achieved in Iowa, USA. This is a better annual average yield than has been achieved with any other woody species and most herbaceous species in the region. However, we believe there is potential for even greater productivity in subsequent coppice rotations if we can learn how to optimize the management of such stands. Therefore, we are quantifying the biological and economic aspects of different coppice rotation management scenarios.

First year re-sprouting can result in over 200,000 stems/ha from a combination of stump and root sprouts with natural thinning starting during the first year. We have begun testing complete harvesting of all re-sprout material on one, two, and three-year rotations. We are also testing row thinning of the coppice stands to speed the development of large, high-yield stems while capturing biomass that might otherwise be lost to natural thinning processes. We have already determined that row thinning at the end of the first growing season results in a new flush of re-sprouting in the thinned area in the second growing season, returning sprout density to near the pre-thinning levels. Also, the growth of the stems left in the first thinning is being compared to un-thinned sprout stands. We will eventually determine how often this short-rotation version of coppice-with-standards can be practiced to obtain economically viable mid-rotation yields.

Yields from harvests of one-year-old root sprout stands are about 3.4 oven dry MG/ha and our first two-year-old sprout harvest averaged about 3.6 MG/ha/yr while sprout numbers had dropped to less than 87,000. When the original plantations are harvested, a combination of both stump sprouts and root sprouts is produced. Observations of such mixed sprout origins suggest that stump sprouts have the fastest growth over the first one to two years, leading to significant mortality in adjacent root sprouts. After about two years, the stump sprouts begin to decline in health, vertical stability, and survival relative to the remaining root sprouts. Therefore, our approach to row thinning is to harvest all sprouts in a band centered on the row of stumps, leaving only root sprouts for longer-term growth. Our results to date indicate that the yield per unit area harvested in a thinning at the end of the first year could be as much as 5.5 MG/ha (root sprouts 3.3, stump sprouts 2.2) with no new establishment costs. Data from the second year of these trials will be included in the presentation and the relative merits of different management strategies will be discussed.

Keywords: coppice systems, sprouting, thinning

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PRO-BIOPA - Sustainable production of biomass from poplar short rotation coppice on marginal land

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PRO-BIOPA is part of the German BMBF programme "Bioenergie2021" aiming (i) to optimize biomass production with short rotation coppice (plantations) of poplar on marginal sites with limited water and nutrient availability and to implement a comprehensive and integrative evaluation of this form of biomass production with respect to (ii) ecologic (GHG balance) and (iii) economic aspects (net energy balance, cost effectiveness). Furthermore, with regard to environmental sustainability we will amend water and nutrient utilization as well as the emission of reactive trace gases (BVOC biogenic organic compounds) by means of biotechnological approaches. The project includes system biological analyses of Arabidopsis and poplar, laboratory studies with transgenic plants in controlled conditions as well as field trials with conventional poplar species/clones in short rotation plantations on marginal land. The different scientific questions are addressed in a multidisciplinary approach by scientists from the Karlsruhe Institute of Technology, the University of Freiburg, the 'Forstliche Versuchs- und Forschungsanstalt Baden-Württemberg', and the industrial partner Netafim.
The presentation gives a project overview and highlights actual results from our poplar SRC.

Keywords: short rotation, coppice, BVOC

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Hybrid poplar suitability for regional deployment as a Bio-fuel feedstock

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Species and hybrids of the genus *Populus* are among the leading woody perennial crops being considered for regional bio-energy programs throughout the western United States. A key component of a successful poplar bio-energy plantation program is the deployment of regionally adapted, pest-resistant, and high-yielding varieties that are also characterized by superior feedstock material chemistry. To test the adaptability of an assemblage of hybrid genotypes across a range of environments, replicated varietal trials were established at eight locations in Oregon, Washington, Idaho, and New Mexico in 2005. All locations were classified according to their climate regime as either mild/mesic or cold/xeric to better match the adaptation of the experimental plant material. Following the fifth growing season in 2009, breast-height stem diameter data were collected. Combined site analyses of variance led to the identification of four genotypes at each site that under went destructive sampling for the development of biomass yield equations. Specific varieties were selected on the basis of their ranking by breast height diameter at each site and their individual contribution to the site-by-clone interaction variance. Wood cores were collected from the selected varieties for specific gravity measurements and the estimation of carbohydrate, lignin, and ratio of syringyl to guaiacyl monolignol content using near-infrared spectroscopy. The presentation will discuss the process and results of varietal recommendations for planting throughout the western United States based on potential biomass productivity, site adaptability, and wood chemical composition.

Keywords: biofuel, feedstock, land suitability

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Biomass production and carbon stocks in poplar-crop intercropping systems: a case study in northwestern Jiangsu, China

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The importance of agroforestry system in CO_2 mitigation has become worldwide recognized in recent years. Poplar-crop intercropping, as one of the traditional agroforestry systems, is the most important management patterns in China. However, little is known about carbon sequestered in poplar-crop intercropping systems. The main objective of this study is to compare the effects of three poplar-crop intercropping patterns (Pattern A: 250 trees·ha^{-1}; Pattern B: 167 trees·ha^{-1} and Pattern C: 94 trees·ha^{-1}) and two double cropping systems (wheat-corn cropping system and wheat-soybean cropping system) on biomass production and carbon stocks in poplar-crop intercropping systems. The experiment was conducted at Suqian Ecological Demonstration Garden of Fast-growing Poplar Plantations in northwestern Jiangsu. A significant difference in carbon concentration was observed among the poplar biomass components ($p \leq 0.05$), with the highest value in stemwood and the lowest in fine roots, ranging from 459.9 g·kg^{-1} to 526.7 g·kg^{-1}. There was also a significant difference in carbon concentration among the components of the crops ($p \leq 0.05$), and the highest concentration was observed in the harvest parts. However, the carbon concentrations, where treatments averaged within crop classes, were 427.3 g·kg^{-1} for wheat, 434.1 g·kg^{-1} for corn and 449.1 g·kg^{-1} for soybean, respectively. Over the 5-year period, the total poplar biomass enhanced as planting density increasing, and the highest total biomass achieved in Pattern A, reaching 15.12 t·ha^{-1} or 12.3%, and 72.4% higher than Pattern B and Pattern C, respectively. Annual biomass production among the crops ranged from 4.69-16.58 t·ha^{-1} at the stand age of 5. Total biomass was significantly different among the 3 crops ($p \leq 0.05$), where 3 intercropping patterns averaged within crop classes were 15.10 t·ha^{-1} for wheat > 10.61 t·ha^{-1} for corn > 5.08 t·ha^{-1} for soybean. A significance difference in carbon stocks was observed among 3 poplar crop intercropping patterns. Overall, Pattern A had the largest total carbon stock, reaching 16.7 t C·ha^{-1} for the wheat-soybean cropping system and 18.9 t C·ha^{-1} for the wheat-corn cropping system. Total carbon stock in the poplar-crop intercropping system was also affected by the cropping systems, where the total carbon stock in wheat-corn cropping system was 13.4% greater in Pattern A, 9.9% in Pattern B, and 23.7% in Pattern C respectively, compared with the carbon stocks in the wheat-soybean cropping system. Results from this case study suggest that Patterns A was a relative optimum poplar-crop intercropping system both for economic benefits and for carbon sequestration.

Keywords: agro-forestry, intercropping systems, C-sequestration

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The influence of a rotation length on production and biodiversity of poplar short rotation coppice

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The contribution comprises the results of evaluation of production and biodiversity of experimental poplar short rotation coppice grown in three harvesting cycles (rotations): one, three and six year long. The 6 year long experiment was established in 9-year old plantation in Central Bohemian Region. Following parameters were measured on trees in testing plots in the end of vegetation period: tree height, stem diameter and number of stems per tree. Harvests were performed of above ground biomass in winter months. Field yield fo fresh biomass was weighted and survival rate was counted in aim to calculate hectare yield of dry biomass. The evaluation of biodiversity was carried out using selected group of biological indicators – ground beetles of family *Carabidae*. Ground traps were used to collect the beetles. Individual collections were assessed and statistically evaluated. The collected individuals were also divided into eurytopic, adaptable and stenotopic groups according to their habitat requirements. The Simpson diversity index and Jaccard index were calculated along with dominance. It is possible to conclude following from collected results: i) length of harvesting cycles (rotation) is influencing strongly all measured and calculated parameters including biodiversity; ii) highest yield of 11,7 o.d.t/ha/year was at longest 6-year rotation; iii) the gradual migration of and colonization by the adaptable and stenotopic carabids to the short rotation plantations may indicate the improvement of microclimatic conditions in these plantations in comparison with annual agricultural crops. Statistical evaluation (ANOVA, Kruskal-Wallis analysis) confirmed differences between most of evaluated growth and yield parameters according to factor rotation.

Keywords: rotation length, biodiversity, *Carabidae*

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Rotation of *Populus deltoides* in agroforestry plantations in India

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Populus deltoides is one of the most popular agroforestry species in north Indian plains. It is grown in irrigated, cultivated land with agricultural crops at rotation of six years. The mean annual increment of plantations is about 25 m^3 per ha per year. The wood is used for veneer, matchstick, paper, etc. The tree is considered to be ready for harvest when it attains about 24 cm dbh. In plantations having greater growth rate of *P. deltoides*, farmers have the inclination to harvest this tree at a younger age once it attains this diameter. A study was conducted to examine the effects of shorter and longer rotations on the economic viability and biomass production of this species. The study revealed greater economic returns with the use of longer rotations in intensively managed plantations. Reduced yield of intercrops and lack of information about economic returns from *P. deltoides* trees in older plantations are considered to be the major reasons behind early felling of trees of this species. Availability of more options in choice of intercrops would favour late harvest of *P. deltoides* trees. High-density plantations of this species are not raised in India, although these plantations can be viable in some sites.

Keywords: agro-forestry, rotation length

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Early effect of water restriction and weeds on *Salix matsudana x Salix alba* `A 13-44´ growth

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The establishment of forest plantations is a critical period where seedlings and saplings performance is commonly hindered by both biotic and abiotic factors. Water stress and weeds are serious constraints that affect the survival and growth. Several willow clones are used in commercial plantations in Paraná River Delta (Argentina) for pulp, harboard and sawmill industries. We evaluated the early effects of water restriction and weed presence in the growth of the clone *Salix matsudana* x *Salix alba* `A 13-44´.

The experiment assessed the responses of willow plants submitted to different weed cover and water availability regimes. Unrooted cuttings (1-year-old) were planted in 15 l containers. Two levels of weed cover were studied: Weedy (We), willow cutting surrounded by 8 sorghum plants and without weeds (NW). Two levels of water availability were applied: Control (C) plants (irrigated every 2 days to field capacity) and Stressed (S) plants (40% of the Control). Treatments were arranged in a completely randomized design with ten replicates. From mid November to the beginning of February diameter and height growth were evaluated weekly. Stomatal conductance (g_s) and leaf relative water content (RWC) were evaluated on several days. Photosynthetic active radiation (PAR) records were taken at full sunlight above the plants canopy and at 0.50 and 0.30 m from the soil level, at 0.10 cm from the willow stems. Willow and weed aboveground and bellowground biomass were harvested and dried at 70ºC. Early negative effects were observed on willow growth. At the end of the experiment, both weeds and water restriction significantly effected willow growth, but a more pronounced restriction was observed in We plants even in Control conditions. Aboveground biomass in NW-C plants was significanlty greater (66%) than in We-C plants; We-C and NW-S plants showed similar values. We-S plants showed the greater reduction in aboveground biomass. Willow root biomass was significantly reduced by water restriction (46 %) and weed presence (27%). Height was significantly greater in NW-C plants (40%) than in We-C plants, while no diferences were observed between NW-S, We-C and We-S plants. Besides, NW-C plants showed greater diameter (30%) than We-C plants, while no diferences were observed in S plants. Leaves production was maintened during the whole experiment in NW-C plants; no leaves production was observed in NW-E and W-C plants since mid January while an intense defoliation ocurred in W-E plants.

Weeds reduced water availability to willow plants. RWC was significantly higher in NW plants than in We plants, and C plants showed higher RWC than S plants. Furthermore, weed presence reduced significantly g_s even in C irrigated plants, therefore NW-C willow plants always showed the highest g_s values. We observed that in We plants PAR was reduced 30 and 70% at 50 and 30 cm respectively from the soil level.

Our results highlight the importance of weed control during the early stages of the establishment period in shade intolerant species like willow. Besides, the knowledge of the different responses (morphological and physiological) of the forest species under weed competition will allow a better undestanding of the vegetation management practices.

Keywords: water stress, weeds

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Short Rotation Forestry of poplar in Italy: current situation and prospect

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The aim of this contribution is summarizing the current status of poplar Srf in Italy, after a decade of commercial deployment of the woody crop; this was done gathering information from academics, involved in research activity, as well technical operators managing commercial plantations across the entire Italian territory, under various pedoclimatic conditions.

In Italy, biomass may potentially cover a significant percentage (up to 10%) of the domestic annual request of energy, reducing imports of fossil fuels, and respecting CO_2 emission levels within the subscribed Kyoto Protocol. Traditionally, most of the used biomass is fuelwood in rural households. But the industrial use of woody biomass, for thermo and/or electric conversion, has rapidly increased in the last few years and it currently has a total power of 500 Mega $Watt_{el}$ with 107 bioenergy plants across the national territory. These plants have been strongly supported by state incentives for energy companies using renewable energies.

Part of the raw material feeding bioenergy plants is supplied by coppice plantations of fast growing trees (poplars, willows, robinia and eucalypts), established by farmers on agricultural lands, and intensively managed, as coppice plantations, with short rotation harvesting cycles (2-5 years). Specific public grants of the European Union (Regional Rural Development Plants) have supported the Italian farmers, during the last 10 years, to establish about 6000-7000 ha of Srf plantations, mostly on alluvial soils in northern-continental Italy, with poplar hybrid clones selected by Italian public and private actors. Cultural operations are fully mechanized, from plantation establishment up to the biomass harvesting. The most used hybrid clones are several Italian ones, such as *Monviso*, *AF2*, *AF8*, *Sirio* and *Pegaso*, and more recently *Imola*, *AF8* and *Orion*.These clones have showed, in experimental trials, yields up to 25 t d.m. $ha^{-1}y^{-1}$, with productivity strongly affected by site characteristics (soil fertility and climatic condition), and water availability (precipitation and irrigation) being the main limiting factor.

In commercial plantations yields are lower, even because the recommended cultural operations such as fertilization and irrigation are rarely applied by farmers. Rotation is generally 2-3 year long and planting densities in the range from 4000-5000 to 6000 cuttings ha^{-1}). Wood biomass, of low quality for high bark percentage (15-20%), is mostly used as chips for bioenergy plants or for co-firing in thermo-electric plants and incinerators; the biomass obtained by poplar plantations with 5 years rotation is preferable used for pellet production. In most of the cases, the profitability of SRF for farmers is currently connected to the public grants, giving the dominating conditions for the farmers of selling the biomass to the energy companies. Pilot experiences of farmers' cooperatives selling the energy obtained by their own produced biomass are showing interesting margins of profitability.

Further development of woody bioenergy crops, especially in peninsular parts of Italy, will be connected to the implementation of the 5 years rotation model, along with improved cultivation techniques (*in primis* micro irrigation and phytoremediation), and finally enlarging the choice of species suitable to different site conditions.

Keywords: short rotation forestry, clonal tests, energy transformation

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Session 4: Poster

Biomass production of poplar hybrids after three biannual coppice rotations

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Short rotation forestry (*SRF*) is based on the use of fast growing species, high planting density and short harvesting cycles spanning, in Italy, from two to five years. The aim of SRF was to produce biomass for energy as an alternative to surplus food crops. Currently, hybrid poplars are the most common used species and new clones are continuously under evaluation, especially for their ability to sustain repeated harvesting. This work has the objective to analyse the biomass production and its components in a short rotation poplar coppice system at the third biannual rotation and compare it to the biomass production of the previous ones. For this purpose, an experimental plantation with a randomized block design and four replicates was established on an alluvial agricultural site with several hybrid poplar genotypes at Mira (northern Italy) in 2003. Experimental data were collected on eight genotypes for several traits involved in biomass production such as survival, diameter, height, biomass allocation in the stem and in the branches. Furthermore, biomass qualitative traits such as wood moisture, wood basal density and wood heating value were also measured. Statistical analysis pointed out the existence of significant differences among genotypes for morphological traits. Biomass production differed among parentages and among rotations. Data analysis from the third rotation showed a decrease of the mean biomass production as compared to the previous rotation, with a value close to 17 Mg ha^{-1} $year^{-1}$. The different parentages also differed in their strategy of biomass allocation but the most performing genotypes maintained the same ranking during the three rotations. These results will be also discussed in relation to survival and production stability of the different genotypes in order to select the best adapted clones to the multiple coppice rotations.

Keywords: biomass production, coppice rotations

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Rooting type, rooting behaviour and post-planting white root development - A key to successful poplar plantations

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Most tree species are highly variable genetically and this variability is the main source for their improvement. Only by selecting and breeding the best individuals we can raise the desired better types. The outer appearance of a species in its native habitat normally gives an indication of an individuals inherent tendencies. Therefore, in improvement programmes it is presumed that the crosses between phenotypically good trees in a population will result in predominantly stable genotypes with inheriting best characters of the parents or sometimes even better than parents. But genetically from wild populations, it cannot be expected, and gain is normally restricted and requires vigorous selection procedures.

In poplars, besides the good growth rate and form, for which the clonal selections have been in practice for almost two centuries, the rooting behaviour is the other neglected but most important character leading to success or failure of plantations. Though some studies on the rooting behaviour are available, but the sustained analysis from rooting of cuttings the field plantation and then to its behaviour after the plantation during its growth period are not available to substantiate that the rooting is a heritable character and helpful in plantation establishments. With these aims in view the studies were conducted to know the behaviour and type of root formation in selected poplar clones of *P. deltoides*, *P. ciliata*, *P. alba* and the hybrids of *P. ciliata* x *maximowiczii* and *P. deltoides*.

Many studies suggest that the rooting in poplars is limited by the level of water table and soil conditions, but it has been observed that rooting in poplars though adventitious in nature is strongly under the genetic control. Studies showed the type of rooting development after 30 days of cutting planting in the nursery is indicator of type of rooting behaviour, whereas after 180 days the rooting pattern can be assessed which remains the same throughout the life of the plant and is consistent for the clone. This rooting pattern can be assessed after 240 days at the time of lifting of plants from nursery for field plantings. After the side roots have been pruned and augur plantings carried out, the pattern of white root development is also consistent and follows the same rooting pattern as has been ascribed to a clone. Thereafter till harvesting this pattern remains the same and is responsible for the overall development, establishment and growth of the poplar plants, which are clone specific. Normally the rooting behaviour has been classified based upon the plunging and anchor root system which develops in poplar clones. But the number of plunging roots and the place from where the plunging roots develop along with the number, and profuseness of the anchor roots is the criteria of classification of roots in poplars into five categories namely A, B, C, D, E and F. The consistency of these categories of root systems has been identified as the criteria for the place and site of planting where a specific clone can succeed. In addition it was found that the rooting behaviour, rooting pattern and rootability in the hybrids of *P. ciliata* x *maximowiczii* was much greater than both the parents i.e *P. ciliata* and *P. maximowiczii* and showed hybrid vigour with thick and vigorous root development. Two clone in case of *P. alba* were also identified with vigorous root development whereas other clones of *P. alba* were found to be poor rooters and poor in growth, development and establishment.

Keywords: Poplars, rooting pattern, hybrid vigour

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Energy crop plantation system development for *Salix* and *Populus* in Michigan, USA

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Woody biomass is potentially the key renewable feedstock for power, heat, and fuel production in the Northeast and Lake States region of the United States. Abundant natural forests and retired agricultural fields offer great potential for the production of these feedstocks which will, in turn, reduce the region's dependence on imported fossil fuels and support rural community development. Demand for biomass from industry in the region continues to grow and landowners increasingly seek advice about sustainable production systems. Michigan State University has built governmental, industrial, and university partnerships throughout the area and across the world and has taken the lead in improving woody biomass production systems and streamlining the feedstock supply chain for Michigan.

Woody crop plantations are not yet used extensively in Michigan but offer the greatest potential for socially, environmentally, and economically sustainable production of biomass for energy. Current research centers on plantation management and sustainability improvements within these systems. A network of five sites throughout the state has been established on which clonal and yield trials, spacing trials, herbicide trials, and measurements of greenhouse gas fluxes are proceeding on taxa from the genera *Salix* and *Populus*. While still in the early stages, preliminary results are presented here that suggest that commercially viable biomass plantations of carefully selected taxa in Northern Michigan will be up to eight times as productive as native forests. The best performing clone (NM-6) averaged 8 dry $Mg \cdot ha^{-1} \cdot yr^{-1}$ after eleven growing seasons in our tests. Future work is examining yield variability across sites and new clones.

In other initial studies we found that conversion of wet, high soil organic matter pasture to bioenergy plantations using conventional tillage regimes resulted in up to a 25-fold increase in soil N_2O flux. Soil CO_2 efflux measurements indicate substantial soil C loss as well, although estimates are complicated by difficulties accounting for autotrophic respiration. These results indicate that, under certain site conditions, pastureland conversion to poplar or willow can incur a significant greenhouse gas debt. Future work is focused on using site selection and novel methods of plantation establishment to reduce these impacts.

Keywords: *Populus*, S*alix*, biomass plantations, sustainability, yield

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An approach for siting poplar energy production systems to increase productivity and associated ecosystem services

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Short rotation woody crops (SRWC) such as *Populus* species and hybrids (i.e., poplars) are renewable energy feedstocks that are vital to reducing our dependence on non-renewable and foreign sources of energy used for heat, power, and transportation fuels. Highly productive poplars grown primarily on marginal agricultural sites are an important component of the future energy strategy in the United States and many other countries. Poplars can be strategically placed in the landscape to conserve soil and water, recycle nutrients, and sequester carbon. Establishing poplar genotypes that are adapted to local environmental conditions substantially increases establishment success and productivity. But, it is difficult to predict field trial success in landscapes where the crop has not been previously deployed. Our overall goal is to merge our knowledge of poplar biology with large-scale spatial analysis to predefine zones of potential plant adaptation that are ecologically sustainable and economically feasible across the landscape. The project builds on SRWC research conducted at the IAES in Rhinelander, Wisconsin, USA since 1968, as well as decades of poplar genetics research in Minnesota, USA that has led to commercial poplar production on >10,000 ha in the state.

We identified in a spatially-explicit manner potential core areas within Minnesota and Wisconsin, USA for potential field testing by combining key climatic and soil properties with land ownership and use constraints. Our approach was to rank lands based on current land use (i.e., open land cover types), land ownership (private vs. public), suitability of soil for agriculture (marginal vs. prime), and economic thresholds. Because the decision to convert lands for SRWC production is an economic decision by most landowners, we incorporated soil rental rates established by the Farm Service Agency, and estimated return on corn yield by county to establish economic thresholds beyond which conversion to SRWC production is not probable. Next, we determined the range of variability (i.e., mean and variance) in climate properties (e.g., growing degree days, temperature, precipitation) and key soil properties (e.g., available water holding capacity, bulk density, pH) for the resulting land base. Additionally, we are currently using a combination of empirical data from prior regional field testing networks (see Coyle et al. – poplar database) combined with productivity models to predict establishment and long-term yield of favorable genotypes throughout the cores areas described above. We will also conduct field reconnaissance and surveys to assess the potential opportunities for maintaining soil health, water quality, and other ecosystem services, assuming poplars are tested and/or deployed within the core areas.

Our presentation will highlight the development of the GIS-based siting protocol, along with results from the productivity modeling and ecosystem services assessment. Our approach is novel in that it integrates genetics and landscape ecology to limit inputs without sacrificing sustainability or productivity of these energy feedstock production systems.

Keywords: energy security, feedstock production, *Populus*

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Early Screening for Poplar Windthrow Resistance Using Low-Energy X-ray Imaging

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The root systems of nine commercial poplar clones were imaged with low energy X-rays once per week over the first four weeks of root emergence from hardwood cuttings. Five ramets of each clone were grown in an inert, low-density substrate (1 mm expanded polystyrene beads) under drip irrigation with a modified Hoagland nutrient solution. Each ramet was grown in a container of dimensions 45mm x 200mm x 500mm. The goal was to investigate whether early root traits, extracted from the X-ray images of trees grown in specialized media, could be used as windthrow screening criteria in poplar breeding programs.

Over thirty-five hundred individual X-ray images were analyzed with digital image processing techniques to extract root system parameters thought to correlate with windthrow resistance. Forty-five thousand root measurements were made from the X-ray images and the data was analyzed using ANOVA techniques. The investigation suggests that varieties which generate fewer but larger early roots from the hardwood cuttings tend to belong to the class of trees thought to be windthrow resistant. This was true for both basal and lateral roots. Trees which generated a larger number of early lateral roots closer to the surface-line also tended to belong to the class of windthrow resistant varieties. Other root traits examined did not show significant differences among resistant and susceptible classes. The results of the X-ray image analysis were compared to the root system traits of five year old poplars harvested at two diverse field sites in the U.S. Pacific Northwest. Six of the clones analyzed with the X-ray technique were in the sample of harvested five year old poplars.

Keywords: wind resistance, X-ray images, root system

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Poplars and Willows in economic and environmental development of India

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Populus deltoides, an exotic poplar, is the backbone of a thriving plywood industry in India. This species is planted in blocks or border rows in irrigated farm lands in sub-tropical areas in the plains and low hills of north India. Harvested at a rotation of 5 to 8 years, about 25 m^3 ha^{-1} $year^{-1}$ mean annual increment is recorded in most of the plantations. Genetic improvement and intensive cultivation have led to realisation of as much as 49 m^3 ha^{-1} mean annual increment. Matchstick and paper industry too consume sizeable quantities of poplar wood, while wood waste is used for burning and making charcoal. The species is planted in different agroforestry models in conjunction with traditional agricultural crops. A wide choice of agricultural crops is available for intercropping with this species, but the choice gets narrowed down in block plantations beyond two years of plantation age, particularly in summer season. Shade-bearing crops have been identified for cultivation with this species. Growing of its nurseries and plantations is an attractive investment, especially among people with large landholding. Planting rates are greatly affected by fluctuations in price of wood in the market. Planting rates have been increasing continuously during the past five years due to consistent rise in price of poplar wood. *P. nigra* is another exotic poplar that has been planted in temperate areas in Jammu & Kashmir state. Some other exotic species of poplar have been planted in this state that have shown fast growth but concerns have recently been raised about their effect on groundwater table.

Indigenous poplars viz. *P. ciliata*, *P. alba*, *P. euphratica*, *P. gamblei*, etc. are important for ecology and economy of the mountainous regions in north-west and north-east India, but their cultivation is limited because of relatively slow growth. Steps are being taken to further promote planting of these species. Native stands of indigenous poplars have already been documented by Indian Council of Forestry Research and Education, Dehradun in Indian Himalayas. Plans are underway for chraracterisation of native populations by molecular markers, selection of superior trees and their clonal evaluation.

Salix alba, *S. angustifolia*, *S. fragilis* and *S. elegans* have been planted in temperate and alpine conditions in Jammu & Kashmir and Himachal Pradesh. Planting stock of *Salix* species viz. *S. fragilis*, *S. vitellina*, *S. matsudhana*, *S. babylonica*, *S. alba* and *S. corrulea* from Jammu and Kashmir has been collected at Himalayan Forest Research Institute, Shimla. Planting stock of various provenances of *Salix* has also been produced at Tabo (Spiti valley) in Himachal Pradesh. The species are much used for domestic consumption by the local communities. Research on value-added products from these species would further promote conservation and cultivation of these species. Native populations are affected by pest problems in recent years. Two species of willow-feeding aphids viz. *Tuberolachnus salignus* and *Pterocomma salicis* have been recorded in Lahaul valley where willow mortality was previously observed.

Though people plant poplars and willows for their direct economic and ecological benefits, these plants also have huge potential for carbon trading under the Clean Development Mechanism (CDM). Awareness about CDM project formulation, execution, monitoring and evaluation is being generated for promoting economic and ecological well-being besides promotion of cultivation of these plants in India.

Keywords: intercropping, wood industry, CDM

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Usage and harvesting situation of poplar plantation in Iran

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Future demand or extreme lack of forest resources is one of the most powerful factors for poplar plantation. The poplar plantation had an enormous influence on the forestry sector and also price of wood and paper products in Iran. Therefore, the main questions are whether, how situation of poplar plantation are? Since, the methodologies of research were descriptive, analytic and approximate. The research questions were tested by statistical and questionnaire analysis. Our results showed that the extreme lack of forest resources and strong demand had an enormous influence on poplar plantation and the situations are impacting directly or indirectly the forestry sector. Increasing demand for poplar timber has encouraged provinces of Iran to expand the fast growing poplar plantations. Approximately, the annual harvesting of potential in Iran is about 10 million cubic meters. If the fast growing poplar plantations propagate, production of Iran is projected to rise further in the long term as wood from existing poplar plantations matures and as new plantations are established.

Keywords: poplar plantations, timber

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Potential of poplar agro forestry for carbon sequestration and livelihood security - a case study from India

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Agro forestry has been recognized as a strategy for carbon sequestration. It has a vast potential for carbon storage in its multiple plant species. The estimated area currently under agro forestry world-wide is 1023 million hectares. The quantum of carbon sequestered in an agro forestry system depends on above and below ground biomass of trees, carbon stored in the soil and the carbon sequestered in wood products. Nevertheless, site-specific edaphic, climatic and biological factors influence the quantum of carbon sequestered in the system. The carbon sequestration potential of agro forestry systems is estimated between 12 and 228 Mg per ha with median value of 95 Mg per hectare. This potential has not been adequately recognized let alone exploited.

Agro forestry also indirectly contributes to carbon sequestration by mitigating demand for wood and reducing pressure on natural forests which are considered to be largest sinks of terrestrial carbon. At the global level, the Inter-Governmental Panel on climate change in its third report estimates that about 100 billion metric tons of carbon over the next 50 years could be sequestered through forest preservation, tree planting and improved agricultural management. The 1997 Kyoto treaty established the global carbon market allowing credits from clean energy and as a result the carbon accounting in wood and wood products gained momentum all over the world.

Through a case study, this paper gives an assessment of the potential of poplar agro forestry plantations raised by the farmers on farm lands to make them competitive in carbon markets for carbon emission reduction credits. It begins with an overview of issues like global warming, climate change and the role of trees in acting as carbon sinks in mitigating climate change. An assessment of the emission abatement has been presented through data collected from farm lands where poplar was grown with wheat as intercrop. The study also shows the trend of emission abatement through trees at different crop ages. With the harvesting of poplar trees raised under agro forestry, the carbon sequestered in trees is not lost; rather agro forestry based wood processing industry takes over the material and facilitates long term locking up of carbon in long lasting wood products besides providing livelihood security to the local people.

Post Copenhagen, the potential of poplar agro forestry for climate change mitigation and human welfare can be realized if the ‘Green Climate Fund’ committed by the developed countries in the summit is made to support projects and programmes of green investors and that with simplified procedures and methodologies. Till such time, the poplar agro forestry will remain little recognized and underexploited in the realm of climate change mitigation and carbon trade.

Keywords: agro-forestry, Kyoto Protocol, global carbon market

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Poplar and hybrid aspen in Sweden - research, practice and progress

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Background

Insistence for using renewable energy sources are increasing. The new requirements from the EU say, among other things, that the share of renewable energy should be 20% of all energy used in the union by 2020. Sweden is well positioned, with a high proportion of renewable sources, 40%, and significant opportunities to further raise the share. One of the sources that we put great hopes for, and which is already significant, is the biomass of woody plants. The Oil Commission pointed out guidelines to reduce the dependence on oil in the country in 2006, and one of the strategies presented was an historical investment in forest and arable fuels. Recently, a commission was given by the Ministry of Agriculture to investigate the potential for intensive cultivation of forest on abandoned farmland and on land with no high environmental values in Sweden, the so-called MINT project. It was concluded that plantation forestry may contribute significantly with bio fuels and raw material for different purposes. Thus, the interest in increasing the production of biomass in an environmentally sound manner is large in the country. Poplars and hybrid aspen are interesting and likely candidates in this effort.

Research and major results

The results to date show that hybrid aspen (the crossing between European and American aspen) and poplars have high growth potentials and that they are suitable for cultivation systems with focus on biomass production. So far they have been tested in plantations with initial densities of 1,100 to 5,000 stems ha^{-1}, which means that the expected rotation period is above 10 years. The productivity of hybrid aspen in those cultivation systems is estimated to be in the span 20–25 m^3 of stem wood ha^{-1} yr^{-1} in southern Sweden, which corresponds to 8–11 tons DM ha^{-1} yr^{-1} including twigs and branches. Hybrid aspen and poplars also differ from *Salix* since they are tree-formed, thus allowing production of other assortments, something that may attract land owners.

In plantation forestry, the next generation after harvest is of utmost interest. Hybrid aspen is relying on root suckers and poplars mainly on stump shoots. There is an option for new planting, which will favour introduction of new plant material, but if natural regeneration is powerful it will most likely be a good economic alternative. Studies have shown that root suckering of hybrid aspen is dense and vigorous. About 50,000 to 100,000 suckers are generally found the first year after harvest. The productivity increases rapidly and the mean annual increment has reached a level of 9–10 ton DM ha^{-1} yr^{-1} in about 4 years on research sites.

Commercial planting material has been selected for hybrid aspen and poplars for the southern part of Sweden, i.e. with mild climate. At the moment the material consists of a batch of 15 clones for both hybrid aspen and poplar. The selection procedure includes vitality, growth and stem quality. Because stem canker in hybrid aspen was earlier a major problem in hybrid aspen plantations, special care has been taken to select clones with large resistance against canker attack. In general, there are big possibilities for improvement of the material within the genus *Populus* since there are significant differences between clones for many important traits.

Wood characteristics have also been studied and, for example, the specific gravity of the wood of the selected clones has been estimated to 369 kg^{-3} for hybrid aspen at 20 years of age and 322 kg m^{-3} for 17-year-old poplars.

Introduction of fast growing species like hybrid aspen and poplar implies that bio diversity and public opinion will be affected. Studies have so far been carried out for willows but not for plantation forestry with *Populus* species.

Practice, performance and economy

The areas planted with hybrid aspen and poplars are currently about 2,000 hectares in Sweden, which could be compared to the c. 15,000 ha of Salix plantations. Thus, so far willows have dominated but the areas with Populus have increased rapidly during the last years.

Inventories of commercial plantations reveal that the high production levels, mentioned above, may be reached with careful preparation, establishment and management on fertile agricultural land. However, there is a risk for lower productivity if care to site selection as well as preparation and management directed towards weed control, and thereby also avoidance of attacks from rodents, are not up to the mark. An observed problem is establishment of poplar on forest land. These plantings have often been unsuccessful but the reason for this is not yet fully understood.

The few examples so far available on economy of commercial *Populus* plantations show good profitability. This is to a large extent dependent on the high productivity with short rotation times, but also on working market conditions and on the possibility to use conventional forestry technique at harvest. However, the technique to manage the natural regenerated stands with high stem density has to be developed. The new rules that allow cultivation on arable land with rotation periods (harvest intervals) of up to 20 years with the energy approach, implies that hybrid aspen and poplar are approved energy crops.

A major problem when growing hybrid aspen is its attraction for deer and moose. This means that there is almost always necessary to fence the stands. Calculations have shown that the cultivations can bear these expenses economically but it reduces the profitability and may constitute a psychological problem for investment in hybrid aspen plantations. Experiences so far reports fewer problems with game in poplar stands although fencing may still be necessary.

Another problem with establishment of hybrid aspen stands in Sweden is the high plant cost. Hybrid aspen plants are currently produced mainly with micro propagation. It would be desirable to use cheap stem cuttings, like for poplars, but so far they have not been successful for producing plants. Poplar stands are mainly planted with rooted cuttings. A drawback for the introduction of *Populus* on larger areas is also that there is yet no approved plant material available for neither hybrid aspen nor poplar for the major northern part of the country.

Progress

Experiences from commercial plantations are highly valuable for the progress of cultivation. The results obtained in well-managed research stands are not fully transmittable to practice. Information and experiences from the practice are therefore desirable and a structured collection of information is also under way.

Future expansion of *Populus* cultivations in Sweden is dependent on approved planting material for the whole country. A new clone test with material available from Sweden and neighbouring countries will be established during 2010. The test includes over 70 and 80 clones of poplar and hybrid aspen, respectively. The test consists of ten sites spread over 5 latitudes from 55˚ to 64˚ N., including both arable and forest land in each latitude. Simultaneously work is going on with molecular biological means to improve poplar material. Since major obstacles for a large-scale introduction of hybrid aspen are the high costs for plant material and fencing, plant propagation with easier techniques is tested, so far with limited success. Due to the large populations of browsing animals in Sweden, the development of fencing, leading to cheaper fences with more efficient set up is most welcome. It is at the moment driven commercially with some contribution from R&D. It is likely that also poplar plantations need fencing for undamaged growth and development.

Although conventional forestry concepts with poplars and hybrid aspen are relatively well-known, the performance in coming natural regenerations is far from solved. The initial phase in those stands may resemble *Salix* cultivations, although shoots are not growing in proper rows and soil conditions are more complicated on forest land. Thus, technical solutions are sought-after. Another important question is production sustainability in natural regenerated

stands. Here, a research project is going on for hybrid aspen where cutting cycles of 4 to 16 years are compared.
The research conducted on high yielding crops has shown that species within the genus *Salix* and *Populus* are highly interesting candidates for production-oriented cultivation. For *Salix* a longstanding research program is in progress with substantial funding from the Swedish Energy Agency. For the genus *Populus* the research in the country has so far been carried out in individual projects of limited duration. In order to find out the need of research and development in Sweden on *Populus* species, an investigation is currently under way which will constitute a decision support for the Energy Agency for future research priorities.
Effects of *Populus* cultivations on environment, cultural heritage and public values are insufficiently known and therefore efforts have started to increase knowledge in these areas.

In conclusion, knowledge of the poplars and hybrid aspen grown under Swedish conditions is insufficient in many areas, but efforts to raise the knowledge level and improve practical performance have begun and are under way.

Keywords: renewable energy, biomass production, rotation lengths

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Energy and economic evaluation of technological innovations in poplar SRC wood chips production

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Mechanization plays a central role in the evolution of energy crops, since the development of technological innovations increases production efficiency, with improvement in economic and energy balances of agro-energy system. The CRA-ING developed technological innovations related to cuttings selection, cultivation and harvesting in SRF plantations, at the same time identifying possible improvement in energy wood chips storage. In this study are analyzed issues related to the cultivation and harvesting of poplar Short Rotation Coppice (SRC) biennal plantations, addressed to energy chips production. The study considered the introduction of innovative machines at different stages, in order to point out the effects on economic and energy balances, when compared to conventional mechanization. Accordingly economic and energy balances of poplar energy wood production chain were compared before and after the introduction of technological innovations. Analysis of results, based on the field tests of machine prototypes designed by CRA-ING, underlined the possibility to reduce biomass production costs and possibility to reach energy sustainability, when compared to mechanization commonly used in energy crop cultivation and harvesting.

Keywords: energy crops, biomass production, harvesting

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Attitude to plywood production of the wood of six Poplar clones ('Brenta', 'Mella', 'Sesia', 'Soligo', 'Taro' and 'Timavo')

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In the present paper we analyze the dendrometric and physical characteristics, and the quality and suitability to rotary cutting of the wood of six new poplar clones ('Brenta', 'Mella', 'Sesia', 'Soligo', 'Taro' and 'Timavo'), comparing them with those of `I-214', the most widely spread clone in the Italian panorama.

In one polyclonal plantation 3 trees per clone were felled. After their dendrometric characteristics were assessed, from every plant we cut some logs for the rotary cutting test, carried out in a plywood industry, and two disks (5 cm thick) per plant, from which we obtained samples for the physical analyses (basic density, fresh weight and total shrinkage).

The basic density of the new poplar clones examined was definitely higher when compared to 'I-214' and so were the quantity and quality of the veneers obtained. In particular 'Taro' and 'Soligo' turned out extremely interesting: the first one for producing the highest number of sheets of excellent quality, the second for both the good quality of the veneer and a growth rate much higher than 'I-214'.

The mechanical characteristics of these clones, which have not yet been determined but which are likely to be superior at least by 15-20 % to those of 'I-214', together with the higher amount of raw material obtainable, make the new clones particularly suitable for all those innovative uses beyond the production of packaging (for instance in the building sector, with or without structural functions), in which the mechanical performance is discriminating.

Keywords: clonal tests, wood properties, plywood

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Innovative system for field dehydration of poplar trees

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An innovative SRF harvesting system based on two steps operation, was developed by CRA-ING 1) the tree felling and inter-row windrowing performed by a feller-windrower equipment; 2) subsequent chipping performed by an harvester equipped with pick-up device. At the harvesting time, the windrowed trees showed a low moisture content affecting their physical qualities and mechanical strength throughout the chipping operation. This work aims to analyze the moisture losses of windrowed trees, in relation to the windrow location, during field storage and weather condition, as well as to characterize chips quality changes during the field storage by the two different harvesting systems comparing the innovative two steps with the traditional single step harvesting systems.

Keywords: energy crops, biomass production, harvesting

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Performance and quality results of a new chipper device for poplar biomass

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The research activities carried out within the wood biomass storage showed that the reduced size of chips had negative effects on the preservation of the stored product. The improving of the machine productivity in the harvesting phase and, in particular, the evaluation of SRF harvesting mechanization systems, highlighted some operative limits due to the cutting system of the maize chopper Class Jaguar. CRA-ING, in order to overpass the mentioned limits, designed a new cutting system. The new rotor was mounted on a Claas Jaguar 890 operating machine, replacing the commercial apparatus and some experimental tests on second-coppicing poplar plantations were performed. CRA-ING designed a rotor of five series of double staggered knives for an amount of ten knives (commercial apparatus of 12 knives). The new rotor developed for poplar chipping improved the chips quality and the machine productivity. The test carried out in the last years demonstrated that in order to have a good air circulation in the chips pile, these must be realized with thin chips particles

Keywords: energy crops, biomass production, harvesting

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Prototype for poplar cuttings optical selection and diametrical classification

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In order to decrease the production cost of poplar cuttings, CRA-ING developed, in collaboration with Agrimat company of Tortona (AL) and Franco Alasia Vivai in Savigliano (AL) under ENAMA support, a prototype able to select mechanically the cuttings in relation to their diameter classes and to count the number of pieces for each class. Having cuttings of the same diameter is the first step to develop a planter totally authomatizated (this activity is under development). The prototype derives from a calibration machine normally used for the asparagus selection. The basic components of the machine are: a V-shaped conveyor belt of 500 cm, taking poplar cuttings from the cutter equipment; a V conveyor belt of 200 cm (variable speed) for aligning and distancing cuttings of about 4-5 cm; an optical electronic device to read the diameter and a pneumatic system to convey cuttings in the proper diameter class vessels. The system is managed by a dedicated software, and the not recognized or too thin cuttings are collected in a specific vessel. In order to evaluate the actual prototype performance, CRA-ING tested it surveying the operation performances. The equipment actually fails around the 3,5% of measurements. After the adjustment of the single components, the prototype will get lower error rates, improving the performances of the cuttings planting operation.

Keywords: energy crops, biomass production, harvesting

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Quality evaluation of poplar and forest species chips produced by an innovative chipper device

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The Research Unit for Agriculture Engineering of the Council for Agricultural Research and Experimentation (Rome) designed a innovative chipper device mounted on a Class Jaguar forage harvester in short rotation forestry harvesting. The new rotor differs from standard rotors as it has a lower number of blades and blade-holders, from 24 to only 10, with different cutting angles and drum insertions. The study evaluates the size distribution of chips produced by standard rotor, compared to those obtained by the experimental rotor. The aim of the study was to compare the particle size distribution of chips produced using the two types of chippers, thereby evaluating a possible influence of wood species in the chips formation process. These tests were carried out using Claas Jaguar forage harvesters series 860 and series 890, both equipped with standard rotors and CRA-ING rotors. Furthermore, studies were performed in poplar cultivations at biennial harvesting and in forest stand, on previously felled trees. The comparison of the innovative CRA-ING rotor with traditional rotors, showed a significant product size increase within particle sizes ranging from 12.5 to 25 mm, and an equivalent product reduction within the finest particle size classes. However, the new rotor tends to concentrate dimensional increments along the longitudinal section of chips, regardless of the tree species. Therefore, following these results, a second rotor was built to reach a cleaner cut on the biomass introduced by the feeding rollers. Thereby obtaining a superior quality of chips in terms of particle size and increasing the size of the chips, even in radial or tangential sections.

Keywords: energy crops, biomass production, harvesting

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Three years researches on poplar chips storage

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In order to identify a storage system able to reduce the fermentative phenomena, in 2007, CRA-ING started a survey on inside chips piles temperatures, moisture content changes, NCV variation and dry substance losses in different operating conditions by the monitoring of four piles (uncovered pile, pile covered with a geo textile sheet, ventilated pile and compacted pile). The final opening of piles revealed that the best solution in product drying, was obtained in uncovered and covered piles. In 2008, CRA-ING started to test the influence of size on the chips final quality, once identified the two most promising storage systems. Aiming to test different solutions, two piles were built: the first pile, the largest one by Spapperi chips, (at Alasia Franco Vivai in Savigliano (CN); the second pile by Claas Jaguar chips (at the power plant of Bando d' Argenta (FE) in San Marco Spa). The results showed a direct relation between drying speed and pile size. In 2009 the trials were carried out on the same site in collaboration with Enervision of Dosolo (MN). The storage of wood-cellulose fibers leaded to the degradation of components with consequent product losses. The different storage techniques could reduce these losses, even if not completely removing negative consequences.

Keywords: energy crops, biomass production, harvesting

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Two steps system in SRF harvesting mechanization

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An innovative unit adapted to the poplar plantation harvesting, growth as SRF, was developed by CRA-ING in order to operate the poplar felling and chipping in two different stages. In particular an harvester for cutting and felling trees in windrows parallel to the movement of the machine, and a chipping machine provided with a pick-up to harvest and chip windrows, were designed. CRA-ING innovative method based on a single operation, allows to work also on wet ground conditions. In addition the heavy units, like mower-chipper and loader, were able to pass on the ground at the end of the winter, in order to chip natural dried products. The new machine showed successful performance reaching operative working capacity of 1,20 ha/h on two years plantations. CRA-ING has also thought to develop a similar characteristic machine working, simultaneously, on coupled rows, thus reducing harvesting times and operating costs. The harvester thanks to a pick-up, still being studied, was able to gather all trees left on the windrow, conveying them to the chipping feed rollers.

Keywords: energy crops, biomass production, harvesting

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Effect of different treatments on crown dimensions and weed covering in poplar plantations and their interactions

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One of the first requirements in poplar plantations is, existence of permanent resources, safe and sufficient water. Poplar varieties have different needs. Water quantities and availability periods effect many changes in their increment conditions. Hybrid poplars are very sensitive to competition from other vegetations, as are many other trees. Establishing a successful hybrid poplar plantation requires effective weed control.

The weed cover in forest plantations can be substantial if unchecked, reducing growth and survival of the tree. Weed control begins with thorough site preparation and continues through 3-4 years. Different varieties of poplars, including open crown and closed crown due to having a canopy with different irrigation periods can affect the amount of weeds. We defined the relationship between canopy of eight clones (top clones of poplars) with different crown dimensions and wet-weight of weed covering in three irrigation treatments (4, 8 and 12-day periods) in one poplar plantation of Tehran province (Iran). This study was done in spilit plot design and three replications. For each clones, diameter and canopy of poplars and wet-weight of weeds (in three locations) were measured and calculated. Examining significant difference of each variable in the trees and different treatments and defining the correlation coefficients of these variables was showed that:
- Between trees and three treatments were significant differences in canopy and weight of weeds.
- A significant correlation between larger crown and less weed was seen.

Keywords: Poplar plantation, canopy, weed.

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Evaluate the potential of evapotranspiration covers as a biomass feedstock in urban southeastern Ontario.

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Shrub willows (*Salix spp.*) and hybrid poplars (*Populus spp.*) are widely acknowledged as potential bioenergy crops, but may also have application as alternative landfill caps. Currently many landfills present in the urban areas of southeastern Ontario do not have properly engineered leachate collection systems or covers to prevent deep percolation that can lead to groundwater contamination. The cost to close existing waste sites with conventional covers is often prohibitive, and the durability of conventional covers composed of compacted fine-grained barrier layers is often insufficient to ensure long-term reliability. Evapotranspiration covers (ET) do not rely on a barrier layer, but instead utilize fast-growing plants and trees to manage water storage capacity and ET to reduce percolation rates. Evapotranspiration covers are a cheaper and more durable alternative and can potentially provide an array of other environmental and community benefits. One environmental benefit of ET covers is the potential to use the biomass generated on the landfill for bioenergy purposes. A major challenge for bioenergy is the realization of sufficient biomass feedstocks that can be provided at low costs. ET covers can be part of the solution and function as a source of low cost biomass feedstock. This study explores the use of *Salix* and *Populus* as the biological component in ET cover systems. Landfills in southeastern Ontario that can potentially be used for ET cover projects have been identified and mapped, and the amounts and costs of potential biomass that might be generated on these sites are estimated.

Keywords: urban forestry, evapo-transpiration, biomass feedstock

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Application of herbicides in production of poplar seedlings

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Weed control is very important, especially in nursery production of seedling stock, because the quality seedling stock is the basic prerequisite for successful establishment of poplar plantation and other forest species. Investigation of the possibilities of applying herbicides in production of poplar plants pointed out to many advantages of their application in relation to mechanical weed control. Usage of herbicides decreases the cost of soil cultivation and competition between weeds and nursery plants, and increases their quality and survival percentage. Investigation efficiency and selectivity of herbicides in the production of poplar plants were performed during the period 2005 – 2007. Trials were set up in two localities which differing in physicochemical soil properties (clay form of fluvisol and sandy form of fluvisol). Cuttings of three poplar clones Pannonia, B229 and Pe19/66 were used for experimental fields establishment. Examined combinations of soil herbicides acetochlor + prometryn, acetochlor + s-metolachlor, acetochlor + metribuzin i dimethenamid + linuron were applied after planting and before the growth of poplar cuttings and weeds. Herbicides fluasifop-p-butil i cycloxydim were applied after the growth of weeds and poplar cuttings to control of grass weeds. Examined combinations of soil herbicides were efficacy in controlling of weed vegetation in studied localities. The best efficacy during the three years on both localities exerted a combination of herbicide acetochlor + prometryn. Herbicides fluasifop-p-butil and cycloxydim have been effective in weed control of grass weeds. Differences in physicochemical properties of soil on the localities beside composition of weed flora also influenced the behavior and herbicides action in such a way that metribuzin applied on soils of lighter mechanical composition (sandy form of fluvisol) in combination with acetochlor exerted phytotoxic effect on poplar plants. Other examined herbicides did not have phytotoxic effect on poplar seedlings.

Keywords: nursery, weed control

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Aspen-Triticale Alleycropping system: effects of landscape position and fertilizer rate

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Short-rotation woody crops offer several key advantages over other potential bioenergy feedstocks, particularly with regard to nutrient inputs and biomass storage. However, a key disadvantage is a lack of income for the grower early in the rotation. Alleycropping offers the opportunity to grow annual crops for income while the trees become established. However, species selection is important to minimize competition for resources between the trees and the annual crops. While winter crops seem well-suited for such purposes, little attention seems to have been devoted to their use in alleycropping systems. In this study, an alleycropping system consisting of the hybrid aspen clone 'Crandon' and winter triticale is evaluated.

The study site is located approximately 15 miles southwest of Ames, IA (USA). During spring 2009, the hybrid aspen clone was planted into a strip-killed cover of triticale (72 trees per plot x 5 landscape positions x 3 replications = 1,080 total trees). Variable fertilizer rates were randomly assigned to a subset of 360 trees (6 trees per fertilizer rate x 4 fertilizer rates x 15 plots). The fertilizer rates were 0, 10, 20, and 40 grams (in the form of 10-gram tablets of slow-release 20-10-10 fertilizer placed in the planting hole). Tree heights were measured at mid-season and at the end of the growing season. Of the trees assigned to variable fertilizer rates, a subsample of 120 trees were harvested (2 trees per fertilizer rate x 4 fertilizer rates x 15 plots) for evaluation of height-to-diameter ratio, taper, and aboveground biomass production. In addition, the roots of 60 trees (1 tree per fertilizer rate x 4 fertilizer rates x 15 plots) were excavated for evaluation of root taper and belowground biomass. Analyses of carbon content are to be conducted on the aboveground and belowground portions of the trees as well.

Data analyses completed thus far indicate a significant effect of fertilizer on tree height growth early in the growing season, with a diminished effect by the end of the first year. In addition, tree height growth was generally consistent across landscape positions, with the exception of the floodplain where weed pressure was especially high. Winter triticale yields were less consistent across landscape positions and appear to be correlated with site quality. Analyses of height-to-diameter ratio, taper, aboveground biomass, belowground biomass, and carbon content are currently underway and will be described in the final version of the poster. Also, preliminary results from second-year tree height growth and winter triticale yields are expected. Finally, some pest management considerations will also be discussed.

Alleycropping, fertilising, biomass production

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Managing hybrid aspen regeneration: novel concepts and practical considerations

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Amid concerns about fossil fuel energy supplies and climate change, an increasing amount of attention has been devoted to deploying poplars as carbon-neutral feedstocks for renewable energy and/or carbon sequestration. However, much of this research has been focused on initial deployment, with comparatively less attention devoted to subsequent rotations. Because hybrid aspen are uniquely capable of re-establishing quickly from both stump sprouts and root sprouts, many uncertainties exist regarding how to best manage these stands beyond the first rotation. Ongoing research at Iowa State University seeks to address several of these uncertainties.

A key challenge is how to effectively capture the energy of these stands, considering that the high density of the stands can result in significant mortality over time from self-thinning. Coppice management offers the opportunity to capture energy which might otherwise be lost to mortality, but may also deplete energy stored in the roots and thereby shorten the lifespan of the system. In order to maintain a renewable supply of belowground energy, a mixed-rotation system consisting of alternating rows of coppice and unharvested sprouts is proposed. Under this system, coppice rows are harvested periodically as the unharvested rows mature, until such a time as the coppice production declines due to shading and/or depletion of belowground resources.

In support of this concept, an equation has been developed whereby the appropriate width for the unharvested rows may be calculated from the current density of the sprouts and the desired future density of the unharvested sprouts. The equation and its practical applications will be discussed. Practical considerations for conducting inventories and estimating the biomass contained in regenerating stands of hybrid aspen will also be discussed. A study of fixed-radius versus variable-radius inventory methods suggests that variable-radius sampling can be used to quickly and accurately inventory sprout stands. Also, a study of the height-to-diameter and taper relationships of stump sprouts and root sprouts suggests that these growth parameters may vary significantly by genotype and across environmental gradients. Estimates of standing biomass will be presented, and analyses of the carbon content of root sprouts and stump sprouts are also planned. The results of these studies and their potential implications for stand management will be discussed.

Keywords: coppice management, sprouts, allometric relations

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Sprouting of willow cuttings in lab conditions

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In many countries the Short Rotation Coppice (SRC) of willows is established on former abandoned land of rural areas. In these areas the seed bank in the soil could be extremely rich and the rhizomes of the perennial grasses forma viable biomass network below the surface. If the chemical weed control before the planting of willow cuttings has not been efficient enough, the wooded biomass yield from SRC can be significantly lower than expected. In order to compete with weeds for light and water successfully it is crucial for willow cuttings to produce the first fine roots and leaves as soon as possible after planting. Beside climatic factors (water availability, soil temperature etc) the speed of bud burst, fine root development and leaf unfolding may depend on the plant genotype and the parameters of the particular cutting (volume, number of buds etc). There are data available about bud burst timing of different willow genotypes in spring. However, less studies have been devoted to the root and shoot production speed of cuttings after planting. In current study we estimated the root and shoot growth of cuttings of two different willow genotypes - 79183 and Tora. We kept the cuttings naturally vertically in water with the upper 5 cm out of water at room temperature for two or four weeks, before careful detachment of all fine roots and burst buds. This method was chosen to simulate stabile sprouting environment without water shortage and to enable to collect all biomass produced by the cutting at the end of the experiment. For biomass measurements all parts of each cutting were dried at 85 ºC for 48 hours and weighted. The results revealed that there was no difference in the shoot+root sprouting speed between two studied clones. Also addition of soil originating from SRC did not increase the production of shoots and fine roots. However, mineral fertiliser in the water caused lower yield of roots and shoots compared with the control version. There was found a positive relationship between the shoot+root growth and cutting diameter ($p<0.001$), but the determination coefficient of the regression line was low ($R^2=0.19$ for cuttings after four weeks). Therefore additional parameters that have an influence on the sprouting speed of the willow cuttings in the lab conditions should be examined in the future.

Keywords: cutting, sprouting, root formation

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Wind damage and subsequent tree growth in clonal poplar plantations

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The effect of wind damage was quantified in terms of increment loss in two 9-year old monoclonal poplar plantations. Wind damage classes were assigned to trees based on their leaning angle. The plots were established before the storm, 100 trees per plot on 12 plots at each plantation. Patches of damaged trees occurred over both plantations with a great variability in recorded damages. There was a positive correlation between mean diameter at breast height (DBH) and the average wind damage class per plot. On each plot however, damaged trees had significantly lower DBH compared to undamaged trees suggesting a domino effect with smaller trees being pulled down by falling larger trees. Basal area increment three years after the storm was studied by means of a covariance analysis and it was found that increment has been strongly affected by increasing wind damage class. However, variation in basal area increment was strongly associated with the basal area recorded before the storm. On the other hand, the plot relative basal area increment was negatively correlated with average plot wind damage class suggesting that, in only three years, trees on strongly damaged plots could use additional growing space within relatively small gaps and compensate for the loss of increment in damaged trees.

Keywords: wind damage, basal area

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Innovations in nursery production for enhancing field performance and acceptability of poplar saplings

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In India, approximately 20 million poplar saplings are planted by an estimated number of over thirty thousand farmers annually. Response of so many growers on planting stock quality which determines field survival and growth is therefore critical and essential for both nursery and plantation production systems. Poplar is traditionally propagated using stem cuttings made from the last year nursery grown saplings commonly called Entire Trans Plants (ETP). The cuttings are planted during winters and ETPs are lifted for field planting next winters when they grow to 5-6 m average height depending upon clone type, growing conditions and cultural inputs. Fast growth of plants in the nursery results in large scale lateral bud sprouting in many clones and such saplings on field planting have been developing foliage only at the top of the plants which are reported to get breaking and stem bending on strong winds during the fresh year of field planting. Further, tall ETPs demand heavily on nursery space and costs on production, lifting, handling and field planting.

We have innovated nursery production by first growing them from mini stem cuttings in tubes anytime after monsoon season to winters and planting the tube plantlets in nursery beds late next year till the beginning of the monsoon season. These plants grow to 3-5 m height based on clone type, site conditions and inputs. The terminal bud set and leaf fall in such saplings get delayed by approximately two weeks when compared with the traditionally grown ETPs. Uniformity of the stock, live buds on the stem, terminal bud size, diameter-height ratio and physical appearance of such stock has shown improvement over the traditional one. The percent infestation of leaf eating Clostera species was also recorded very low on such plants. Field performance of these saplings from experiments and from farmer's fields is also encouraging. The demand for such saplings is significantly increasing in some locations where farmers have been planting them in their fields for a couple of years now. The presentation includes detailed experimental data and photos confirming the above observations.

Keywords: nursery production, field performance

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Use of contrasting branching habit and spring phenology to reduce core defect and pruning cost in Short Rotation hybrid poplars

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A significant investment of time and money is expended on repeated pruning of short rotation poplar plantations that are managed for high quality peeler- and sawlogs. This effort is in large part focused on reducing the number and size of branch defects in the sawlog. A trial was planted by GreenWood Resources on the Lower Columbia River Tree Farm to investigate the effect of polyclonal deployment on stem diameter growth, branching, and stem form of crop trees destined for sawlog production. The experiment was established using a polyclonal deployment of paired clones - one a crop clone, the other a trainer clone - planted at a relatively tight spacing of 0.9 × 3.7 m. The control treatment consisted of the crop clone planted at a wider, 3.7 × 3.7 m spacing without intercropping with trees of the trainer clone. The intercropped/polyclonal plots were thinned of the trainer trees at age five to an equivalent spacing of the crop trees in the control plots. Two separate pairings of trainer and crop clones were studied; each pairing represented a contrast of branch phenotypes and spring phenology in which the trainer clone exhibited greater branching vigor and earlier spring phenology. The hypothesis is that intercropping with the trainer clones has an inhibitory effect on the frequency of branching and/or the diameter of the branches of the crop trees through shading and competition during the five years before thinning occurs. Moreover, intercropping may lead to an extremely cost-effective method of producing energy feedstock: The production of the high-value saw logs will carry a disproportionate share of the growing costs of the trainer clone that is delivered as energy feedstock at minimal cost on the occasion of the mid-rotation thinning. Data collected at the end of the fifth growing season show that ($p \leq .05$) variation in diameter and number of branches in the first proleptic whorl as well as the number of sylleptic branches between the ground and the first proleptic whorl were significantly affected by the intercropping treatment. Diameter at breast height was recorded for the trainer clones and converted to biomass using yield equations developed for this taxon in this area. For the two separate pairings of trainer and crop trees, the first trainer clone averaged 94 dry metric tons/hectare yield, while the other averaged 67 dry metric tons/hectare.

Keywords, branching, phenology, pruning

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Aminoacids composition and fodder value of poplar leaves by minirotation cultivating

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The contents of amino acids in some clones of black and balsam poplars and their hybrids were investigated. The average size of amino acids summary contents in absolutely dry proteins of leaves from 1-year old shoots of black poplars hybrids was 11.46 %. The same one in balsam poplars was 7.83% and in intersectional hybrids of *eupopulus* – 9.41 %. The common contents of irreplaceable amino acids were accordingly 5.81 %, 3.94 % and 4.74 %. The total contents of lysine, methionine and tryptophane were at black poplars hybrids – 0.81 %, at balsam poplars – 0.67 % and at intersectional hybrids of *eupopulus* – 0.78 %. So it is established that green mass of poplars contains significant amount of amino acids which is comparable to the contents them in green mass of lucerne.

The fodder values of absolutely dry green leaves in 8 clones of poplars were varied under the contents of so-called fodder units from 0.22 to 0.28 kg/kg, on the digestible proteins from 23.6 to 30.7 g/kg and on the crude proteins from 3.6 to 5.6 %. The same parameters at lucerne were accordingly 0.24 kg/kg, 34 g/kg and 4.2 %.

The quantities of biochemical elements in absolutely dry masse of leaves varied by amount of crude fat from 4.0 to 5.4 %, on crude cellular issue from 14.5 to 17.6 %, on crude ashes from 8.6 to 11.3 % and on biological active substances from 49.2 to 57.3 %. The contents of these elements in lucerne were accordingly 2.4, 17.7, 9.1 and 50.5 %.

The contents of carotene varied in raw mass of leaves from 55.0 to 98.0 mg/kg and in absolutely dry mass from 176.8 to 324.0 mg/kg. The content of carotene in raw mass of lucerne was 46.0 mg/kg.

The contents of macro elements in poplar leaves also varied essentially. The contents of calcium changed from 4.52 to 7.91 g/kg in raw mass and from 16.5 to 28.1 g/kg in dry mass. The lucerne had accordingly 5.04 and 19.3 g/kg. The phosphorus varied from 0.66 to 1.02 g/kg in raw mass and from 1.90 to 3.81 g/kg in dry mass. The lucerne had accordingly 0.64 and 2.5 g/kg. The potassium varied from 0.12 to 0.30 g/kg in raw mass and from 0.44 to 0.90 g/kg in dry mass. The lucerne had accordingly 0.38 and 1.46 g/kg. The nitrogen varied from 0.68 to 0.90 g/kg in raw mass and from 1/9 to 3.0 g/kg in dry mass. The lucerne had accordingly 0.69 and 2.7 g/kg.

As a whole it is possible to see, that the fodder value of poplar leaves by minirotation cultivating also close to lucerne.

The comparison of these indexes in different clones of poplars allows choosing best from them under the contents of amino acids and fodder value.

Keywords: biochemical elements, carotene, fodder value, irreplaceable amino acids, leaves, clone of poplars, macro elements.

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BSBEC-BioMASS – Optimising biomass yield of SRC willow

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Climate change is one of the most pressing issues facing society today and one of the main contributors is our continued use of fossil fuels, therefore alternative forms of energy need to be developed. One method is to use perennial bioenergy crops to derive lignocellulosic fuel. BSBEC-BioMass is the Perennial Bioenergy Crops Programme of the BBSRC Sustainable Bioenergy Centre (BSBEC). The aim of BSBEC-BioMass is to underpin the development of a sustainable biofuels sector by optimising biomass from perennial biomass crops whilst maximising energy savings and reducing greenhouse gas emissions. Work stream 1 of BSBEC-BioMASS will focus on increasing biomass yield in willow through the identification and manipulation of key processes involved in dry matter production and partitioning. BSBEC-BioMASS will utilise existing crop resources alongside a new dedicated trial planted in 2009 which contains four genotypes of willow showing large variation in physiology and morphology. The trial has been designed to provide intensive destructive and non-destructive measurements of numerous plant traits. Work stream 1 will aim to optimise biomass by addressing 3 routes: First, extending canopy duration and improving cold tolerance by looking at the timing of budflush and start and duration of senescence. Second, maximising carbon fixation by altering crop morphology/architecture; for this different architecture strategies will be investigated by measuring traits including: the extinction coefficient (K_{ext}), leaf area index (LAI) and specific leaf area (SLA). Finally, selection of an optimal allocation ratio of above (harvested carbon) and below ground (reserve carbon) will be investigated by profiling the forms and locations of stored carbon over the growing season in photosynthetic and non- photosynthetic parts alongside a $^{14}CO_2$ labelling trial. Key traits associated with biomass yield will then be identified for detailed genetic analysis using existing mapping populations. This information will be used to select for improved varieties of willow via marker assisted selection. This work is also being carried out in parallel in *Miscanthus* grass.

Keywords: climate change, willow trials, biomass production

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Comparison of three models for estimation of poplars stem taper in poplars

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In Sweden, poplar has until now only been planted on small areas. The plantations have been established mainly in 1980-1990 when some of the farmland areas were laid down. Most of the planted areas have been arranged for exposing the capacity of the specie. Today there are about 300 hectares of poplar plantations in Sweden. The plantations are dominated by the clone OP 42 (*P. maximowiczii x P. trichocarpa*). Due to high and increasing demand for biofuel in Sweden, the interest for poplar as suitable short rotation specie has increased. However, equations for prediction of volume for individual poplar, taper equations and yield tables for poplar stands for Swedish conditions are missing. In a newly started project dealing with poplar as promising specie for future production of biomass, mainly for pulpwood and bio fuel, the present study is one important part. Construction of tables and equations is of great importance as base for presenting practical recommendations aiming an efficient forest management for high biomass production combined with an economical outcome.

The objective of the study was to develop a taper model easy to use, not segmented, yet applicable for all stem sections, predicting diameter at a given height or a height at a given diameter. Field data were collected from 33 stands where 68 trees in total were sampled. The stands were localized on three areas in central and southern Sweden (Lat. 55-60˚ N.).

Most of the stands have not been managed properly. As damages by wild games are intense in Sweden, fencing the plantation is an important way to decrease the damages. The stands have not been thinned in time, which cause strong competition and in some cases self thinning. The sampled trees were felled and diameters, tree heights and crown heights were measured. The total age and age of different segments of the stem was defined through annual ring counting. Stem number per hectare ranged between 155 and 3493, tree age between 14 and 73 years, tree height between 10 and 32 m and the stem diameter at breast height between 124 and 512 mm.

The output of a four degree polynomial taper equation (Hjelm 2010) was compared and analyzed with two published equations (Kozak et al 1969, Benbrahim & Gavaland 2003) for predicting stem form. The results show that Kozaks quadratic equation had less adaptation for predicting diameter in the lowest neiloid shaped stem section than the other equations. The polynomial equation in the study showed a significantly better adaptation along the main stem with parabolic form than the other equations. The correlation was high for all three equations with $R^2 = 0.99$ for Kozaks and Benbrahim & Gavalands equations respectively and $R^2 = 0.98$ for the polynomial equation. The root mean square error (Rmse) was 0.0718 for Kozaks equation and 0.0601 for Benbrahim & Gavalands equations and 0.0494 for the constructed polynomial equation.

Data from the present study will be used in a later study on stem volume and yield tables for poplar. Taper equations and form factors from the present study will be important inputs for the construction of the volume equations. The forest owner will then have a tool for estimating the future yield of poplar plantations on former farmland.

Keywords: stem taper, yield tables, thinnng

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Increasing interest in the production of willow for bioenergy in Québec, Canada

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Our research team has been conducting studies of willows in short rotation culture (SRIC) in Eastern Canada for more almost two decades.

Over the last five years, technological innovations have encouraged several Québec land owners and industrials to establish commercial activities that today represent hundreds of hectares of willow under SRIC. Partnerships between land owners and scientists have provided an operational base for performing original research activities. Such projects involve the recycling of waste water on willow plantations, the use of pork manure as fertilizer and the implementation of willow cultivation in the very brief growing season characteristic of Québec's northern regions. In some cases, very high yields have been measured, while simultaneously generating numerous environmental benefits for the region. These results suggest a promising future for the development of SRIC of willows in Québec.

Keywords: bioenergy, production system

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Sex related variations in the dimension of wood elements and specific gravity in *Populus deltoides* Bartr. ex Marsh cultivars

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The present study deals with intra-ramet and inter-clonal variations in the wood elements dimension and specific gravity in female (G48) and male (G3) clones of *Populus deltoides* at the harvesting age of 6 years in the plantation wood. The spacing of ramets in plantation is 5x5m. Variance ratio test (F) revealed that both G48 and G3 clones were significantly different for fiber length, fiber diameter, vessel element diameter and specific gravity. G48 clone showed higher wood elements' dimensions and lower specific gravity than of the G3 clone. The fiber length, fiber diameter, vessel element length and wall thickness is higher in G48 clone than G3 and vice versa for vessel element diameter and specific gravity for both the studied clones. It showed that female clone have better fiber dimensions while male have better specific gravity. Intra -ramet variation for height was significant for vessel element diameter and specific gravity. Wood elements dimensions were generally higher for basal disc while specific gravity increased with the height. Specific gravity and vessel element dimension were significantly varied from pith to periphery radial locations. Increasing trend from pith to periphery was observed for wood element dimensions and specific gravity. The fiber length ranged from 1030.09 -1073.74 µm (mean=1051.92 ± 157.41µm) for both the clones. The fiber diameter, wall thickness, vessel element length,vessel element diameter and specific gravity ranged from 25.61- 34.57 µm (mean=30.224 ± 5.21µm), 3.68 -3.87 µm (mean = 3.748 ± 1.32µm), 518.92 -546.52 µm (mean = 532.76 ± 98.30µm), 128.84- 120.00 µm (mean = 124.42 ± 26.47µm) and 0.38- 0.44 (mean = 0.41) respectively. It is concluded from the work that fiber dimensions and vesel element length were higher in female (G48) while specific gravity and vessel element diameter was in male (G3)clone.

Keywords: wood elements, specific gravity

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'Tailor-made' wood for biofuel production

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The shift from traditional fiber offtake from the forestry industry to offtakes such as woodchip for combustion to produce biopower or cellulose for conversion to liquid fuels requires a new perception of wood characteristics. A major challenge in this context is presented by the biofuel sector. Cellulose, a glucose polymer which is a major component of plant cell walls, is one of the most abundant polymers in nature and has thus attained increasing prominence with the evolution of the biofuel industry. Successful progress towards efficient saccharification and production of biofuels from lignocellulosic biomass relies on our ability to reverse and overcome the natural recalcitrance of plant cell walls. The conversion of biomass to biofuels requires disassembly of the cell wall and efficient removal of the lignin polymers. We have developed several transgenic concepts for dealing with cell wall recalcitrance. We have engineered foreign soluble polysaccharide polymers into the cell wall during its normal development. When processed, the polymers intercalated in the cell walls of these plants serve as "Trojan Horse" polymers and dissolve upon mild pretreatment, opening channels for cellulases and other catalysts during saccharification. The transgenic plants exhibit significantly enhanced cellulose hydrolysis upon mild pre-treatment compared to wild type plants. In addition we successfully introduced transgenic enzymes into model plants to disrupt the cellulose microcrystalline lattice. The activity of these enzymes results in the reduction of cellulose crystalinity. These transgenic plants exhibit a significant increase in saccharification compared to wild type plants.

Keywords: Bioenergy, Ligno-cellulosic biomass, Saccharification

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Foliar application of ethylene inhibitors stimulate rooting in black poplar rooted cuttings

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Cobalt ions (Co^{2+}) and 1-aminoisobutiric acid (AIB) are well known inhibitors of ethylene accumulation. The effects of foliar treatment by 100 µM solutions of these substances in the early phases of cuttings rooting on thirteen morphological traits of shoot and root system growth and development in seven genotypes of eastern cottonwood and two genotypes of euramerican poplar were examined. The effect of the treatment and its interaction with genotype was significant for the most of examined traits. Better results were gained in some difficult-to-root genotypes suggesting the usefulness of foliar treatment with examined substances, especially cobalt. The best reaction by root initiation and formation was observed on the basal part of cutting, but strongly influenced by genotype. These results suggest that the ethylene accumulation could be a problem in the early phases of cuttings' rooting, especially in the difficult-to-root genotypes. The obtained results could be implemented in the design of cultivar-adjusted nursery and plantation establishment technology.

Keywords: Rooting, Cutting, Ethylene inhibitors

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Session 5: Poplars and the environment: how much green is green?

POPFULL: Toward a full balance of all greenhouse gases, of energy and of the economic profitability of an operational SRC plantation of poplar and willow

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One of the strategies for mitigation of anthropogenic greenhouse gas emissions that is receiving a lot of attention in this post-Kyoto era, is the use of bio-energy as a replacement for fossil fuels. Among the different alternatives of bio-energy production the use of biomass crops – such as fast-growing woody crops under short rotation coppice (SRC) regimes – is probably the most suited, in particular in the EU. Two issues need to be addressed before the efficacy of bio-energy for carbon mitigation can be conclusively assessed, i.e. (i) a full life cycle analysis (LCA) of the global warming contribution of SRC, and (ii) and an assessment of the energy efficiency of the system in combination with a full economic balance. In this presentation the POPFULL project, incl. some first results will be presented. This project is one of the most ambitious in Europe in terms of the operational scale (19 ha) and the overarching research questions it is addressing.

The project involves both an experimental approach at a representative field site in Flanders, Belgium and a modelling part. For the experimental approach a 19 ha SRC plantation of poplar (*Populus*) and willow (*Salix*) is being monitored during the course of 2+2 years, harvested and transformed into bio-energy using two alternative techniques. Eddy covariance techniques are being used to monitor net fluxes of the most important greenhouse gases (CO2, CH4, N2O, H2O and O3) and to make a full LCA balance of the warming potential. These flux measurements are combined with common assessments of all biomass pools (incl. soil) and fluxes. For a full energy accounting and a full economic balance of the SRC plantation we use life cycle analysis and energy efficiency assessments over the entire life cycle of the SRC plantation until the production of electricity and heat. A significant process based modeling component integrates the collected knowledge on the greenhouse gas and energy balances toward predictions and simulations of the net reduction of fossil greenhouse gas emissions (avoided emissions) of SRC over two rotation cycles.

The POPFULL project is receiving funding from the European Research Council under the European Community's Seventh Framework Program as ERC Advanced Grant Agreement nr. 233366 and the Research Center of Excellence ECO.

Keywords: Greenhouse gases, LCA balance, Eddy covariance

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Linking wood bioenergy production in poplar and willow plantations with soil and wastewater phytoremediation in Italy

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Main results of research activity of CNR-IBAF-Italy on phytoremediation are here reported. Worldwide, rising interest on bioenergies (biogas, biofuels and wood), in alternative to fossil fuels, deserves a great attention on all the energetic uses of wood (fuelwood, pellets, cellulosic ethanol) for major environmental benefits. In Italy, a part of this wood could be provided by short rotation, coppice plantations (Srf) of poplar and willow trees. Using Srf for phytoremediation is a novel technique, combining high level of biomass yield (up to 25 t ha-1y-1 in dry matter according to clone selection) with high removal of contaminants. Srf plantations can be used for disposal of livestock wastewaters, with significant concentrations of Nitrogen (N) and heavy metals; with volumes of disposable wastewaters similar to those of food crops, but with minor contamination risks of the food chain. For N phytoremediation, a useful parameter for clone selection is N use efficiency (NUE, t of dry matter per Kg N in harvested biomass) that was found negatively correlated to clone yield. For Cadmium (Cd), the translocation factor (Tf, the percent of the ratio of Cd concentrations in aerial over the root plant materials) was used to select clones for phytoextraction and phytostabilisation.

Keywords: SRC; Heavy metal; Nitrogen; Livestock slurry

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Physiological response of poplars and willows grown on crude oil contaminated soils

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Phytoremediation of crude oil contaminated soils is one of the most challenging phytoremediation applications. Complexity of this process refers to various hydrocarbon compounds, plant-microorganism interaction and phytotoxicity of contaminants. High concentrations of crude oil affect viability of plants, while presence of microorganisms moderates environment for plants with the aim of mutual benefit. Although signs of phytotoxicity are most often easily visible, sometimes plant physiological processes can indicate stress in plants due to the presence of xenobiotics in cases without visible signs. This paper presents investigation of the potential of various poplar (*Populus sp.*) and willow (S*alix sp.*) clones for phytoremediation of soils contaminated with crude oil through assessment of physiological parameters. Three poplar clones: (I) *Populus deltoides* cl. PD2;(II) *Populus x euramericana* cl. EA8 and (III) *Populus nigra x maximowiczii x Populus nigra* „Italica“ cl. NM3 and three willow clones: (I) S*alix alba* cl. SA/H/1; (II) *Salix alba* cl. SA/I/1 and *Salix alba* cl. SA/K/1 were grown in pots with soils containing six different levels of crude oil contamination. Physiological parameters included were: (I) nitrate reductase activity, (II) net photosynthesis/dark respiration, (III) proline content and (IV) chlorophyll fluorescence. Proline content varied in poplars from 9.90 to 58.50 µg/g while in willows variation was smaller and proline content ranged from 4.52 to 23.19 µg/g. Crude oil contamination affected photosynthetic processes in willows where chlorophyll fluorescence (Fv/Fm) decreased from 0.744 to 0.588 in clone SA/K/1, while in poplars values were increased for clone PD from 0.652 to 0.756.Nitrate reductase acivity increased at lower contamination level in willows while on the other side enzymatic activity in poplars was decreased. In general, results showed effect of contamination on physiological parameters, although there was no uniform effect on all investigated species and clones.

Keywords: Poplars, Williws, Phytoremediation, Plant Physiology

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European aspen (Populus tremula L.) - Targeting the improvement of its water deficit tolerance

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European aspen is one of the most important pioneer tree species of ruderal and segetal succession ecosystems in Germany. Its clonal growth provides the opportunity for a single individual to establish many stems subsidized by a common root system of the mother organism. This allows an efficient utilization of locally disturbed soils on degraded or temporarily dry lands. In Germany, with the exception of fast growing hybrid aspens, aspens are not as important for short rotation coppicing systems on agricultural lands as balsam or black poplar (*Populus* spp. sectiones Tacahamca, Aigeiros). Species of the latter sections usually show a faster juvenile development and higher / earlier yield. Due to the aspens' better acceptance of unfavourable soil and / or water supply conditions we suggest this species, indigenous to Germany, for biomass cropping on degraded or temporarily dry sites, e.g. on set aside railroads, mining dumps etc. In this context, we investigate aspen with regard to its water deficit tolerance, water use and long term reaction to moderate water deficits. In a narrow sense, breeding for a higher water deficit tolerance means breeding for the reduction of yield losses under dryer conditions. This breeding goal is essential for the fulfilment of economic requirements concerning the establishment bio-energy poplar cropping systems in Germany that provide CO_2-neutrality and a low energy-input crop production. For instance, the intrinsic water use efficiency of poplar is usually lower than that of maize or other C4 crop plants. Therefore, the main objective of our previous and future research projects (www.tu-dresden.de/forst/isowood, www.fastwood.org) is the combined investigation of the physiological and genetic background of the aspens' water deficit reaction and water deficit tolerance (WDT) in comparison to other poplars. Physiology of water deficit reaction (WDT) Unfortunately, WDT is not a directly measurable trait. One of our research emphases is the evaluation of water deficit tolerance using wood physiological proxy traits of the tree ring archive. We combine traits with different relationships to the water deficit tolerance. The first type of traits we use simply reflects the trees' water deficit reaction, like for instance yield related traits. The other traits are, at least partially, related to specific water deficit adaptations of the trees. Among others, we use carbon / oxygen isotope ratios of the annual tree ring wood ($\delta^{13}C$, $\delta^{18}O$), cell length of fibre and vessel elements, vessel lumen cross sectional area and X-ray wood density as WDT proxy traits. Our results demonstrate plant and genotype specific differences in coping with a severe drought impact in 2003 and also differences in the sensitivity of the proxy traits to atmospheric drought in combination with good soil water supply. We suggest the combination of different traits to derive a drought tolerance index for ranking the genotypes for breeding purposes. Genomics: Targeting the improvement of water deficit tolerance The second emphasis of our research lies on the detection of quantitative DNA polymorphisms possibly valuable for marker assisted breeding of new cultivars. First we are going to search for appropriate genomic regions that are responsible for the genetic differentiation of WDT in dependence of age, i.e. that are significantly related with the investigated physiological traits and indexes during the juvenile stage of the life cycle. We investigate a crossbred F1-aspen family grown in two different plantations in a QTL-mapping approach that should help to delimit the genomic regions of interest. In addition, we select candidate genes with the help of database and literature search and we evaluate the genes' natural SNP diversity with respect to local and water availability adaptations. For that reason we establish a collection (≈300) of aspen from natural succession and of conventional poplar cultivars in the greenhouse and additionally in a field trial for intense physiological investigations. For SNP detection, we follow an EcoTILLING approach in comparison with

DNA sequencing to investigate approx. 60 amplicons. This work is aimed at the preparation of a population suitable for an association based mapping approach and well characterised in terms of physiological differences and population structure. Effects of cyclophysis and topophysis have to be regarded carefully. Present results (preliminary QTLs and maps) as well as experiences with the methods and the sampling design will be discussed.

Keywords: Water Deficit Tolerance, Genomics, Populus tremula, Wood Anatomy

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Whole-plant and xylem hydraulics in poplar: insights gained from Populus deltoides - Populus nigra hybrids

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We present results and insights gained from the characterization of the hydraulic architecture of eight field-grown *Populus deltoides* × *Populus nigra* genotypes, already known to differ in terms of growth performance, water-use and xylem anatomy (Marron *et al.* 2005, Monclus *et al.* 2005, 2006; Fichot et al. 2009). Under well-watered conditions, we provide evidence that (1) there are wide genotypic variations for hydraulic efficiency inferred from the leaf specific hydraulic conductance (k_{SL}) and for resistance to drought-induced cavitation inferred from the water potential inducing 50% loss in hydraulic conductance, (2) a strong trade-off between water transport efficiency and xylem safety occurs at the whole plant level, indicating that genotypes with lower k_{SL} apparently compensate the greater risks of embolism by building a safer xylem, (3) higher relative growth rate is tightly associated with lower whole-plant hydraulic efficiency and higher resistance to cavitation, (4) estimates of leaf water-use efficiency (intrinsic water use efficiency and carbon isotope discrimination against ^{13}C) are weakly associated with whole-plant hydraulics. Under limited water supply, the characterization of xylem resistance to cavitation provides additional evidence that (5) xylem safety acclimates to moderate drought in a genotype-dependent manner, (6) there is no clear relationship between xylem safety and either xylem water transport efficiency or xylem biomechanics, contrary to what is observed at inter-specific scales. Our results provide interesting insights for the possible role of whole plant hydraulic architecture and xylem hydraulics in mediating key aspects of whole-plant physiology in poplar, such as growth and water-use efficiency. Finally, our results also indicate that there may be great opportunity for intra-specific comparisons to unravel specific issues that cannot be fully addressed by inter-specific comparisons.

Keywords: Carbon Isotope Discrimination, Growth Performance, Hydraulic Architecture, P. x euramericana, Trade-offs, Water-deficit, Xylem Anatomy

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Flora-diversity in Swedish willow and poplar stands: Woody energy crops can improve biodiversity in agricultural landscape

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The use of biomass derived from fast-growing trees grown on agricultural land is common practice in parts of Sweden, where plantations of *Salix* sp. today cover about 14 000 ha in total. Floristic diversity in short-rotation willow *(Salix)* and poplar *(Populus)* plantations was compared with adjacent agricultural fields of various crop types. The plantations were grown on former agricultural land and most of the study sites were located in southern and central Sweden. Surveys of ground vegetation were carried out by using sample plots located along transects running perpendicular to the habitat boundaries. The surveys were carried out partly within the ERA-NET Bioenergy project Rating-SRC (http://ratingsrc.slu.se/). Mean cumulative species number across sites (all transects and plots included) was similar (between 20 and 30 species) in tree stands (*Salix* and *Populus*) and arable fields. Fields of conventional crops (e.g. cereals) had lower species number, whereas grasslands sometimes had higher species numbers compared to the tree stands. The number of joint species in the two vegetation types (tree stands and arable fields) was mostly low, which indicates different types of flora. Species number decreased clearly from edge towards central parts of tree plantations. The results were interpreted in a landscape context. Mainly based on the low quantity of species shared by tree stands and adjacent arable fields, the results support the hypothesis that small-scale plantations of fast-growing trees increase floristic diversity in landscapes dominated by agriculture. We conclude that woody energy crop plantations can contribute to increased biodiversity in agricultural landscapes, although species richness within the tree stands usually is low. Since the area of energy plantations in Europe is anticipated to greatly increase in the near future, we need to discuss how these plantations should be arranged and managed in order to favour increased biodiversity in the best way possible. A high number of edge habitats and small plantation size can, for example, support increased biodiversity.

Keywords: Biodiversity, Energy Crop, Flora Diversity, Land Use, Salix

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The potential of willow and poplar as carbon sinks

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A large share of the annual anthropogenic greenhouse gas emissions is attributed to global deforestation. Increasing forested areas therefore has a positive impact on carbon sequestration and mitigation of high atmospheric CO_2 concentrations. In Sweden recent reports suggests that about 300 000 to 500 000 hectares of arable land, and abandoned not yet afforested arable land, may be cultivated with energy crops and fast-growing tree species in the future. Fast-growing species such as willow and poplar are of high interest, mainly as producers of biomass for fuel, but also for sequestration of carbon. The present study calculates the rate of C sequestration in biomass and soil in willow and poplar plantations. The overall aim is to assess the potential for C sequestration in those plantations and, in this context, find out any knowledge gaps during the process. Carbon is sequestered in woody biomass, but also accumulated as organic matter in the soil by leaf litter fall and by root litter from fine root turnover. The woody biomass pool possesses a longer turnover time compared to leaves and fine roots, which are producing litter quite immediately after they are formed and contribute to the accumulation of C in soil organic matter (SOM) from the first year and onwards. In this study accumulation of C from leaf litter and fine root litter to (SOM) was estimated to 8.3 and 9.5 Mg C ha^{-1} over the first 20 - 22 years in willow and poplar plantations, respectively. The calculated average rate of C sequestration to SOM in a willow plantation was 0.38 Mg C ha^{-1} yr^{-1} and the corresponding rate for poplar was 0.48 Mg C ha^{-1} yr^{-1}. Those rates of sequestration were in range with findings from several other studies, both in forests and on afforested land. The rates of sequestration of C in above ground biomass were 3.2 and 3.8 Mg C ha^{-1} yr^{-1}, respectively, in willow and poplar plantations. If 400000 ha of abandoned arable land in Sweden were planted with willow and poplar, about 1.5 Tg C would be sequestered annually in woody biomass, including above and below ground production, and 0.2 Tg in soils, over a 20-year period according to these calculations. This shows the potential for willow and poplar plantations on arable land to mitigate the effect of high CO_2 concentrations over a short time span. Further research areas of interest are the effects of former land use, climate and management on C sequestration rates in fast growing plantations on arable land, as well as changes in soil C stocks following afforestation and the magnitude of the contribution of root litter to soil C sequestration.

Keywords: C Sequestration, Production, Biomass

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Do FSC guidelines work? Evaluating terrestrial insect communities in Pacific Northwest hybrid poplars and adjacent natural areas

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Terrestrial arthropod communities are an integral part of a properly functioning ecosystem that is often overlooked in short-rotation woody crops (SWRC). These communities can act as harbingers of ecosystems overall health.Hybrid poplars in the Pacific Northwest are a unique SRWC grown under drip irrigation on a 12-yr rotation within stringent Forest Stewardship Council (FSC) guidelines.The FSC emphasizes maintaining environmental quality and habitat when growing certified products.Because management decisions (crop, pest, etc,) are continually evaluated during the 12-year rotation, a premium should be placed on identifying the components of this agroecosystem, specifically the insect community.We conducted a four-year investigation of the hybrid poplar terrestrial arthropod community prior to and following harvest to examine the impact of this planned catastrophe on the local insect community composition.Identifying the community composition, including the presence of pests and beneficials, allows for the development of management strategies that minimizes the overall environmental impact through pesticide application and planting strategies.Pitfall traps were utilized to monitor insect populations in newly planted and mature poplar stands, as well as the surrounding riparian and sagebrush habitats.Abiotic differences between these communities were quantified by recording above and below ground temperatures at the midpoint of each transect.
Surveys show that the terrestrial insect community consists of a number of pestiferous and beneficial insects; with a small suite of species dominating all observed communities.This suite of species includes both an omnivorous ground beetle (*Calathus ruficolis* Dejean, Coleoptera:Carabidae) and an ant (*Tetramorium caespitum* L, Hymentoptera: Formicidae).Newly planted stands and natural areas had the highest insect diversity (1-yr H'=1.74; Poles H'=2.36; Sagebrush H'=2.30; Riparian H'=2.52) and the mature stand the least diverse (H'=0.56).A similar trend in regard to evenness was also noted.The surface temperature within each community was closely tied to its apparent physical structure. Throughout the study sagebrush was the warmest habitat and closely mirrored by 1-yr old plantings while the mature stands were the coolest.Our findings suggest that the current management of hybrid poplars in the Pacific Northwest under the FSC guidelines does provide suitable habitat for the local insect communities, although the suitability of these habitats may depend on age structure of the poplar stand.Further, this understanding of the insect communities of both the surrounding habitat and poplar stands has led to the development of a more complete integrated pest management strategy within this silvaculture.

Keywords: Insect, Community, Diversity, Habitat, Riparian, Sagebrush

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Session 5: Poster

Full greenhouse gas balance of a bio-energy plantation (POPFULL)

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The ecosystem scale research project we just started in Flanders(Belgium; POPFULL) will provide a full accounting of the greenhouse gas balance of a 19 ha short rotation coppice (SRC) plantation of fast-growing poplar and willow. The ultimate goal is to examine the potential of SRC cultures to reduce atmospheric CO_2 concentrations in Europe –through fossil fuel substitution – and to mitigate climate change. As atmospheric CO_2 concentrations will inevitably increase further from 370 ppm at present to values between 490 ppm (best case scenario) and 1260 ppm (worst case scenario) at the end of this century (IPCC, 2007), we test the potential of SRC plantations to sequester CO_2 from the atmosphere and also investigate the emission/uptake of the other most important greenhouse gases (H_2O, CH_4, N_2O) from the plantation and their environmental controls. We will measure the different greenhouse gases exchange of this high-density SRC culture will be monitored for four years using eddy covariance techniques. This would represent the first project in which all these greenhouse gases are investigated at the same time in a SRC coppice plantation. Moreover, as poplar is a sensitive species to O_3, a strong pollutant in many European areas, we also investigate the effect of ozone damage to CO_2 uptake and on the net exchange of H_2O, CH_4, and N_2O from the SRC plantation. Within the framework of the POPFULL project we are also quantifying the complete energy balance and the full economic balance in line with a full life cycle assessment. This research receives funding from the European Research Council under the European Community's Seventh Framework Program (FP7/2007-2013), ERC grant agreement nr. 233366 (POPFULL).

Keywords: Climate Change, Short Rotation Coppice, Greenhouse Gasses Exchange.

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GEI for some poplar clones in the early stage of growth in eastern Croatia

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Research into phenotypic stability, adaptability and productivity of poplar clones at plantation age 2 + 3 was conducted in the lower course of the River Drava and the tributary of the River Danube in the east of Croatia. In this area, the construction of river infrastructure has led to severe changes in the water regime. It is for this reason that the selected poplar clones should be adapted to the specific new condition of low groundwater levels. The parameters of dbh and survival were measured in eight poplar clones planted in three experimental plots. Measurements included the clones *P.* ×*canadensis* (’Pannonia’, ’Bl Constanzo’, ’I-214’, ’M 1’), and the *P. deltoides* clones (’710’, ’S 6-36’, ’S 6-20’, ’S 1-8’). According to the obtained results, the locality 25b in the Forest Office of Valpovo had the poorest site quality, followed by the locality 7k, while the locality 5g in the Forest Office of Osijek was found to be the best. The interaction of a genotype and the environment (GEI) in which a particular genotype (clone) may exist results in different phenotypes. This in fact represents the extent of reaction of a particular genotype to the intensity of external factors. The extent of reaction of a particular clone is genetically determined and so is phenotypic stability of this genotype. A phenotypically stable genotype is a clone in which the phenotypic value of a particular trait is very similar in different environments. Its usable value does not depend only on its maximal productivity, but also on its ability to retain certain traits (e.g. increment) at a relatively high level and in different environments. The results of this research confirm that even at such a young age the quality of a particular habitat has conditioned modifications in average clone values of dbh and survival. They also indicate the amount of production to be expected from the mixture of these clones or from the cultivation of a particular clone. As a rule, the most suitable clones that cause optimal modifications, from a silvicultural aspect, are those with high phenotypic instability, whose cultivation is justifiable in optimal habitats, but also in less suitable habitats provided that agrotechnical measures and adequate plant protection are applied. The obtained results and the value of the regression coefficients and regression analysis in particular show that at this plantation age the tested clones may be divided into two groups in terms of phenotypic stability, adaptability and productivity: a) Phenotypically very unstable clones of high productivity with specific adaptability to optimal sites/environment (’S 1-8’, ’710’ and ’Bl Constanzo’); b) Phenotypically very stable clones with medium or low productivity with the tendency to adapt to all sites/environment (’Pannonia’, ’I-214’, ’M 1’, ’S 6-36’, ’S 6-20’).

Keywords: GEI, Poplar Clones

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Variability of antioxidant activity of Populus x euramericana (cl. M1) cultivated at two different forms of fluvisol

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In this study, drought induced oxidative stress and antioxidant activity were examined and compared inleaves of *Populus x euramericana*clone M_1. which has grown at two granulometrically different forms of fluvisol (sandy and loamy). Biochemical *in vitro* tests such as FRAP (ferric reducing ability of plasma/extract) and DPPH test were applied for tracking of total antioxidant activity and free-radical capacity, respectively. Also, some plant metabolites such as free proline as a drought indicator and malonil-dialdehide (MDA) as a main product of lipid peroxidation, were quantified. These two parameters have been expressed per mg of soluble proteins whose content has been determined as well. The examination has been conducted during June, July and August 2009. According to results gained by DPPH method, poplars cultivated at sandy fluvisol have shown higher scavenger capacity then poplars cultivated atloamy fluvisol with its maximum in June (RSC=90.171 ± 0.906 %). Results gained by FRAP test follow the same trend as DPPH method and both antioxidant activitiesmhavemdramaticallymdescendedmaftermJune. The highest rate of lipid peroxidation (31.027 ± 0.449nmol MDA per mg of proteins) was found in June while the largest amount of free proline was found in July (121.782 ± 0.651nmol /mg proteins) both in poplars grown at sandy fluvisol. All the results regarding higher oxidative and drought stress, thus higher antioxidant activity in poplars cultivated at sandy fluvisol, may be attributed to lower humidity content insandy comparing to loamy fluvisol and other stressful environmental conditions especially emphasized during the summer.

Keywords: *Populus x Euramericana*, Antioxidant Activity, Fluvisol

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Clonal response to defict irrigation in hybrid poplar plantation for biomass production

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Under the current scenario of climate change, with the likelihood of prolonged drought periods and rising temperatures, the sustainability of hybrid poplar plantations aimed at biomass energy production must be studied. Irrigation is required for the viable cultivation of poplar in much of the Mediterranean area since low water availability is a limiting factor to production. Given the relative scarcity of this resource, it is important to use it wisely. This can be achieved, on the one hand, by employing irrigation methods appropriate to the context of the crop, and on the other, by selecting the genetic material which proves to be most efficient as regards water use. To study the performance of the different clones in terms of production and water use efficiency under different irrigation scenarios, a trial was established in the fertile lowlands of the river Henares (Madrid) in a high density plantation (20.000 trees/ha). Four clones belonging to different hybrids were included in the trial ('I-214', 'AF2', 'Pegaso' and 'Monviso'). These were watered automatically using 8 different watering regimes: 100%, 130%, 90%, 80%, 70%, 65%, 50% and 30% of the field capacity, controlled using tensiometers and humidity sensors. Another trial was set up in parallel under controlled greenhouse conditions where the clones were grown for four months in individual pots and were watered automatically to field capacity (100) and to 80%, 60% and 40% of field capacity. Growth and production were evaluated by recording height and diameter growth, number of leaves, number of branches and total biomass (in the greenhouse trial) and also gas exchange measurements were recorded. Significant differences were observed in growth and production of the different clones in both trials, those displaying the greatest growth under optimal watering conditions being those which showed the most pronounced decrease in production when irrigation was restricted, although their growth was still significantly higher. Between the wilting point (situated at 65%) and 50% of the theoretically optimal amount (f.c), the production falls by half for all the clones. Very restrictive watering (30%) reduces the production by up to 80% although no signs of mortality or decline were found in any of the clones. There are also differences in terms of water use efficiency (Wi), inferred from gas exchange measurements, in adult plants, significantly increasing efficiency under water stress conditions leading significantly to stomatal closure (cc -0.85). At clonal level differences are observed in Wi at field capacity and when a medium stress was applied but not under more restrictive watering conditions. Strategies as regards differences in efficiency seem to differ at clonal level, being more related to the photosynthesis rate /A) in some cases and to stomatal conductance in others.When there were differences in W i this was significantly higher in the more productive clones, showing a significant positive correlation between them. The results for four month old plants in controlled conditions as regards gas exchanges measurement are, however, not so clear and therefore must be repeated over time. By determining the clonal performance in terms of production and water use efficiency under water-limited conditions, the most appropriate materials can be selected for plantations aimed at biomass energy production in semi-arid zones. Furthermore, this information provides an additional selection criterion for improvement programs centred on attaining greater sustainability of plantations.

Keywords: Hybrid Poplar, Biomass, Watering, Water Use Efficiency.

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Influence of drought conditions on morphological and physiological attributes of Populus deltoides clones

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Populus deltoides is one the most important forest species planted in Argentina. The high growth rates of clonal plantations allow to provide a big quantity of raw material for sawmills, pulp and particle board industries. For a successful biomass production poplar crops need adequate water supply. Although plantations located in the Paraná River Delta have adequate precipitation, they are exposed to soil and atmospheric water deficit periods during the growing season, which restrict photosynthesis and growth limiting forest productivity. Unfortunately, in the actual scenario of climate change new challenges are expected to come out and compromise plants performance. There are consistent differences in the productivity of cultivated clones under adequate water supply. However less information is available about their responses under low water availability. Poplars relay in various drought tolerance mechanisms to cope with drought, which vary according to genotype. Considering the necessity to select drought tolerant material, the purpose of our study was to evaluate the capacity to tolerate water stress conditions of four clones of *P. deltoides*, mainly focusing on mechanisms that can allow the maintenance of plant growth.

The experiment was established under controlled conditions in September 2009. Twenty cuttings per clone were planted in 15 l plastic pots filled with organic soil, and irrigated daily to allow sprouting. At the beginning of December young plants were submitted to two irrigation regimes (well watered -WW- and water stressed -WS-) during four months. To avoid the access of water form precipitation plants were covered when necessary with a removable polyetilene roof. Morphological measurements included: height and diameter of the main stem, leaf number and leaf abscission and total leaf area, while physiological attributes: water potential, stomatal conductance and tissue water relations.

Water restriction significantly reduced water potential, stomatal conductance and modified tissue water relations. Osmotic potential decreased with drought, but a low osmotic adjustment capacity occurred only in clone '20-82'. In addition plants under stress showed an increase in tissue elasticity, which could also help them to maintained turgor. Significant differences were observed among clones. 'Stoneville 67' which showed low osmotic potential, had generally low water potential and high stomatal conductance. At the same time leaf abscission occurred in most of the clones except in '129/60', which maintained a significantly higher leaf number, and hence, higher total leaf area. Morphological attributes were significantly changed in WS plants. But the effect of drought was genotype dependent. Clones '129/60' and 'Stoneville 67' had greater ability to maintain growth under conditions of limited water availability. The maintenance of high growth rates and reduced leaf senescence (20%) under drought conditions was the strategy observed in '129/60'. In contrast 'Stoneville 67', with similar growth rate showed 60% of leaf senescence, but exhibit lower osmotic potential, lower water potential and higher stomatal conductance. Our results indicate that both morphological and physiological mechanisms contribute to increase the drought tolerance but the relevance of them varies according to genotype.

Keywords: Poplar, Clones, Drought Tolerance

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Productivity and structural characteristics of some black poplar and white willow stands in the Danube inundation

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Soils in the inundations of the great rivers are natural habitats of poplars and willows, and are mostly intended for wood production. In such habitats poplars can achieve significant growth elements of trees (diameter at breast height, height), and stands (basal area, volume), which significantly exceed the potential of other tree species. White willow stands achieve lower productivity compared to poplar stands, and are establishedinmoister habitats, where poplars can achieve modest returns. However, the habitats in which poplars can achieve significant productivity, some willow clones can also achieve significant elements of tree and stand growth. The results of such experiment are presented in this paper.

The stand trial was established with two black poplar (*Populus* × *euramericana* (Dode) Guinier) clones (Konstanca, I-45/51), and three white willow (*Salix alba* L.) clones (351, 285, 182) on fluvisol soil type, at a distance between planting of 4 × 4 m.The random distribution with four replicates of 42 trees in each replication was used. Twenty nine years after, the breast diameters and height of each tree were measured, after which the trees were cut, and some of them were measured in order to establish a volume curve for each studied clone.

Upon that time some 246 to 428 trees·ha^{-1} were determined in the stands of studied poplar and willow clones, with the variation coefficient between replications ranging from 20 to 55%, which revealed the lack of significant differences between clones. Poplars clones had significantly higher top (33,3-36,0 m), and mean heights (32,6-34,5 m) compared to willow clones, revealing the high production potential of studied habitat. Willow clone 351 had significantly higher top (27,5 m), and mean heights (26,9 m) compared to other two poplar clones with heightsranging from 21,7 to 22,2 m.

Poplar clone I-45/51 had significantly greater dominant (54.1 cm) and mean breast height diameters (42.6 cm) compared to poplar clone Konstanca. Willow clone 351 had dominant (44.9 cm) and mean breast height diameters (36.9 cm) which were not significantly different compared to poplar clone Konstanca, but were significantly higher than breast height diameters of other two willow clones having dominant diameters ranging from 35,1 to 36,1 cm and mean diameters from 29,8 to 30,4 cm.

Comparison of height distribution cumulative curves using non-parametric Kolmogorov-Smirnov test confirmed significant differences between all studied clones, while comparison diameter distribution cumulative curves confirmed non existence of significant differences only between poplar clone Klonstanca and willow clone 351, and between willow clones 285 and 182. Models of height and diameter structure according to Weibull distribution, obtained using percentile method, revealed good agreement with empirical distribution, which was confirmed in all studied clones using Kolmogorov-Smirnov test.

Poplar clones I-45/51 and Konstanca, and willow clone 351 had basal areas ranging from 35.70-44.92 m^2·ha^{-1},which was significantly higher compared to willow clone 285 and 182 with their basal areas ranging from 17.03-17.82 m^2·ha^{-1}.Stand volume per hectare ranged from 550-675 m^3·ha^{-1} in poplar clones, and 678 m^3·ha^{-1} in willow clon 351, which differed significantly from the volumes achieved by other two willow clones ranging from 165 to 190 m^3·ha^{-1} .

Presented results showed high production potential of willow clone 351 in alluvial habitats, in which poplar clones I-45/51 and Konstanca had also achieved the high production potential.

The obtained results revealed that white willow is a significant resource from the aspect of production of significant quantity of technical wood, possibilities of storing carbon, and "improving" the gene pool of cultivated clones with the aim of reducing the monoclonal stands on alluvial terrains.

Keywords: Poplar and Willow Clones, Volume Productivity, Stand Structure

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Association study between eco-physiological and genetic UV-B response among different *Populus alba* L. clones

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Over the last ten years poplar has become the model plant, amongst forest trees, to be investigated for functional genomic studies. In Mediterranean regions, *Populus alba* has drawn much interest being a fast growing species with a good potential for biomass production. Nevertheless, it is crucial to achieve a better understanding of the molecular genetic basis controlling the response to environmental stimuli and stresses in forest trees. This has also important consequences in the context of global change. In this work we report a study about the different responses of five poplar clones - collected from different latitudinal and altitudinal areas - to elevated levels of UV-B, jointly analyzing molecular and eco-physiological parameters. Three years old plants were acclimated in two identical phytotrons, except the presence of a UV-B lamp array just in one of them. Plants were treated for 16 hours with a UVBbe (UVB biologically effective radiation) supplementary dose of 6 KJ/m2/day and allow to recover during the night (8hours). The polymorphism of four genes (chalcone synthase (*chs*), caffeic acid *O*-methyltransferase (*comt*), RuBisCO (*rbcL*) and photolyase), putatively related to UV-B response has been investigated, as well as their expression levels. qRT-PCR was used to analyze gene expression of plants treated for 3,6,12 and 36 hours with elevated levels of UVB, after RNA extraction from the clone's leaf tissue. *Chs*, *comt* and *photolyase* gene expression levels were different among the five poplar clones. The *rbcl* qPCR analyses showed no significant difference in the expression pattern among the clones. The sequence polymorphism analyses showed an absence of missense mutations. The maximum PSII quantum efficiency, Fv/Fm, was measured after 12 h of dark recover before the UVB were switched on, at the beginning of the experiment and after 24, 48 and 72 hours of UVB by PAM-2000 Chlorophyll Fluorometer (Walz, Germany). During the treatment, Fv/Fm in the stressed clones decreasedsimilarly and progressively already after 24 h, reaching values of about 0.65 after 72 h of UVB treatment, indicating only a slightly permanent photo-inhibition. The last day of the experiment, chlorophyll fluorescence was measured in plants acclimated to saturating light, during1 h dark recovery time and after 16 h of darkness, allowing to differentiate between fast and slow relaxing NPQ components. Fast relaxing NPQ clearly differed between cv. ´Val Bormida´ (the highest latitude clone) and cv. ´Policoro´ (the southernmost clone) on one side and the other *P. alba* clones, indicating that clones from the highest natural UV-B irradiance environment, showed a different (stronger) ability to recover the functionality after the ultraviolet radiation treatment by non photochemical quenching. Interestingly, the same two clones showed a different expression pattern for two genes involved in the production of screening pigments (flavonoids) and in the modification (thickening) of cell wall (lignin), suggesting that the adaptation to high UV-B irradiances is related to an active acclimation process rather than a constitutive resistance.

Keywords: UVB Stress, Poplar, Ecophysiology, Gene Expression

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Treatment capacity of slurry composting and biofiltration liquid fertilizer and short-rotation biomass production of fast-growing tree Species - Second-year Production

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Slurry composting and biofiltration (SCB) liquid fertilizer is made from swine wastewater and has importance in respect to waste recycling. To know both the possibility of SCB liquid fertilizer replacing with chemical fertilizer and the effect of it on short-rotation biomass production, we treated SCB, general liquid fertilizer, chemical fertilizer, ground water and no treatment as a control on an experimental site where fast-growing tree species such as poplars, willows and yellow poplar (*Liriodendron tulipifera* L.) were planted. The same amount of total nitrogen was applied in each treatment. An experimental site having density of 10,000 trees/ha was established in spring 2008 with a block design composed of 15 clones of poplar, 2 clones of willow and yellow poplar in which five individuals were used in each clone and species within each of 3 replications. The trees were one-year-old rooted cuttings and were cut at the height of 10 cm above ground after planting to induce coppices. We measured total amount of liquid fertilizer and aboveground biomass every November for 2 years.
Survival rates of all clones were favorable showing 94 and 92% in 2008 and 2009, respectively. Actual amount of treated liquid fertilizer was 29.8 ton in first year and 32.4 ton (810 ton/ha/year) in the following year. Annual biomass production was 1.7 ODT/ha/year in 2008 and 7.7 ODT/ha/year in 2009 showing drastic increase. Biomass production is expected to boost due to the development of root stumps for next one or two years. Biomass production by treatment in the second year was the best in chemical fertilizer (10.6 ODT) and followed by general liquid fertilizer (10.5 ODT), SCB liquid fertilizer (7.5 ODT), ground water (5.8 ODT), and no treatment (4.2 ODT). In respect to species, hybrid poplar (*Populus alba* × *P. glandulosa*) showed the most biomass production (17.2 ODT/ha/year) and white willow (*Salix alba* L.) was the least (1.8 ODT/ha/year). The best clone was Hyunsasi No.4 (*Populus alba* × *P. glandulosa*) producing 20.1 ODT/ha/year biomass and the worst was 131-25 clone of white willow showing 0.6 ODT/ha/year. For short-rotation biomass production by fast-growing tree species, selection of suitable clones is necessary. Moreover, SCB fertilizer can be used to increase the production substituting for chemical fertilizer. For annual biomass production, hybrid poplar, especially Hyunsasi No.4 will be the best choice.

Keywords: Slurry Composting and Biofiltration (SCB), Liquid Fertilizer, Swine Wastewater, Biomass Production, fast-growing Tree Species

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Effects of water availability on Populus nigra L. genotypes in nursery

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Although European black poplar (*Populus nigra* L.) can be found nearly all over Europe, it is one of the rarest and most endangered trees. For this reason, it was chosen as a case study in order to evaluate the effects of climate change on the vegetation health. In Casale Monferrato (Northern Italy) a nursery was established using different genotypes of *Populus nigra* coming from different latitudes of its distribution area (north, centre, south) and hybrid clones as controls, in order to carry out biometrical, physiological and spectral measurements. Two different water regimes were applied to simulate water deficit ("dry test") and water surplus ("wet test") for comparison with environmental conditions ("ambient test"). The study aimed to determine whether different water availability might cause physiological modifications in poplar and if these could be ecotype-related. Soil moisture, meteorological parameters and stem growth were recorded in continuous. The biometric parameters were measured at the beginning and at the end of each growing season. Leaf chlorophyll content, fluorescence, predawn water potential and spectral measurements were performed at the same time on the same leaves. Finally the susceptibility to some pathogens was evaluated among the genotypes. The analysis of predawn water potential showed stress only when soil moisture was lower than 10%, while the florescence measures gave no indication of stress for the different tests. Between the genotypes of the same geographical area wide variability related to water stress was found: it depends probably on adaptations that single trees developed in their site of origin thanks to intra and inter specific competition for the resources.

Keywords: Water Stress, Ecology, Climate Change

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Bioenergy production and environmental effects of Short Rotation Coppice (SRC) cultivation

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Short Rotation Coppice (SRC) with willow (*Salix sp.*) was introduced in Sweden after the oil crisis in the 1970s as one of the means to replace fossil fuels by renewable energy sources. Today, about 14 000 hectares of SRC with willow are commercially grown on Swedish arable land using mainly different clones and hybrids of *Salix sp.* SRC cultivation is fully mechanised from soil preparation, planting and management to harvesting and combustion. SRC willow fields in Sweden are harvested every three to five years during the non-growing season and the above-ground biomass is chipped on-site and directly transported to and burned in combined heat and power plants. The average production of commercial SRC willow plantations in Sweden nowadays is ca 5-6 tonnes dry matter per hectare and year depending strongly on site conditions, although much higher biomass production from well-managed sites can be achieved. After harvest, the plants coppice vigorously, and replanting is therefore not necessary. The estimated economic lifespan of an SRC willow stand is 20 to 25 years. The recent sharp increases of cereal prices have resulted in a slight decrease of the agricultural land cultivated with SRC. Also the higher fertiliser prices have resulted in fewer SRC fields fertilised and therefore in less biomass produced by SRC. However, an increased supply of willow chips for energy purposes is still predicted and favoured by Swedish stakeholders and decision-makers. Since the current trends for a surface increase of SRC are not favourable, different drivers need to be addressed for a potential increase of interest by farmers for SRC. For this reason, an evaluation of the potential impact of SRC cultivation on water, soil and biodiversity in comparison to other agricultural crops, and to what extend such effects can contribute to an increase of biomass produced for energy, is of highest importance. SRC is a perennial crop that differs from arable crops in a number of traits: SRC plantations remain productive for a number of years (20-25), harvest occurs in most cases every 3-5 years in winter, the plants are deeper rooted and are much taller at harvest than other arable crops. Additionally, SRC requires no annual soil cultivation, considerably less agrochemical inputs when once established, and much less nitrogen fertilizer, therefore it is a crop of low intensity concerning management compared to the other arable crops. As a result of all the above, SRC has a much lower carbon footprint compared with food or biofuel production from annual arable food crops. Concerning impact on water issues, although evapotranspiration rates for willow and poplar SRC seem to be a bit higher than for arable crops, effects on the catchment level (large-scale) have not been identified or anticipated. This has to be combined with the proved better groundwater quality achieved when SRC is cultivated instead of arable crops. Similar comparisons concerning phytodiversity indicate an increase if SRC is planted in areas dominated by agriculture or coniferous forest. Animal diversity in SRC in terms of invertebrates and birds is considerably higher in SRC in comparison to arable crops, but it seems to be highly site-specific, therefore the decision where to establish SRC is of high importance to achieve environmental goals. The various effects of SRC on soil issues illustrate the multiple function that SRC might have when planted in a certain area. Soil organic carbon seems to be increased when SRC is cultivated instead of other crops, and the amount of heavy metals, especially of Cd, is decreased due to elevated uptake in willow shoots. Therefore, SRC plantations seem to be able to play the role of a carbon sink and simultaneously to clean soil from hazardous compounds. In all, SRC cultivation seems to offer advantages to reduce negative effects on the environment due to other land uses, and careful planning for SRC establishment will be proved beneficial to take maximum advantage of the positive SRC effects on the environment. However, an increased SRC cultivation area can be only achieved by involving farmers to replace "conventional" arable crops with SRC, e.g. by offering incentives to farmers to be compensated for the environmental services served by growing SRC instead of other crops.

Keywords: Bioenergy, Biomass, Energy Crops, Energy From Agriculture, Environmental Decision Support Tools, Environmental Impact, Poplar, Willow

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Impact of Short Rotation Coppice (SRC) cultivation on water quality

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The surface of agricultural land cultivated with short rotation coppice (SRC) using willow and poplar for production of biomass for heat and/or electricity is expected to increase in the short-term in a range of European countries. This will have implications for various environmental issues. As a perennial crop, SRC differs from arable crops in physical traits and management practices. Concerning groundwater quality, results so far imply low nutrient leaching when SRC is cultivated compared to other "conventional" crops, but effects in the long-term concerning SRC plantations cultivated for several years applied with recycled by-products as sewage sludge and wastewater should be also considered for a general evaluation of the SRC impact on groundwater. Extensive sampling of groundwater from several SRC fields in Sweden cultivated for several years has been conducted, and results of the obtained results concerning N and P leaching are discussed in this paper. These results are compared to respective N and P in the groundwater of adjacent to SRC "conventional" crops. With the help of these results, the overall environmental impact of SRC on water quality will be evaluated.

Keywords: Bioenergy, Biomass, Energy Crops, Energy From Agriculture, Environmental Impact, Poplar, Willow

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Identification of the main environment factors and cultural practices affecting biomass productions in Short Rotation Coppices (SRC) plantations

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Biomass from short rotation crops (SRC) is one of the possible alternatives to fossil fuels in Europe. During the last 7 years more than 6000 hectares of energy plantations (Short-Rotation-Coppices -SRC)have been established in Italy, withpoplars (*Populus* spp.) and willows (*Salix* spp.) clones. The biomass productivity of these plantations appeared much variable, ranging from 4-6 to 20-25 oven dry t•ha^{-1}• year^{-1} (Odt•ha^{-1}• y^{-1}) in the first rotation cycle also with the same genetic material. This wide variability suggests that the importance of factors different from the planting material genetic characteristics has been underestimated: site characteristics, as local climate and soil, influence tree growth; besides cultural practices (irrigation, fertilization, weed control) may be crucial for attaining high yields. Purpose of this paper is to report the results of observations carried out in SRC experimental plots in Italy, in order to get information, through stepwise regression analysis and principal component analysis, on theenvironmentaland cultivation factors affecting plants survival and stand productivity in the first two years of growing. Data collected were subjected to statistical analysis; independent variables were: annual and vegetative season rainfall, annual mean air temperature, soil texture, N and organic matter content, and cultivation practices (fertilization, irrigation, weed control). Dependent variables were: a) mean annual yield (Odt ha^{-1} y^{-1}); b) plant survival at the end of each year (%). One of the main output of this researches is to provide information on productivity in time, will serve as a base for giving precious technical and economic indications on this new woody crop. Water (as rainfall andirrigation) resulted the main variable affecting plant survival and biomass production in poplar and willow clones. Water resources will be important as climate change further complicates issues (change in seasonal distribution and amount of precipitation, increased evapotranspiration and reduction in soil moisture). Efforts such as breeding for temperature/drought tolerance or alternative management strategies (drip irrigation, phytoremediation) will be required to allow SRC to adapt to the challenges caused by global warming.
Based on the first results the development of predictive models useful to estimate biomass production in SRC plantationson the basis of easily measurable site features appears feasible.

Keywords: Poplar, Willow, Short Rotation Coppice, Environmental Factors

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PRO-BIOPA: technical and economical potential and harvest possibilities for poplar and willow short rotation coppice in south-west Germany

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The project "PROBIOPA" (Sustainable production of biomass with short rotation plantations of poplar on marginal sites) aims at optimizing the biomass-production of poplar in short rotation coppice (SRC) on sites with limited nutrient and water-supply and the comprehensive, integrative economical and ecological assessment of SRC. The poster will give an overview about the work package in which both institutes are involved. The primary objectives of this work package are the assessment of the technical and economical area potential for SRC in Germany, a life-cycle analysis for the management of SRCs and the comparison of different supply chains to show the impacts of different utilization and transport systems. To this end, an experimental short rotation plantation was established and its annual growth and the cultivation of further SRC are being monitored. The methodology used in this study includes the accomplishment of time studies on harvest processes as well as a literature research to identify system relevant processes. With the help of GIS computation, using digital site maps, climate data and a Digital Terrain Model (DTM), the biomass potential will be estimated, considering restrictions by protected areas. For the life-cycle-analyses a professional software is used which allows accounting all impacts between the change of land use to the end of life of the products, with the main focus on different harvesting operations. One of the main results expected will be a detailed estimation and classification of which sites should be preferentially used for SRC cultivation and information about their technical, economical and ecological biomass potential. A model for a sustainable, site adapted supply chain as well as innovative and efficient harvesting and transport concepts will be developed and their impacts will be identified.

Keywords: Short Rotation Plantations, Short Rotation Coppice, Bioenergy, Biomass Potential, Life-cycle Analysis, Supply Chain

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A simplified CO_2 balance in two case studies of river restoration

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The Kyoto Protocol promotes afforestation and reforestation in order to foster sustainable development and recognizes to forests an important role as carbon sinks. River restoration projects aiming at restoring riparian habitats can be considered as part of reforestation activities. The CRA-PLFtogether with the Po River Fluvial Park and other river parks carried out several pilot trials (on about 150 hectares) to convert areas dedicated to conventional crops or intensive poplar cultivation into floodplain forests and to recover degraded areas on the upper part of the Po River basin. The main aim of these projects was to restore floodplain forests for recreational purposes and to actively contribute to the conservation of native poplars genetic resources, black poplar in particular, by creating a network of artificial *in-situ* gene conservation units to support a dynamic evolutionary process in a short time. For each trial plantings and maintenance practices were recorded, under the supervision of CRA-PLF. The present paper compares two cases set up in 1996: one along an old meander to recover a gravel quarry and to create a conservation buffer strip in an agricultural landscape; the other to create an open parkland for recreational activities and touristic exploitation and to improve the environmental value of a densely populated area subject to flooding. The two plantations were characterized respectively by a major reduction of cultivation practices and, in the second case, by the application ofintensive cultivation techniques. A simplified CO_2 balance was calculated comparing the emissions generated during the cultural practices and the C fixed in the biomass yielded to evaluate the sustainability of plantations.

Keywords: CO_2 Balance

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Evaluation of adaptive response of Populus nigra L. genotypes during successive drought cycles

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The changes in the distribution and intensity of rainfall due to global warming influence the ability of plants to adapt to the changing environment. The main objective of this study was to observe the influence of water stress on three *Populus nigra* genotypes coming from different latitudes (north, centre, and south) of the natural distribution area of this species. For this purpose, cuttings of these genotypes were planted in one cubic meter boxes and managed with two different irrigation regimes: well-watered (wet) and water deficit (dry). Dry plants were subjected to two cycles of irrigation cessation, during early spring and mid summer 2008 respectively. The stress intensity was estimated by measuring soil water content, predawn leaf water potential and relative water content. At the end of each cycle ($Tmax_1$ and $Tmax_2$), the osmotic potential at full turgor and the cell wall elasticity were estimated by freezing-point osmometer and pressure-volume curves respectively. During the first cycle, dry plants were subjected to a mild stress (Ypd < - 0,4 MPa and RWC > 95%), showing no significant differences among genotypes. At $Tmax_2$, genotypes showed different drought responses: in northern and central ones Ypd dropped to - 0,9 and -1,7 MPa respectively, with a decrease of the leaf RWC, while in southern ones it did not change significantly (- 0,6 MPa). After resumption of the irrigation, the osmotic potential at full turgor showed significant changes only in the southern genotypes (0,6 MPa), while no changes were recorded in the modulus of elasticity (e). The decrease of Yp_{full} of southern genotypes in response to drought may beassociated with the accumulation of soluble compounds, improving turgor maintenance under low soil water content.

Keywords: Water Stress, Ecology, Climate Change

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Clonal differentiation in root cadmium accumulation and distribution in Salicaceae exposed to cadmium by energy dispersive X-ray microanalysis (SEM-EDXMA).

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Knowledge of the distribution of Cd in roots is necessary in order to understand the mechanisms of accumulation and translocation in *Salicaceae*. Four clones of poplars and 2 of willows, characterized by Cd tolerance, accumulation and translocation ability in a companion study, were grown in nutrient medium (hydroponic culture) containing 50 µM of Cd. The study aimed at mapping this element, through energy dispersive X-ray microanalysis (SEM-EDXMA), to assess whether the concentration and the short-term exposure to Cd had any influence on its distribution in the roots. The study was to establish the link between the distribution of heavy metal and chemical elements at different root levels, from apex to distal zone, and into tissue sections, as central cylinder, cortex and epidermis. The element mapping showed higher Cd contents in poplar than willow. Cd was found differently distributed across root levels and sections. Overall, observations suggested differential patterns between species and clones in accumulating Cd within the root profile. The possibility that Cd accumulation and translocation patterns arise from differences in uptake processes and structural properties is discussed and related to tolerance mechanisms. A different Cd localization was measured in each clone, both at cross-section and root level. The localization of Cd in the section would point at the mobilization of metal across the root. However, considering A4A and 58-861, Cd was not found in internal tissues, which could be related to the lack of anatomical barrier, such as the Casparian strip. This could give control to mineral uptake, while modulating tolerance strategy in phytoremediation systems.

Keywords: Phytoremediation, Cadmium, Roots

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Influences of soil physical properties on growth of poplar stand in north of Iran

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In present study, the influences of some soil physical properties on the growth of *Populus deltoides* (clone 79.51) were investigated in Guilan province Guilan/Iran, where two low and high quality plantations were observed. In each poplar stands, fifteen sample plots were chosen and diameter at breast height (DBH) and height for all trees within them were determined. Soil samples were taken from all sample plots of both stands. Soil texture, saturated moisture content, bulk and particle density (B.D and P.D) as well as porosity were examined. For comparing two plantations t-test analysis was used. The result showed that among soil physical properties, percentage of clay, sand, B.D and saturated moisture content were significantly different between two stands.
Results indicated that heavy textured soils with high B.D are undesirable for growing *Populus deltoides* (clone 79.51) in Guilan plain and therefore planting of it, is not recommended on these soils.

Keywords: Soil Physical Properties, *Populus deltoides*, Poplar Stands, Plantation

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The effect of poplar plantations on soil chemical properties in North of Iran

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Iranian commercial forests of 1.8 million hectares are insufficient to support the fibre industries, therefore to provide the wood demand, poplar planting have developed in plain areas of north of Iran. In this study the comparison of the productivities of two low and high quality poplar plantations grown on adjacent areas, and the influences of these stands on chemical soil properties were investigated in Guilan province/ north of Iran. In each poplar stands, fifteen sample plots were chosen and diameter at breast height (DBH) and height for all trees within them were determined. Soil samples were taken from all sample plots of both stands. Chemical soil properties of C, N, C/N, P and K were examined. T-test was used for analysis with help of SPSS software. The results show that all of the soil chemical properties differed significantly between two stands. Results indicate that poor quality stand has negative influence on soil nutrient and reduce its fertility and the other hand reduction in availability nutrient elements have had negative effects on growth and quality of stand.

Keywords: Poplar Plantation, Chemical Soil Properties, Low And High Quality

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Production characteristics of *Populus* x *euramericana* I-214 clone on loamy form of fluvisol in a flood and protected part of the Danube alluvial plain in the Central Danube Basin

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Black poplar (section *Aigeiros*) has a *relatively* long history of growing in Europe. In Serbia, as in the most European countries poplar tree is grown for the purpose of mechanical processing. Successful poplar growing depends on three factors presented in a high quality habitat of a tested (selected) variety and intensive processing.

Protected alluvial plain of plain rivers presents a special problem. Regulation of rivers flow significantly influences the hydrological soil characteristics.

After the construction of embankments this part of alluvial plain is additionally moisturized only by the underground waters, and poplar productivity in protected part of alluvial plain also depends on the annual flow of underground waters level.

From the above mentioned reasons the characteristics of habitat conditions on loamy form of fluvisol, and productivity of *Populus x euramericana* cl. I-214 in *flood-protected part* of the Danube *alluvial plain* in the Central Danube Basin were analyzed in this paper.

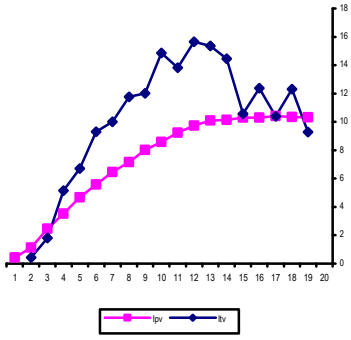
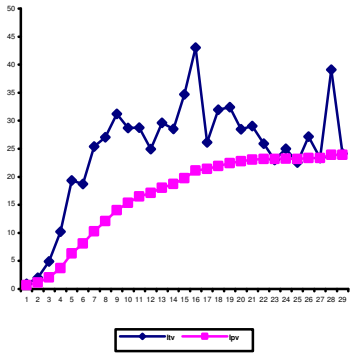
Morphological structure of studied undeveloped soil (fluvisol) profile was similar to the depth of 75-80 cm, and thus the granulometric composition and textural class were also similar.

The profile in protected part of alluvial plain below this depth was of „a lighter “mechanical composition, as the consequence of smaller content of silt+clay fraction.

Differences in humus content and reaction of soil solution were observed; the humus content was higher in the profile of the protected part of alluvial plain. This trend was probably due to the absence of flooding and possibility of organic matter deposition.

Dynamic of average and current volume growth calculated on the basis of average tree stand in flooding area is given in graph.1. Maximum current growth of avearge tree stand was recorded in a 19 year old stand.

Graph. 1 - Average and current volume growth of a stand (m^3ha^{-1})

Protected part	Flooded part
	

On the basis of previous investigations it was determined that dependence of volume stand in relation to silt+clay fraction had the shape of a parabola, and that the greatest stand volume was achieved with the content of silt+clay fraction ranging from 30 to 45%. In both cases the content of silt+clay remained in these limits, and maximum stand production in these cases was also obtained when the content of silt+clay fraction ranged from 30 do 45%. Maximum current stand volume growth, and the volume growth were higher in flooding area. Analysis of edaphic conditions confirmed that there was an unsignificant difference between trials, so the difference between maximum current growth and culmination time could be explained mainly by the absence of additional stand flooding in the protected part of Danube alluvial plain.

Studies were done in two field trials in the Central Danube Basin in the *flood* and *protected part* of the Danube *alluvial plain* in the Central Danube Basin.
Morphological profile construction of studied undeveloped soil was similar to the depth ranging from 75-80 cm, and thus the granulometric composition and textral class were also similar.
Analysis of edaphic conditions confirmed that there was insignificant difference between trial areas, so the difference between maximum current growth and culmination time could be explained mainly by the absence of additional stand flooding in the protected part of Danube alluvial plain.

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Underground water dynamics in hidromorphic soils of protected part of alluvial plain of Danube in southern Bačka

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The work presents basic types of hidromorphic soils in protected area of middle part of Danube alluvial plain in Republic of Serbia. Four soil types were examined within the hidromorphic order of soils: fluvisol, humofluvisol, humogley i eugley. The most important characteristics of these soils were presented in this work. The hydrological characteristics were particularly described, especially dynamics of underground water during the growing period in two years. Considering the influence of the water level of the river Danube on the underground water level dynamics, the distance of examined sites from the river Danube and altitude were examined. The distance from the river Danube varied from 1916,00 – 4161,00 m, while altitude varied from 73,00-75,50 a.s.l.m. The average underground water level related to the soil surface varied in fluvisol from 258-301cm, in humofluvisol from 234-260cm, in humogley from 142-167 cm and eugley from 38-51cm above soil surface. The amplitude of the variation of underground water level varied from 94-120 cm in fluvisol, 138-256 cm in humofluvisol, 105-134 cm in humogley and 43-55 cm in eugley. There was high correlation between underground water level and water level of the river Danube.

Keywords: Underground Water, Hidromorphic Soils, Aluvial Plain, Danube

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Wood production determinants in poplar: where are we going?

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Because world resources of fossil fuels are rapidly decreasing, it is necessary and urgent to find alternative sources of energy. By 2020, the European Union aims at reaching 20% of the share of total energy produced by renewable energy. One way to achieve this is to use crops specifically dedicated to the production of woody biomass. Currently, woody biomass comes mainly from logging residues or from the waste wood industry. However, cropping systems specifically dedicated to the production of woody biomass as short rotation coppice are presently gaining in importance.

In a context of large-scale production of biomass produced from fast growing woody species, it is important to optimize the production by selecting species and genotypes adapted to local conditions. Plant material can be selected either directly for biomass production or for potential determinants of wood production such as phenology, ramification, leaf growth and composition, or water- and nutrient-use efficiencies (WUE and NUE). Then, wood production can be maximized by playing either on the choice of the plant material (species and genotypes within species) for a given pedoclimatic context and/or on the adopted cultural system (notably through planting densities / rotation length). The prerequisite is to evaluate the strength of the relationships between wood production and its potential determinants, and their reliability (plasticity) for the different species, cultural systems, and pedoclimatic contexts.

Studies have already been conducted to analyze the effect of the interaction between genotype and environment. The present study will provide new information about this interaction as it takes into account factors that were not studied concurrently in previous studies. Our experiment proposes to study the influence of genetic and environmental factors (and their interaction, GxE) on phenological, architectural, functional (WUE and NUE), and foliar determinants of wood production. The genetic factor is considered at inter-specific level (comparison of three fast-growing species, poplar, willow and black locust) and at intra-specific level (clonal tests for each species). The environmental factor consists of different pedoclimatic contexts in France and different cultural systems (more or less intensive).

Two experimental sites at the West and East sides of France are used in this experiment. These sites are located (1) in French Brittany (47°60'N, 1°91'W) with an oceanic climate and (2) in Burgundy (47°18'N, 5°19'E) with a continental climate. Determinants of productivity such as NUE, WUE, bud and leaf phenology, ramification, growth in height and girth, leaf increment, and foliar structure and function will be analyzed throughout the growing season with repeated measurements and observations (from March to October 2010) at the two sites, for the different clones of the three species, and for different planting densities. This monitoring is done under the framework of a French project called SYLVABIOM (2008 to 2012). The SYLVABIOM project is supported by the Bioenergy thematic of the French research national agency (ANR).

Keywords: Short Rotation Coppice (SRC), Water-use Efficiency (WUE), Nutrient-use Efficiency (NUE)

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Two dimensional liquid chromatography technique coupled with mass spectrometry analysis to compare the proteomic response to cadmium stress in poplar

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Plants are useful in studies of metal toxicity, because their physiological responses to different metals are correlated with the metal exposure dose and chemical state. Moreover a network of proteins and biochemical cascades that may lead to a controlled homeostasis of metals has been identified in many plant species. This paper focuses on the global protein variations that occur in a *Populus nigra* spp. clone (Poli) that has an exceptional tolerance to the presence of cadmium. Protein separation was based on a two dimensional liquid chromatography technique. A subset of 20 out of 126 peaks were identified as being regulated differently under cadmium stress, and were fingerprinted by MALDI-TOF. Proteins that were more abundant in the treated samples were located in the chloroplast and in the mitochondrion, suggesting the importance of these organelles in the response and adaptation to metal stress.

Keywords: Protein Biomarkers, Cadmium, Phytoremediation

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Soil carbon assessment as part of a total carbon balance of a bio-energy culture with fast-growing poplar (POPFULL)

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The POPFULL research project aims at (i) providing a full accounting of the greenhouse gas balance, (ii) the quantification of energy efficiency and (iii) an analysis of the economic balance of a short-rotation coppice (SRC) culture with poplar and willow trees. Dit is noodzakelijk om de efficiëntie van bio-energie voor koolstofmitigatie in relatie tot de milieu-impact met zekerheid te kunnen vaststellen.The ultimate goal is to examine the potential of SRC cultures to reduce atmospheric CO_2-concentrations in Europe, through fossil fuel substitution, and to mitigate climate change. Within the scope of the project, this study focuses on the determination of productivity and making up the carbon balance of a large, multiclonal poplar plantation.

In April 2010, a short-rotation coppice plantation of 19 ha was established in Lochristi near Ghent, Belgium (51° 07' N; 03° 51' E) on a former agricultural site consisting of pasture and maize fields. Twelve poplar (*Populus*) and three willow (*Salix*) clones, belonging to different species and interspecific hybrids of various origins, were planted in replicated monoclonal blocks. The general idea is to make up the full carbon (C) balance of the SRC culture by quantifying all carbon pools and fluxes on a yearly basis. C-pools include all above- and belowground plant biomass (leaves, shoots, stump and roots) and soil carbon. Measured C-fluxes are photosynthesis, and both autotrophic and heterotrophic respiration of the ecosystem components. Measurements at leaf or plant level will be restricted to four representative clones and all data will be scaled up in place and time to determine the productivity and net carbon exchange of the total ecosystem on a yearly basis. The obtained production fluxes will be validated using flux measurement data of the entire ecosystem obtained by eddy-covariance techniques from a meteorological measurement tower in the plantation.

Assessment of initial soil carbon has been made just before establishment of the plantation, i.e. in March 2010. Soil samples were collected within the framework of a complete soil survey including former pasture and maize field soils. Both loose and bulk density soil samples were taken at 0-15, 15-30, 30-45, 45-60, 60-75, 75-90 cm below the soil surface, at 100 sample locations spatially distributed over the site. Carbon and nitrogen (N) contents were determined by dry combustion for all samples, which allowed to perform a study of the spatial variability of C and N contents and hence compare the two initial types of land use. Furthermore, differences in nutrient contents per 15 cm depth increment were examined and in combination with bulk density data, the total initial soil carbon pool was calculated. After the first rotation period of 2+2 years, an analogous soil survey will take place, allowing to determine the changes in carbon content of the soil.

Keywords: Soil Carbon, Populus, SRC

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Visual preferences for poplar plantations on roadside landscapes in Turkey

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From past to present in Anatolia (Turkey), poplars as plant materials are commonly used in both rural and urban landscaping. They are possible found in different areas of use that such as easy producing, fast growth, easily adapt to all kinds of ecological conditions, the availability of almost any nursery with some of the features. Especially in the rural landscape, the new residential areas around, limiting agricultural areas, planting of traditional crops, commercial usage and are used for roadside vegetation. In this study, the visual structure and composition of poplar plantations which play an important role in shaping the way the rural roadside landscape were investigated. For this purpose, the compositions of the poplar samples in the selected routes were photographed. In order to determine visual preferences for poplar's roadside compositions, semantic differential technique (SDT) was applied. As a result of the research, it has concluded that poplar tree plantations as a single specie or their compositions in which they were both with other plant types showed significant visual diversity. Some suggestions about using poplars for rural roadside landscape planning have been made.

Keywords: Poplar Plantations, Plant Composition, Semantic Differential Technique.

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Clonal variation in growth and productivity of poplar and willow in a bio-energy plantation (POPFULL)

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The POPFULL research project aims at (i) providing a full accounting of the greenhouse gas balance, (ii) the quantification of energy efficiency and (iii) an analysis of the economic balance of a short-rotation coppice (SRC) culture with poplar and willow trees. Dit is noodzakelijk om de efficiëntie van bio-energie voor koolstofmitigatie in relatie tot de milieu-impact met zekerheid te kunnen vaststellen.The ultimate goal is to examine the potential of SRC cultures to reduce atmospheric CO_2-concentrations in Europe, through fossil fuel substitution, and to mitigate climate change. Within the context of POPFULL, clonal variation in production-determining characteristics is studied, in relation to biomass yield for energy production.

A 19 ha high-density SRC plantation with fast-growing poplar and willow clones was established on a farmland in Flanders (Belgium) at the beginning of April 2010. Twelve poplar (*Populus*) and three willow (*Salix*) clones were used, representing different species and interspecific hybrids of *P. nigra*, *P. trichocarpa*, *P. deltoides*, and *P. maximowiczii* for poplar and *S. alba*, *S. dasyclados*, *S. schwerinii* and *S. viminalis* for willow. After soil preparation by ploughing and tilling, 25 cm long dormant and unrooted cuttings were planted, that had been soaked in water for 24 h. They were planted following the Swedish double-row system with alternating inter-row distances of 0.75 m and 1.5 m and a distance of 1 m in the row. This corresponds to a planting density of 8900 cuttings per hectare. The planting was performed with a converted agricultural leek planting machine. The plantation is designed in large replicated clonal blocks, which cover different types of previous land use. The spatial pattern of clones, that has been mapped, is used for a correct interpretation of the continuously measured greenhouse gas fluxes above the plantation. Assessment of mortality and initial growth has been executed, indicating rooting and establishment capacity of different clones in the given environmental conditions.

Both structural and ecophysiological growth characteristics are examined for a period of 2 + 2 years. Phenological patterns, leaf area index (LAI) and crown architecture, which represent the total photosynthetic area, will be measured in detail including seasonal and annual variation. An ecophysiological characterization of photosynthesis will be performed at the leaf level. Structural measurements provide height and diameter data, used to estimate biomass yield through allometric relations. Significant rust infection and/or pests will be scored to evaluate clonal variation in tolerance level. After the first harvest, coppice ability of the different clones will be quantified by monitoring mortality, number of shoots per stump and self-thinning.

Keywords: Production Physiology, SRC, Clonal Variation

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Carbon dioxide assimilation and productivity of poplars

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Poplars productivity, like other trees and plants, is determined by an integration of factors affecting photochemistry, biochemistry, physical diffusion of carbon dioxide for chloroplasts, leaf cells and single leaves, along with activities of non-photosynthetic tissues. During our studies significant differences in the productivity of the selected 29 poplar clones was observed to be correlated with many morphometric traits, therefore they were analysed for, leaf size, angle of insertion, CO_2 uptake and other related characters.

The studies reveal that the normal angle of leaf insertion varied between 42-52^o to receive the normal incidence of illumination, and the size of the leaves varied with the productivity level of the clones.Since different tissues of the plant contribute to varying degrees to their organic nutrient supply, major among them being leaves which determine its growth and yield potential. The study showed that clones having larger leaves also had other promising morphometric and productivity traits probably due to the larger amount of$CO_2$and PAR fixed by enzymes and chlorophylls with corresponding higher rate of photosynthesis.

Photosynthetic rates were closely associated with PAR and stomatal conductivity. Photosynthetic rates were found to be maximum in larger leaves where CO_2(IN) was also higher showing the significant positive relation between the photosynthetic rate, leaf area and CO_2(IN).

Differences in the photosynthesis appeared to be caused by the differences in the CO_2fixation by the photosynthetic process rather than by differences in the amount of CO_2evolved in respiration. The clone 63-N had the higher values for the morphometric traits, leaf area, productivity as well as the photosynthetic traits compared to the poor performance shown by clone T1-6 (91). These clones also showed the CO_2output at 145.37 ppm and 256 ppm, respectively. The widest gap with highest value of CO_2(IN) and lowest value of CO_2(OUT) was observed in best performing clones and the lowest gap in the input and output value of $CO_2$was in the poor performing clones, where the utilized CO_2value was minimum.

Keywords: Poplars, Photosysnthesis, Productivity

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Extension of cultivation of *Populus deltoides* to outside the traditional planting zone in India

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Populus deltoides (poplar) is among the most popular and prominent tree species in agroforestry scenario of India. It is commonly planted in fertile and irrigated lands in plains of Punjab, Haryana, western Uttar Pradesh and adjoining areas of Uttarakhand, Himachal Pradesh and Jammu & Kashmir states. Planting of this species in conjunction with agricultural crops gives huge dividends to the farmers in this region. It is generally believed that this species is not suitable for planting in areas south of 28°N. Scattered studies in India have indicated that it might be possible to grow it at lower latitudes too if adequate management practices are enforced. Indian Council of Forestry and Education, Dehradun has therefore embarked upon an initiative to extend the area under its cultivation and plant it on a large scale in areas of Bihar state located at below 28°N. The plan is being implemented with help of Bihar government under a project funded by the Planning Commission, Government of India.

In the first phase, 6.10 million plants of poplar are scheduled to be planted by March 2011 in one district, Vaishali, located at 25°N. Lessons from this exercise will be used to replicate similar planting effort in suitable sites in other parts of this state. Planting stock for this project is being raised in farmers' nurseries. Plantations are being raised exclusively in association with local agricultural crops. Wherever farmers have carried out cultural operations as per recommendations, outplanting success and initial growth have been comparable to good quality plantations existing in the traditional planting zone of poplar.

Results from first phase augur well for the second phase of the project. Besides providing economic returns to growers, raw material to industries and employment to people, it will give environmental benefits and add to the carbon sink. It will be possible to judge the success of this unique model within few years, as poplar is harvested in India at rotation of 6 to 8 years. If successful, it would serve as the basis to extend this model to other species as well.

Keywords: Agroforestry, Adaptation, Cultivation

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Seasonal acclimation of leaf morphology and water use efficiency (WUE) show different adaptation strategies in two opposing genotypes of *Populus nigra* L.

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Species like black poplar characterised by a continuous growth, can acclimate to the changing environmental conditions during the seasons through a modification of morphological and physiological features. Of particular importance is the acclimation to the increasing evapo-transpirative demand and water availability. In this perspective, each generation of leaf becomes a sort of valve regulating the whole tree water use efficiency and determining the short-term plasticity of a certain genotype to different environmental conditions.
The main objective of this work was to asses the seasonal changes on morphological and physiological features of leaves of two contrasting genotypes of *P. nigra*, growing in a common environment. The *58-861* genotype (North Italy) reacts to the increasing dry conditions decreasing leaf conductance and increasing the intrinsic water use efficiency at leaf level, without any other significant changes. On the other hand, the *Poli* genotype (South Italy) showed constant values for all the observed parameters, demonstrating a higher adaptation to a relative drier environment.
The leaf dimensions as well stomatal density, were the main morphological driver of the different responses.
This work demonstrates that the seasonal (short term) plasticity of morphological and physiological features could play a significant role for the success of a continuous growth genotype (provenance?), with implication for breeding strategies.

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Keywords: Poplar, WUE, Plasticity

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Differences in the ability of Salix clones to cope with water and light restriction

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Willow plantations in the Parana River Delta (Argentina) are one of the largest areas in the world cultivated with this short-rotation species. They are established in the lowland areas of the islands and are managed mainly for pulpwood and particleboard industries. Plants are exposed frequently to multiple stress factors during the establishment. In fact, the native vegetation exerts intense competition, restricting the availability of above and belowground resources compromising willow performance. Under co-occuring stressful conditions plants can change their physiology and/or morphology allowing resource acquisition and improving their competitiveness and productivity. The way in which plants respond to environment can be used to predict the result of new forest management treatments.

The aim of this study was to assess the effects of water and light restriction on physiological and morphological responses of young willow plants. The experiment, established under controlled conditions, was a factorial design of two clones: *Salixmatsudana* x *Salix alba* 'A 13/44' and *Salix alba* x *Salix babylonica* 'A 131/25', with three levels of irradiance: full sunlight, moderate shade and severe shade (100%, 25% and 10% of light availability respectively), and two water regimes: with and without water limitation. On September 2006, cuttings were planted in plastic pots filled with organic soil, maintained under full sunlight conditions and irrigated daily. At the beginning of December the resource restriction was initiated. The interactive effects of shade and drought were evaluated measuring physiological and morphological attributes tightly linked to stress acclimation. To compare the magnitude of the plastic responses we calculate a plasticity index for each characteristic.

Resource restriction caused significant changes in most of plant characteristics. Both shade and drought reduced water status and gas exchange, but the effect of drought was alleviated under shade. Plants growing under full sunlight and moderate shade showed osmotic and elastic adjustment in response to drought. Shade increased leaf area and shoot/root ratio, while drought reduced both characteristics. Plants can generally handle with one environmental stress but their capacity to tolerate more than one distinct stress seems rare due to trade-off and constraints. Our results showed that the physiological and morphological adjustments observed in both clones increased their tolerance to co-occurring resource stresses, and enabled them for a more efficient resource acquisition. These responses are not consistent with the trade-off hypothesis, which postulates a conflict between drought and shade tolerance. However while stressful conditions did no affect plants survival it decreased growth. The analysis of plasticity indexes indicated that both morphological and physiological changes to cope with severe shade were more pronounced than those which contributed to alleviate water restriction. Clones exhibited different responses. For the morphological traits studied here, 'A 13/44' showed higher phenotypic plasticity to both drought and shade than â€˜A 131/25'. But for physiological traits 'A 131/25' showed higher phenotypic plasticity to drought. We suggest that these clones would show a similar capacity of acclimation when growing under the multiple stressful conditions generated by herbaceous vegetation, and similar mechanisms could be triggered, but the responses would be linked to genotype.

Keywords: *Salix*, Drought, Shade

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Carbon sequestration potential of poplar energy crops in the midwest, USA

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Energy use and climate change mitigation are closely linked via ecological, social, and economic factors, including carbon management. Energy supply is a key 21st century National security issue for the United States; identifying and developing woody feedstocks for transportation fuels and combined heat and power operations are a crucial component of the future National energy strategy of this country. When strategically placed in the landscape,purpose-grown trees such as short rotation woody crops (e.g., poplars) exhibit high productivity levels and ecosystem services such as soil and water conservation, nutrient recycling, and carbon sequestration. Given the demand for carbon estimation methods and tools, we are using a combination of expert knowledge, literature review and database enhancements, and field testing to assess the carbon sequestration potential of poplar feedstock production systems in the Midwest, USA.

We are acquiring knowledge from existing published manuscripts pertaining to poplar productivity potential, genotype × environment interactions, and other pertinent silvicultural aspects that are important for deploying short rotation woody crops (see Coyle et al. – poplar database), along with standard forest carbon modeling methods, to develop baseline carbon estimates for standing poplar biomass. For the field testing, 4 trees of 10 clones (C916000, C916400, C918001, Eugenei, NM2, NM6, NC13563, NC13624, NC13649, NC14018) belonging to 4 genomic groups (*P. deltoides*; *P. deltoides* × *P. nigra*; *P. nigra* × *P. suavelons*; (*P. trichocarpa* × *P. deltoides*) × *P. deltoides*) were selected during September 2009 based on productivity from previous trials and the need for adequate genetic variation to characterize the variability in carbon sequestration potential. All clones were present in 10-year-old plantations established at Escanaba, Michigan, USA and Ames, Iowa, USA. Trees of most clones will also be tested from a 22-year-old clonal orchard in Rhinelander, WI, USA, with potential future testing at Arlington, Wisconsin, USA and Waseca, Minnesota, USA. The Escanaba trees were harvested in October 2009, while all others will be collected during summer 2010. At all sites, diameter at breast height will be recorded before harvesting. Then, trees will be felled, total height measured, and cross-sections (i.e., cookies) will be collected at breast height and at one- and two-thirds the height of the tree (i.e., approximately mid-height and just below the live crown). The cookies will be sanded and digitally scanned for growth ring analysis. In addition, specific gravity will be determined and wood from each ring will be sampled and analyzed for total carbon content. Using volume estimates based on data collected above and stand productivity estimates from process-based modeling (e.g., 3PG), yearly carbon sequestration potential will be estimated within a rotation, as well as rotation-age stand-level carbon sequestration rates. A carbon sequestration yield table will be developed for appropriate clones and sites, with the long-term objective of developing a regional table. Preliminary results from Escanaba, Ames, and Rhinelander will be presented in Orvieto.

Keywords: ecosystem services, *Populus*, sustainability

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Production of Short Rotation Woody biomass with and without irrigation

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At Whitecourt, Alberta, a short rotation woody crop (SRWC) plantation is being grown with and without irrigation using treated municipal sewage wastewater. This project is part of a nation-wide Canadian Biomass Innovation Network study, led by Natural Resources Canada, which is investigating growing of SRWC's as a bioenergy feedstock. The Whitecourt site was chosen because of its accessibility for demonstration purposes, its proximity to a wastewater treatment facility, and the fact that a potential end user of the wood fibre produced (a waste-wood fired power plant) is located in the community. Five willow clones and two poplar clones are being monitored for their performance with and without irrigation. Growth, survival, biomass yield, insect and disease issues, heavy metal uptake by the willow, accumulation of heavy metals in the soil and ground water are being monitored. The use of wastewater for irrigation offers the opportunity to increase yields of willow biomass by augmenting low rainfall in western Canada, to reduce environmental impacts of waste water disposal and to decrease the need for manufactured fertilizers. This has the potential to reduce operating costs and improve the net carbon budget of plantations. At the end of the first rotation, yield increases up to 30% have been measured for some clones. No adverse affects on soil chemistry has been detected.Ground water sampling is underway.Four new irrigation installations and two biosolids installations are being developed to further test the concept of utilizing SRWC plantations for waste treatment in Western Canada.

Keywords: Short Rotation Coppice, Willow, *Salix*, *Populus*, Poplar, Irrigation, Sewage Wastewater, Biomass, Biosolids

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Intraspecific variation of physiological and molecular response to cadmium stress in *Populus nigra* L.

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In the last twenty years, cadmium (Cd), which is relatively rare in its natural form, has become one of the most widespread hazardous pollutants due to its wide use in industrial, agricultural and domestic applications. Among the advanced and innovative treatment methods, phytoremediation is one of the least expensive. Several studies showed that the Salicaceae family, with its genera *Salix* (willows) and *Populus* (poplars), is well suitable for phytoremediation. The genus *Populus* presents a wide natural variability due to its adaptation to contrasting environments. Recent works concluded that *Populus nigra* (*P. nigra*) holds interesting potential for phytoremediation. Little is known about the variability of the response to heavy metal stress within the species, while it could be a key for a better understanding of tolerance mechanisms and for breeding. The aim of the present study was to characterize the natural variation of response to Cd in *P. nigra* in order to understand the different mechanisms of Cd tolerance. Two *P. nigra* genotypes, originating from contrasting environments in northern and southern Italy, were submitted to Cd stress in hydroponics during three weeks to enhance the transpiration-driven Cd translocation to leaves. Biomass production, photosynthetic performance and Cd concentration in leaves were measured at the end of the experiment. Furthermore, some candidate genes, involved in response to heavy metal stress, were characterized by expression pattern and genetic linkage analysis. Biomass production and photosynthesis were significantly affected by the treatment in both clones but the southern clone was markedly more tolerant to Cd stress than the other despite the Cd concentration of leaves was not significantly different between them and was quite low compared to other species. Thiol and phytochelatin content, associated to the transcription profile of glutathione S-transferase gene, indicated relevant differences in the use of phytochelatin pathway under Cd stress that could explain the different tolerance to Cd. The northern clone accumulated thiols (dire avec precision lesquels), but down-regulated GST gene, whereas the southern clone accumulated phytochelatins and up-regulated GST genes, which can be useful to complex and detoxify Cd. This response suggests that the gluthatione pathway can be alternatively targeted at detoxifying oxidative stresses or complexing of Cd ions. The natural germplasm of *P. nigra* represents a valuable resource for understanding tolerance to Cd and for selecting of plant material for phytoremediation.

Keywords: *Populus nigra*, Cadmium tolerance, phytoremediation

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Poplar trees, a key to remediate Fluoride rich groundwater: a case study from India

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So far, Poplar trees have been known for their timber values such as for meeting the domestic needs or for making commercial cricket bats, matchwood, tool-handles, fuel andfodder etc. but the present case study shows that the poplar trees can also be used to remediate the fluoride rich ground water to resolve the environmental pollution problem. One of the major problems of water quality in India is the presence of excessive fluoride, arsenic and nitrate inground waterin many parts of the country. However, fluoride problem is wide spread in nine sates of the country. It is manifested in the form of'endemic fluorosis' disease which is an acute public health problem in India. Nearly 66 million people face the risk, of which an estimated six (06) million are children.World Health Organization (1994) recommends the drinking water should not contain more than 1.5 ppm of fluoride. The planting of Poplar trees (species - *Populus deltoides-used for match wood, pulpwood, light timber and fuelwood*) which is a very common tree planted in North India specially in Haryana as a part of agroforestry along with wheat, sugarcane, pulses and fruit trees acts as a catalyst to alleviate sodicity in soils in the high concentration fluoride affected areas. The state of Haryana is growing maximum Poplar trees.On one acre of land, nearly 200 Poplar trees are grown without affecting other crops. That is why despite having number of irrigation canals which are well known for increasing the sodicity, now shows less concentration of fluoride in its ground water due to the presence of Poplar trees and makes the water potable. These tree species, not only keeping the surroundings environment green but also helping in mitigating and solving problematic societal excess fluoride problem, in those part of country where there is a luxuriant growth of Poplar trees.

Keywords: Fluoride, Fluorosis, Agroforestry, Irrigation, Sodicity

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Application of Eddy Covariance Technique for Assessing the Carbon Capture Potential of Poplar (*Poplar deltoides*) in Uttarakhand, India

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Poplar is largely grown in the Tarai area of Uttarakhand. Being a fast growing species, it has tremendous potential of carbon capture in a short span of 10 -12 years. Therefore assessing the net carbon exchange in the Poplar forest or plantation is of paramount importance in the validation of carbon capture estimates. Use of eddy covariance technique has accelerated in recent years.Eddy covariance is a micrometeorological technique that allows a non-invasive measurement of exchange of carbon dioxide across vegetation –atmosphere interface.

The eddy covariance method is most applicable over flat terrain when the environmental conditions are steady and the underlying vegetation upwind of the sensors is horizontally homogeneous for an extended distance of several hundred meters. Tarai area of Uttarakhand is fulfilling the required conditions of this method and therefore systematic research study is urgently warranted in this direction for assessing the carbon capture potential of Poplar. Moreover Eddy Covariance method can also provide relevant information on inter-relationship between carbon fluxes and phenology.This paper is to reviews the potential applications of Eddy Covariance method as a tool for validating carbon dioxide exchange between ecosystem and the atmosphere with special reference to Poplar.

Keywords: Poplar Forest, Eddy Covariance, Carbon Fluxes, Phenology

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Environmental conditions influence distribution of two willow (*Salix*) species and their hybrid at relict site in Hrubý Jeseník Mts. (Czech Republic)

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The genus *Salix* (willow) is represented by four relict subalpine species in Czechia. All ones have limited distribution and they are threatened there. One of the potential threats is intensive hybridisation and possible gene-flow. Therefore, we addressed the question to what extent are relict subalpine willow taxa threatened by hybridisation with common mountainous species. In the first part of the project, we studied morphological and ecological overlap between *Salix hastata*, *S. silesiaca* and their hybrid *S.* × *chlorophana*. Two study plots were established in the Velká Kotlina fold (Hrubý Jeseník Mts, Czechia), where is the most stable population of *S. hastata* in CZ as well as is known frequent occurrence of the hybrid there. We recorded the taxon, the size of plant, morphological key characteristics, biotope and main physical conditions on both plots. The data revealed expected morphological overlap between studied three taxa. Additionally, we found that the hybrid is the most frequent taxon at the locality (56.18 % out of 372 plants), whereas the most rare one is *S. silesiaca* (14.52 %). However, these taxa are ecologically and spatially well separated: *S. hastata* significantly prefers subalpine springs and its ecotons, *S. silesiaca* grows mostly in subalpine *Vaccinium* vegetation and finally the prevailing biotope for the hybrid is the wind-swept alpine grasslands. Although is the hybrid the most frequent taxon at locality, environmental preferences of each taxon cause spatial separation of the endangered species *S. hastata* from the hybrid and therefore is not directly threatened by the hybridisation with *S. silesiaca*. This project recently continues by the analysis of genetic structure of the population, frequency of hybridisation events and the nature of the gene-flow among taxa using appropriate molecular markers.

Keywords: Environmental conditions, willow, Salix, tree morphology

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Plant-Microbe Interactions: The Role of Plant Genotype and Phenotype in Regulating the Symbiotic Microenvironment

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Genotypic variability in plant carbon and nitrogen metabolism greatly alters both the intra- and extracellular metabolite profiles and thus determines the biochemical microenvironment in which microbial (fungal and bacterial) symbionts exist. Our previous biochemical analyses of black cottonwood (*Populus trichocarpa;* T), eastern cottonwood (*P. deltoides;*D), their inter-specific hybrids, other *Populus* sp. and their transgenic and cisgenic mutants provide a diverse variety of clones with tailored differences in primary and secondary metabolism that can be exploited to probe plant-microbe interactions. We hypothesize that enhanced production of metabolites of primary C and N metabolism in poplar roots, promotes colonization by ectomyccorhizal fungi and endophytic bacteria, whereas the production of unique secondary metabolites function as selective agents that both promote and inhibit specific microbial species. Here we present the current status of poplar genetic resources that have been created through transgenesis to provide the basis for future studies on poplar-microbial associations.

Populus trichocarpa and *P. deltoides* differ in their profiles of secondary metabolites and these metabolic differences segregate in successive generations, such that quantitative trait loci associated with metabolite production (mQTL) can be mapped. We have mapped several hundred mQTL for both leaf and root metabolites in two pseudo-backcross (TDxD') pedigrees. Five candidate genes were selected from within an mQTL hotspot region, and constructs were designed for their up- and down-regulated expression. These cisgenic transformants with putatively increased and decreased secondary metabolism will be characterized and used to test the effects of altered metabolism on plant-microbe associations. Additionally, the F2 backcross progeny with extremely high (and low) production of secondary metabolites that led to the identification of the mQTL will be selected to determine the effects of altered metabolism on plant-microbe associations. We have also shown that a key difference in secondary metabolites among poplar species is the nature and concentration of hydroxycinnamate-quinate/shikimate esters that are present. The role of such metabolites in microbial colonization can be assessed by the selection of poplar species with diverse profiles of such metabolites.

Recent reports suggest that root growth induced in poplars interacting with *Laccaria bicolor* required polar auxin transport, as well as auxin signaling through poplar auxin response regulator proteins. Although many microbial genomes carry genes of the auxin biosynthetic pathway, it is unclear to what extent these signaling pathways are universal or specific in establishing a symbiotic relationship between a specific microbe and host genotype. We hypothesize that certain auxin response factor proteins belonging to the *Aux/IAA* and *ARF* families play a direct role in establishment and /or signal transduction post-establishment. We are testing this hypothesis by co-culturing specific microbial strains with PCR-confirmed poplar RNAi lines specific to genes from the *Aux/IAA* and *ARF* gene families. A micropopagation protocol established to generate whole plantlets from *Populus* shoot tips will be used to test candidate endophytic and rhizosphere microbes, which will be tracked by imaging and molecular profiling methods to determine where, when, and how the microbe associates with the host plant.

Keywords: Genotype, Phenotype, *Populus trichocarpa, P. deltoids*, plant metabolism

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Plant Microbe Interfaces: Defining and understanding the relationship between *Populus* and its microbiome

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Rapid progress in biological and environmental sciences has been enabled by the availability of genome sequences and the tools and technologies involved in interpreting genome function. As our understanding of biological systems grows, it becomes increasingly clear that the functional expression of individual genomes is affected by an organism's environment and the community of organisms with which it associates. For example, 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 the greatest 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. Our goal is to understand the genome-dependent molecular and cellular events involved in establishing and maintaining beneficial interactions between *Populus* and its associated microbial community. To achieve this goal, we are focusing on 1) characterizing the natural variation in *Populus* microbial communities within complex environments, 2) elucidating *Populus*-microbial interactions at the molecular level and dissecting the signals and pathways responsible for initiating and maintaining microbial relationships, and 3) performing metabolic and genomic modeling of these interactions to aid in interpreting the molecular mechanisms shaping the *Populus*-microbial interface. Recent results related to defining the natural diversity of microbial associates of *Populus deltoides* and elucidating explicit signaling mechanisms between *Populus* and its microbial associates will be described.

Keywords: Populus, genome, microbies

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Olive oil mill wastewater application in a poplar plantation: perspectives for fertilization, fertirrigation and phytoremediation

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The olive oil mill wastewater (OMWW), i.e. vegetation water, derives from the extraction of olive (*Olea europaea* L.) oil and contains suspended matter consisting of olive pulp, mucilage, pectin, oil, etc., in a relatively stable emulsion. In the Mediterranean area OMWW is produced in large amounts, causing environmental hazards. The use of these wastewaters on soils may enhance their fertility, thanks to the fertilizing properties of the waste, as organic matter, P, K and N contents. However, the use of OMWW as fertilizer may lead to some disadvantages because of its acidity, salinity and the accumulation of lipids, organic acids and phenolic compounds. These later compounds, in particular, having bactericide and phyto-toxic properties, may cause N cycle alterations and changes in the soil microbial activity with contamination of surface and ground waters. For all these reasons in the main olive oil producers in the world the agronomic use of OMWW is regulated. In Italy a maximum application rate of 80 m3 ha-1 year-1 is permitted. In the Mediterranean countries the most challenge concerning OMWW is the great amount produced in a short period (three-four months) during fall and winter, when natural precipitation can leach the OMWW below the active root zone of the plant towards groundwater. Land application of OMWW on soils cultivated with high evaporative demand tolerant plants such as poplar could enhance the biological and chemical degradation of the effluent and solve its disposal problem. The main aim of this research is to understand how negative effects of OMWW application on soil physiochemical characteristics can be reduced by fast growing poplar plantation. With this purpose, a pilot system of Short Rotation Forestry (SRF) poplar plantation has been planted (spring 2009) in an olive farm in Pisa, Tuscany, where OMWW has been distributed at agronomical rate. To better evaluate the poplar and OMWW interactions, through the complete water and solute mass balances, lysimeters (1 m3) were assembled adjacent to the experimental field with SRP plantation and filled with silica sand. Four poplar cuttings belonging to the same clones used in the field (I-214 and Monviso) were planted in each lysimeter. An initial characterization of the site was done by electromagnetic induction (EMI) technique and chemical analyses by standard methods, as well as OMWW composition. The effects of OMWW application will be discussed in terms of modifications in soil and substrate physiochemical properties and plant growth analyses.

Keywords: Olive oil mill, waste water, fertilization, irrigation

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Session 6: Plant metabolism and plant-pest interactions: lessons to improve pest and stress resistance

The role of Isoprene in stress tolerance: (eco) physiological studies in transgenic poplars

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Plants not only exchange water and CO_2 with their environment, they also emit a variety of biogenic volatile organic compounds (BVOC). Isoprene (2-methyl 1,3-butadiene) is the dominant compound from all plant BVOC. The versatile influence of isoprene on the chemistry of the atmosphere constitutes for the remaining high importance in atmospheric and environmental research. But also in plant science isoprene plays a special role because it is still under discussion why plants emit so high amounts of isoprene.
Different studies provided details that the emission of isoprene protects plants against high temperature episodes and oxidative stress. Recent results of our group present a relevant contribution to clarify the functions of isoprene emission in poplar. We conducted several experiments with transgenic non-isoprene emitting *Populus x canescens* plants where isoprene synthase (*ISPS*) gene expression was knocked-down by RNA interference (RNAi) technology. Studies of transient temperature and light stress demonstrate that for poplar leaves the ability to emit isoprene is crucial to maintain photosynthesis when exposed to sunflecks. Metabolite and transcript analyses revealed that switching off isoprene biosynthesis in poplar leads to comprehensive shifts in different metabolic pathways and an adjustment of the antioxidative system in the non-isoprene emitting poplars indicating direct and indirect roles of isoprene in oxidative stress defense. Furthermore, with a long-term outdoors study it turned out that the repression of isoprene emission results in implications on poplar-fungi and poplar-insect interactions and therefore a new ecological function of isoprene in poplar arose. Overall, the transgenic approach allowed to support discussed functions of isoprene and to address novel issues.

Keywords: Isoprene, Stress tolerance, *Populus x canescens*, RNA

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Broad-sense heritability of a pathogen community in a hybrid cottonwood system

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Pathogens exert selection for resistance on host plant populations, which results in corresponding selection for virulence in pathogen populations. Host plant hybridization can disrupt such tightly coevolved host-pathogen interactions by creating a genetic continuum between parental host species. However, few studies have explored how pathogens respond to the genetic diversity of closely related species and their naturally occurring hybrids. Here, we report on the abundance and distribution of fungal leaf pathogens infecting *Populus fremontii*, *P. angustifolia* and their naturally occurring hybrids in stands along the Weber river in northern Utah, and in common gardens containing replicate clones of individual tree genotypes. We found that: 1) different tree genotypes support different fungal pathogen communities, 2) there is significant broad-sense heritability of plant resistance to the fungal pathogen community, and 3) fungal pathogens have shifted from native to non-native hosts. Our results suggest that plant hybridization enables host shifting by providing pathogens with a genetic continuum for adaptation. To our knowledge, our results are the first to demonstrate the broad-sense heritability of plant resistance to natural pathogen communities. Furthermore, these findings suggest both inter- and intraspecific genetic variation in foundational hosts determine the evolutionary trajectory of pathogen communities.

Keywords: Heritability, Fungal Pathogen, Genotypes, *Populus*

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Towards broad-spectrum resistance to *Melampsora larici-populina* rust in poplar: new genetic constructions and potential adaptation of the pathogen

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Broad-spectrum resistance to *Melampsora larici-populina* (Mlp) leaf rust is a major challenge for poplar breeding in Northern Europe. When no rust epidemic was evident in the natural Populus nigra populations, cultivation of inter-specific clones carrying qualitative resistances have strongly modified both qualitative and quantitative pathogenicity components of Mlp populations in cultivated areas. In monoclonal poplar plantations, durability of rust resistance will depend strongly on (1) resistance mechanisms pyramided in a given genotype, (2) genetic diversity present in Mlp populations submitted to these high selection pressures and (3) possibility of recombination rate associated with sexual reproduction on larch.

Recent results on genetic and molecular bases of Mlp-poplar interaction and multidisciplinary research projects in development will be presented.

Although quantitative resistance (QR) is often considered more durable, a multidisciplinary approach is being conducted at INRA not only to identify optimal genetic constructions for broad spectrum resistance but also to predict potential adaptation of the pathogen. Starting with a single Populus deltoides x P. trichocarpa F_1 pedigree, much attention is being paid to two major loci, R_1 and R_{US}, that both map on LG XIX. Dominant allele R_1 is inherited from P. deltoides. It controls a qualitative resistance to M. larici-populina, and it was found to be statistically associated with QR levels in several P. deltoides x P. trichocarpa F_1 progenies. Dominant allele R_{US} is inherited from P. trichocarpa. It has major effect of uredinia size and other QR components in both intraspecific and interspecific F_1 progenies. Fine mapping of R_{US} is being conducted using a 1410 genotype F_1 host progeny, and a BAC library was constructed to generate a physical map of 2000 kb around R_{US}. A promising BAC clone has been identified and sequenced. In parallel, an expressional approach based on the analysis of R_{US} and r_{US} infected material generated transcriptional and translational candidates.

Opportunities offered by genetic variability available in Populus nigra, the natural host of Mlp, are currently explored in inter-specific crosses and in a new F1 mapping pedigree. Moreover, a collection of more than 1100 P.nigra genotypes collected in different natural European populations has been recently evaluated for rust resistance to initiate an association genetic study.

On the pathogen side, several populations of M. larici-populina that underwent contrasted selection pressures by R_{US} are being characterized for their phenotypes and for their genotypes at several microsatellite loci. The first results demonstrate the existence of pre-adaptation in “wild” pathogen populations and a clear selective pressure by the host.

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

Keywords: Poplar rust, *Melampsora larici-populina, Populus nigra*

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Variation in leaf damage in a segregating hybrid *Populus* family identifies genomic regions associated with insect and rust damage

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Pest damage to *Populus* crops reduces the amount of biomass available for bioenergy use. Genetic variation in leaf physical and chemical properties of trees is thought to influence the extent of pest damage, e.g. by influencing palatability to herbivores. Understanding the genetic and genomic mechanisms underlying palatability and resistance to pest species will provide tools for marker-assisted breeding of Populus varieties that minimize biomass to forest pests.

In order to identify genomic regions associated with susceptibility to insect and rust damage, parents and progeny of a mapping population of poplars (*Populus trichocarpa* × *P. deltoids*) were assessed for seven categories of leaf damage, namely that due to chewers, skeletonizers, leaf miners, leaf rollers and sap suckers, leaf galls and that caused by rust infection. Trees were scored in early (June) and late (August) summer. Significant variation in damage levels was observed among genets (genotypes), and greater levels of damage were observed later in the season (Table 1). Each category of leaf damage was treated as a quantitative trait in a QTL mapping approach.

A total of 17 QTL were identified for eight of the season-trait combinations assessed (Table 3). No significant QTL were associated with leaf rollers, gall damage, or sap suckers on stems. A smaller number of QTL were identified for the June data than August data, consistent with the higher levels of damage observed later in the season. For the June data, one QTL was identified for each of three damage categories: chewer, skeletonizer, and leaf miner. Each of the June QTL were located on a different linkage group. These QTL explained between 3.0 and 7.7% of the phenotypic variance observed in these traits.

For the August data, 14 QTL were identified for five damage categories: chewer, skeletonizer, leaf miner, sap suckers on leaves, and rust infection. One QTL was identified for skeletonizer damage, four QTL identified for chewer damage, and three QTL for each of the other categories. The single QTL identified for August damage levels explained between 2.9 and 9.3% of the phenotypic variance observed in these traits. In total, the QTL observed for each trait explained 24% of the phenotypic variance in chewer damage, 5.8% in skeletonizer damage, 11% for leaf miner damage, 14% for the presence of sap suckers on leaves, and 15% for rust infection.

Different QTLs were identified for skeletonizers and leaf miners from the June and August data, indicating potential facultative defence mechanisms being induced later in the season. Alternatively, the causal insect species may have been different between these two time points. For the August data, QTLs for some insect damage types (chewing and leaf mining) co-located in a single position, indicating this genomic region may contain genes related to damage or defence. The limited co-location between QTL for different damage types indicates the insect community may respond to genetic variation underlying different polygenic traits depending on the type of herbivory and time of year. These QTLs will be the basis of a bioinformatics study to identify potential chemical mechanisms underlying the variation in insect and rust damage in this hybrid population.

Keywords: Populus, leaf damage, poplar pest

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The effect of the local environmental conditions on associated organisms of Lombardy poplar (Populus nigra cv 'Italica')

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Lombardy poplar (*Populus nigra* cv 'Italica') has been planted all over Europe, mostly as single trees or in rows along roads or around squares. This clone represents a unique opportunity to study local effects (e.g. associated community diversity) on a single genotype. We investigated the effect of local environmental conditions (biogeographical species pools, site conditions, surrounding vegetation) and clines in environmental conditions (climate, latitude) on the diversity of associated organisms, notably insect species. Pairs of trees were selected at least on five urban and five rural sampling sites in each of the following countries: France, Germany, Hungary, Italy and Poland. Fifty leaves per tree were selected randomly once a month from May until September and evaluated for damage by six functional groups of insect: chewers, skeletonizers, leaf miners, sucking and sap feedings, gall makers and leaf rollers. Insect species were also determined for leaf miners and gall makers. There was no significant difference in damage levels between pairs of trees at each site, but significant differences were found among sites and among countries for the means of leaves damaged by each functional group. The same leaf miner species were found in the five counties but at different frequencies. The dominant gall-maker species was the aphid, *Pemphigus spyrothecae*, in all countries; however both species composition and dominance varied strongly among sites. Remarkably, species diversity (Shannon-Wiener Index) of gall makers and leaf miners was higher on urban sites than on rural sites.

Keywords: insect associates, local environment, *Populus nigra* cv 'Italica'

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Atmospheric ozone removal from *Populus nigra* leaves exposed to realistic atmospheric ozone concentration, and the effect on phenolic and volatile organic compounds along the plant profile.

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Poplar plants, especially those cultivated in urban areas, are exposed to increasing levels of tropospheric ozone concentrations. This pollutant penetrates in leaves through stomata and quickly reacts inside leaves, thus making poplar plantations valuable ozone sinks, but at the same time triggers oxidation processes which lead to leaf injuries and a decrease in productivity. To counteract these negative effects, poplar leaves produce an array of antioxidants which react with ozone and reactive molecules which ozone generates in the leaf tissues. In particular, poplars produce considerable amount of isoprene, a volatile organic compound which provides enhanced leaf thermotolerance and scavenge ozone in the intercellular spaces before the leaf tissues are damaged, and contribute to ozone removal reacting with ozone when released in the atmosphere. In different experiments, we exposed plants of the widespread agroforestry species Populus nigra for several weeks to an ozone concentration which is likely to be attained in many areas of the world in the near future (80 ppb) and measured 1. the capability of poplar leaves to remove atmospheric ozone, 2. the effect of ozone exposure on leaves along the vertical profile in terms of changes in physiological parameters (photosynthesis and stomatal conductance), emission of volatile organic compounds (VOCs, i.e. isoprene, methanol and other oxygenated compounds), concentration of antioxidant surface compounds, concentration of phenolic compounds. We show that ozone uptake is largerly driven by stomatal opening, and the exposure to a realistic level of ozone concentration did not produce visual injuries and major changes in physiology. The emission of isoprene and oxygenated six-carbon (C6) volatiles were inhibited by ozone, whereas methanol emission was increased, especially in developing leaves. We interpret these results as suggesting an ontogenetic shift in ozone-treated leaves, leading to a slower development and a faster senescence. Most surface and phenolic compounds showed a declining trend in concentration from the youngest to the fully expanded leaves. Ozone reduced the concentrations of chlorogenic acid derivatives at the leaf surface, whereas in total leaf extracts a metabolic shift towards few phenolics with higher antioxidant capacity was observed.

Keywords: Populus nigra, Ozone, Phenols

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Session 6: Poster

Minimizing pest damage to poplars grown under FSC certification guidelines through clone selection, management practices, and pest specific control strategies.

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Avoidance of clones susceptible to endemic pest species in western North America is complicated by abiotic conditions. High desert regions of eastern Oregon and Washington frequently experience winds exceeding 80 – 95 kph. Native *Populus trichocarpa* has a deep rooting system to withstand high winds and periodic droughts, but native riparian areas harbor pests attacking *P. trichocarpa* and various species of *Salix*. Clones with *trichocarpa* parentage chosen for their rooting structure are targeted by all endemic pest species. Trees older than 3 years are attacked by *Prionoxystus robiniae*, and younger trees are more susceptible to *Paranthrene robiniae*. *Cryptorhynchus lapathi* were significantly more successful at establishing a population in clones with *P. trichocarpa* x *P. deltoides* (TxD) parentage (♀ x ♂) than in either of clones with *P. deltoides* x *P. nigra* (DxN) parentage (♀ x ♂), or a clone of *P. deltoides* x *P. maximowiczii* (DxM) parentage (♀ x ♂)[1].

Trees with wind damaged tops are rapidly colonized by *C. lapathi* and should be removed to reduce the pest population. Timing of harvest prior to pest emergence reduces the pest population. Chipping operations should be centralized in one location to reduce point sources of plant volatiles that are attractive to *Pa. robiniae females*. Pruning activities in months when pests are dormant minimizes release of attractive plant volatiles.

We have conducted research supporting the new registration of eight insecticides for the protection of hybrid poplars grown under Forest Stewardship Council's certification program. These protection strategies include insect growth regulators, pest specific systemic insecticides delivered through the drip-line, and chemicals with novel modes of action. Sex pheromones are used to removed the male population through mass-trapping efforts, or disrupt mating of specific pest species. A serious outbreak[2] of *Pa. robiniae* was reversed by aerially dispersed microcapsules (Checkmate WPCM-F) containing the female sex pheromone, resulting in male confusion.

Keywords: Poplar pests, FSC certification, insecticides

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A rare major Quantitative Trait Locus determines survival of a gall forming insect in willow

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Biotic interactions, such as herbivory, can have important impact on the ecology and evolution of plant. Plants as a resource for growth and reproduction of insects can vary depending on chemistry, phenology and development, due to either genetics or environmental factors. Reduced relative performance of the herbivore defines if plants are resistant.

Great genotypic variation in resistance is documented among natural growing *Salix viminalis* against the gall midge *Dasineura marginemtorquens* (Diptera, Cecidomyiidae). On resistant *Salix* genotypes neonate larva die and no galls are formed. The willow-gall midge interaction is complex and includes symptoms both known to enhance (gall formation) and reduce (hypersensitive response, HR) insect performance.

By means of quantitative trait locus (QTL) analysis we investigated the genetic architecture of traits determining larval survival and HR. The parents to the experimental population were selected because i) there was documented variation in the effect on larval survival ranging from 0% to 100% among siblings and ii) they show different plant responses associated with resistance, i.e., both HR and a symptomless response.

Presence of one major QTL as well as two minor QTLs determining larval survival were revealed in this population as well as several QTLs for HR response.

To identify putative genes in the major QTL, the homologous region in the *Populus* genome was searched for annotated genes. Based on candidate genes the mechanism of resistance was suggested to be associated with absence of growth and not presence of defence.

Keywords: Willow pests, Genotypic variation

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Photosynthesis and isoprene relationships across a range of poplar genotypes

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Poplars (*Populus sp.*) are among the strongest isoprene-emitting plants. Ten poplar genotypes belonging to four different species, were grown under the same environmental conditions in a common garden experiment, to study the influence of the genetic variability on isoprene emission and on the relationship between isoprene and photosynthesis. Photosynthesis was not significantly different between species but isoprene emission was significantly higher in *Populus trichocarpa* than in the other poplar species. Isoprene emission ranged from 18.2 to 45.2 nmol m–2 s–1 in the different plants and was more variable than photosynthesis. There was no clear association between isoprene emission and photosynthesis. In most genotypes photosynthetic capacity developed earlier than isoprene emission capacity throughout the leaf development. The emission of isoprene was inversely correlated with the intercellular CO_2 concentration (Ci) which drives photosynthesis. A positive linear correlation between the fraction of recently assimilated carbon emitted as isoprene and isoprene emission rate was found. The slope of this relationship indicated that each nmol of isoprene emitted requires a fixed fraction of photosynthetic carbon regardless of the intra and inter-specific variability in the *Populus* genus, and of leaf ontogeny. A comparison with data of recent studies showed that the slope of this relationship doubles in drought stressed leaves. However, this might be explained by an increasing contribution of carbon sources for isoprene biosynthesis that comes from stored photosynthates. If this is true, then the amount of carbon directly shunted from photosynthesis into isoprene is constant in all poplars.

Keywords: Photosynthesis, Isoprene, Poplar genotypes

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Comparative analysis of the photosynthetic characteristics in 3-year-old *Populus tomentosa*

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Populus tomentosa is a native species in China. Owing to its rapid growth, excellent wood quality, it is being widely employed for forestation and landscape in north China. Photosynthetic characteristics of 30 *P.tomentosa* clones were measured with lico-6400 photosynthetic instrument, and the relationship between the environmental factors and physiological indicators was studied by correlation analysis and stepwise regression equation. The results showed that the dirunal variation of Pn of *P. tomentosa* clones presented a typical double-peak curve, and that stomatal limitation was a major regulatory factor of decreased photosynthesis. Pn-Par and Pn-Ca curves of five *P. tomentosa* clones presented a "S"-like pattern, and also accorded with quadratic equation. Under saturated conditions of the Ca and luminous intensity, Pn, lcp, csp of the five clones showed significant differences. In addition, with the luminous intensity saturated, rankings for Pn were:
BL204(24.64)>BL206(23.76)>BL30(21.50)>BL207(19.54)>BL63(18.64μmol·m^{-2}·s^{-1}).
Whereas with the Ca saturated, BL206 was the maximum value: 30.15 μmol·m^{-2}·s^{-1}, and BL63 the minimum value: 20.34μmol·m^{-2}·s^{-1}. Meanwhile, for the five clones, lcp ranged from 33.08μmol·m^{-2}·s^{-1} to 81.17μmol·m^{-2}·s^{-1}, and csp ranged from 74.03μmol·mol^{-1} to 93.35μmol·mol^{-1}. The values of Pn, Gs, Ci and Tr of 30 poplar clones were significantly different.

Keywords: Photosynthesis, *Populus tomentosa*

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Constitutive and induced resistance between a susceptible and resistant aspen clone and transcriptional difference towards *Melampsora megnosiana*

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Plants are exposed to a variety of pathogens in their natural habitats. To understand the key processes of defense responses in aspen (*Populus tremulae*) at the transcript level two clones C72 and C23 with differential level of resistance from the SwAsp collection were inoculated with a foliar rust (*Melampsora magnusiana Wagnar*). Leaf samples were collected from adjacent areas of the inoculation site to examine the long distance (systemic) defense responses at day1, day3 and day14 post treatments. We performed microarray experiments on the biothrophic interaction, on comparison with the healthy controls we found that the two clones respond in a widely different fashion to the rust. Clone C23 showed almost no response to biotroph after 24 hours while clone 72 gave a clear defense response to the pathogen. Quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR) showed a significant differential expression patterns in susceptible and resistant colnes. Chitinase, cinnamic acid reductase and the iaa genes showed signification up-regulation in resistant clone. The level of expression was 5.9 delta threshold cycles in chitinase gene at day14. Data analysis from extracted total phenolics and condensed tannins verify the results of cDNA arrays and qRT-PCR.

Keywords: Aspen, *Melampsora megnosiana*

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Mechanisms through which poplar rust affects yield in intensive production systems

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Economic yield is based on the conversion of light energy into the economically important part of the crop, i.e., the woody stems in short rotation coppice used as a renewable energy crop. Even when the stool, root system and leaves remain unharvested, all these components have a crucial importance in yield determination. To maximize economic yield the negative biological effects of diseases must be controlled.

To assess the effects of rust caused by *Melampsora medusae* on yield of poplars, we established a factorial experiment, with two clones of *P. deltoides*: 'Australiano 106/60' and 'Onda', and three levels of disease: without rust (sprayed periodically with 25,8 g ai/ha of tebuconazole during two consecutive years); with rust only during the first year, and with rust (not sprayed in both years). Disease progression was monitored every fifteen days by counting the number of uredia per square centimeter on a random sample of ten heavily infected leaves per plot. Leaf dynamics was evaluated every fifteen days by counting the number of new and fallen leaves, and leaf area was estimated using a model based on length and maximum width of the leaves. The fraction of photosynthetically active radiation (PAR) intercepted by the canopy was measured at different heights several times during the growing season with a ceptometer. Photosynthesis at light saturation (Asat) was measured during the peak of the rust attack with an IRGA, and electron transport rate (ETR) was measured through chlorophyll fluorescence (FMS2, Hansatech). Stem dry matter per plant was evaluated at the end of the two consecutive growing seasons and in December of the second year, just before a new rust attack.

The first pustules were observed during the last week of December. 'Onda' was more attacked than 'Australiano'. In 'Onda', diseased plants had less leaf area and dropped their leaves about fifteen days earlier than healthy plants. 'Australiano' showed the same trends but the differences between diseased and healthy plants were not significant. PAR transmittance was higher inside the rusted canopy, with plants intercepting only about 50% of incident light. In 'Onda', Asat and Agross (Asat – respiration) were lower in leaves with uredia but leaf respiration was higher. The decrease in Asat by rust in 'Onda' was not only due to higher respiration, indicating that the photosynthetic system was damaged, as shown by the drop in ETR. Asat in leaves with uredia was not affected in 'Australiano'. This behavior negatively affected the accumulation of nutrients and root development and, therefore, the leaf area developed, chlorophyll content of the new leaves and growth was significantly reduced in 'Onda' since the beginning of a new growth season even before the crop is attacked again by rust.

Both leaf dynamics and photosynthesis indicate that the damage produced by rust is related to the genotype, and that growth reduction is explained by a decrease in leaf area and also by a drop in photosynthesis of the remaining leaves.

Keywords: Poplar rusts, production systems, Photosynthesis

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Application of herbicides in production of poplar seedlings

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Weed control is very important, especially in nursery production of seedling stock, because the quality seedling stock is the basic prerequisite for successful establishment of poplar plantation and other forest species. Investigation of the possibilities of applying herbicides in production of poplar nursery plants pointed out to many advantages of their application in relation to mechanical weed control. Usage of herbicides decreases the cost of soil cultivation and competition between weeds and nursery plants, and increases their quality and survival percentage. Investigation efficiency and selectivity of herbicides in the production of poplar plants were performed during the period 2005 – 2007. Trials were set up in two localities which differing in physicochemical soil properties (clay form of fluvisol and sandy form of fluvisol). Cuttings of three poplar clones Pannonia, B229 and Pe19/66 were used for experimental fields establishment. Examined combinations of soil herbicides acetochlor + prometryn, acetochlor + s-metolachlor, acetochlor + metribuzin i dimethenamid + linuron were applied after planting and before the growth of poplar cuttings and weeds. Herbicides fluasifop-p-butil i cycloxydim were applied after the growth of weeds and poplar cuttings to control of grass weeds. Examined combinations of soil herbicides were efficacy in controlling of weed vegetation in studied localities. The best efficacy during the three years on both localities exerted a combination of herbicide acetochlor + prometryn. Herbicides fluasifop-p-butil and cycloxydim have been effective in weed control of grass weeds. Differences in physicochemical properties of soil on the localities beside composition of weed flora also influenced the behavior and herbicides action in such a way that metribuzin applied on soils of lighter mechanical composition (sandy form of fluvisol) in combination with acetochlor exerted phytotoxic effect on poplar plants. Other examined herbicides did not have phytotoxic effect on poplar seedlings.

Keywords: Poplar seedlings, Herbicides, Weed control

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Insect herbivory response to Populus nigra genetic diversity

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Recent research suggests that genetic diversity in tree population may shape associated species assemblages and then drive ecosystem processes. Trees can support large numbers of insect species and some functional traits of trees have been shown to have major influence on insect communities and resultant damage. Studying the response of insect herbivore populations to the genetic diversity of tree populations is therefore a relevant approach to test community genetics hypotheses.

The objective of our study was to evaluate how insect herbivory is affected by genotypes of *Populus nigra*, a dominant native poplar species in riparian forests of Europe. In a field experiment, we planted eight native *P. nigra* clones in plots of increasing genetic diversity with combinations of one to six clones. Insect herbivory was measured using visual estimation of leaf damage by six functional guilds of insects: chewers, skeletonisers, leaf miners, gall makers, leaf rollers, sap suckers and sap feeders.

Response of insect herbivory to increasing genotypic diversity was analysed by considering the net diversity effect which was further divided in a complementary and a selection effects. Overall we observed slightly higher insect herbivory in poplar clone mixtures than in clone monocultures. This pattern was due to two opposite mechanisms. There was a positive complementary effect, with higher associational susceptibility in more genetically diverse mixtures. On the other hand, the selection effect also increased with the genetic diversity as the more diverse clone mixtures were more likely to comprise insect resistant clones.

Keywords: community genetics, insect herbivory, *Populus nigra*.

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Investigating variation of disease resistance and wood formation genes in willow (*Salix spp.*)

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The aim of this poster is to give an overview of the project started in October 2009.

Willow, used as a biomass crop in short rotation coppice, has the potential of producing energy that is greater than carbon neutral, and has the ability to grow fast without high maintenance or fertilizer input.

Several species are native to Ireland but very little is known about their genetic diversity and nothing is known about gene diversity within coding regions of their genome in populations distributed throughout the island.

Therefore, the overall objective of this project is to investigate genetic diversity of neutral markers and adaptive genes in populations of willows (mainly *Salix viminalis* and *Salix caprea*) in Ireland (natural and commercial clones) through the study of molecular genetic markers (Single Nucleotide Polymorphisms; Amplified Fragment Length Polymorphisms). The candidate gene approach is being used on some genes related to leaf rust resistance and lignin pathway, genes which have been studied on poplar, a close relative of willow.

A previous project, supervised by Colin Kelleher from the National Botanic Gardens, has studied genes of the lignin biosynthesis pathway within the genus *Populus*. These genes have been analysed on 42 samples of willows collected at the National Botanic Gardens and at Teagasc Kinsealy Research Centre, representing the three subgenera of *Salix*, and including nine commercial clones and six old varieties from Irish basket makers. The other samples are from Ireland, Germany, and the United Kingdom. The DNA of these samples has been extracted and 27 primer pairs corresponding to 27 different genes studied in Populus have been amplified by Polymerase Chain Reaction. These genes are involved in the lignin, cellulose and sucrose biosynthesis, but also in a putative lignin regulation, in growth hormone regulation, and in transcription factors.

Six amplified loci showed one clear unique band after agarose gel electrophoresis, but the PCR conditions on seven other primers need to be improved by increasing the temperature and decreasing the time of the annealing step. The amplicon is the same length as poplar in almost every case, from 411 bp to 1204 bp. Further analysis will not be carried out on the 14 other genes. Among them, nine didn't amplify and five showed multiple bands.

The genes that presented a strong unique amplification are sequenced using a kit and automated DNA sequencer. These genes will be analysed on natural Irish populations of willows.

At the same time, samples of leaves of natural populations of *Salix caprea* and *Salix viminalis* from all over Ireland are collected including 10 populations of each species and around 30 individuals per population. *S. caprea* is native from Ireland and grows in woodlands with other deciduous trees whereas *S. viminalis* is an alien species found mainly along rivers. Because of this, the sampling strategy won't be exactly the same.

Keywords: Disease resistance, Wood formation, *Salix*, Willow

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Ozone pollution compromises *Populus* transcriptome responses to insect herbivore attack

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Ozone is considered one of the most severe air pollutants in forest ecosystems around the world, particularly downwind of industrialized areas. In *Populus* species, ozone causes various symptoms of oxidative stress. Similar signalling pathways may be activated in response to both biotic and abiotic stresses; therefore, we used genomic resources of *Populus* to investigate the influence of ozone pollution on plant responses to herbivore attack. In separate experiments, we used three genotypes of hybrid *Populus* that varied in their susceptibility to ozone pollution. Experiments were conducted in Continuously Stirred Tank Reactor fumigation chambers. We used ozone concentrations of 80 ppb to reflect spring and summer conditions in central Pennsylvania. Half of the plants were in control chambers where no ozone was added. After one week, all plants had portions of their stems enclosed in soft mesh bags and gypsy moth (*Lymantria dispar* L.) larvae were added to half of the bags. After 24 hours, leaves with similar amounts of damage and undamaged controls were collected and subjected to microarray hybridizations using either a custom array containing approximately 6500 unigenes or a commercially available full genome array. Ozone exposure severely compromised the dynamic responses to insect herbivore feeding. While approximately 20% of the *Populus* transcriptome was responsive to herbivory under control conditions, only approximately 4% of the transcriptome responded to herbivory after 80 ppb ozone. Moreover, the magnitude of expression of the genes under ozone exposure was mostly lower than under control conditions. Of particular interest, expression of genes associated with defence signalling and induced resistance was compromised or strongly reduced under ozone exposure. Consistently, concentrations of signalling compounds and defence metabolites were significantly reduced despite extraordinary accumulation of fatty acid precursors resulting from ozone-mediated destruction of cell membranes. We will discuss our results in the context of this study and other related work that shows effects of ozone pollution.

Keywords: herbivory, hybrid poplar, microarray, ozone, *Populus*

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Session 7: International cooperation for poplar research and applications

Poplars and Willows in the world – A comprehensive synthesis

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The FAO of the United Nations has authorized the International Poplar Commission to produce a major update of the 1980 FAO publication entitled 'Poplars and Willows'. Our goal is to provide a synthesis of the latest worldwide knowledge and research on poplars and willows for a general audience. The electronic publication provides an up-to-date treatment including more emphasis on taxonomy and environmental benefits with a worldwide scope.

Completed chapters include the cornerstone chapter of the book on the systematics and nomenclature of poplars and willows by D.I. Dickmann and J. Kuzovkina that is arguably the most comprehensive treatment of that subject ever. The chapter on genetic resources and conservation of poplars by B. Stanton et al. includes coverage from 23 countries from each region of the world. The chapter on abiotic stresses by N. Marron et al. gives worldwide coverage of stresses including desertification, salinity and atmospheric conditions. The chapter on properties, processing and utilization by J. Balatinecz et al. gives an update of the latest utilization opportunities for poplars and willows.

Chapters on environmental applications, diseases, animal pests, markets, and rural livelihoods and sustainability are in progress at the time of this writing. The completed chapters are available at: www.fao.org/forestry/ipc/en. Click on 'Poplar & Willow Book'.

Keywords: Poplars, Willows, FAO, International Poplar Commission

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The contribution of poplar-science and technology to sustain rural livelihoods in developing countries

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Poplars are multi-purpose species. They provide a nearly endless list of of wood and fibre products, non-wood products, and are grown increasingly in bio-energy plantations for firewood. They have a positive role in the rehabilitation of degraded lands, forest landscape restoration, and climate change mitigation. These versatile attributes make poplars ideally suited for supporting sustainable livelihoods and contributing to rural development, particularly in developing countries.

The presentation will illustrate by means of practical case studies from around the world how science and technology and the transfer of knowledge related to it can contribute to sustain rural livelihoods and have an impact on food security, poverty alleviation and sustainable land-use.

Keywords: Poplars, Willows, FAO, International Poplar Commission, Sustainable livelihoods, Forest restoration

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The Italian National Poplar Commission and its international cooperation activities

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Italy officially ratified the Convention placing the International Poplar Commission (IPC) in the framework of FAO through the law 3 December 1962 n. 1799. The establishment of the Italian National Poplar Commission (CNP) followed in the year 1969 by the Decree 1 August 1969 linked to article V of the Convention itself. The NPC, having very mixed and balanced composition public-private, has been put in the framework of the Italian Ministry of agriculture food and forest policies (MiPAAF) and hosted by the State Forest Service (CFS), where currently it is still based.

Founded firstly to promote the national cultivation, use and research of poplars – where Italy traditionally represented the world excellence - and other planted trees outside forest, the CNP in its statute also recognises the importance of international cooperation carried out under the umbrella of FAO-IPC, both on applications and research. Therefore the CNP has promoted, participated in or contributed to some activities having international dimension, some of them described in this paper.

One of these relevant initiatives was the First international Conference on the future of poplar culture, held in Rome-FAO on 13-15 November 2003. It registered some 170 participants from all around the world and managed to jointly underline the global potential of poplar cultivations from the econonomic, social and environmental sides according to the concept of sustainable development.

The CNP also significantly supported and participated in the sessions of the IPC describing, for instance, the advance of poplar clones research in Italy and the state of play of Italian industry and design in the processing of poplar wood. Between them the 22nd IPC session (Chile and Argentina on November 2004) and 23rd IPC session in China, Bejing, on October 2008.

Concerning Europe, the CNP is near to the activities developed by the association "Pro-Populus", established in Brussels on 5th December 2008: it aims to support the use of poplar as ecologic and renewable resource and to corporate poplar cultivators and industry in order to overcome the growing crisis of the sector, due to international economic problems and competition.

The CNP also promotes the development of international projects in the field of cultivation of poplars and similar species outside Europe, for instance the FAO project GCP/INT/059/ITA financed by the Italian Minister of forest affairs. Under the title of "Improve the contribution of Poplars and Willows in meeting sustainable livelihoods and land-use in selected Mediterranean and Central Asian Countries" it aims to transfer the Italian knowledge on poplars and willows into selected countries of Central Asia and mediterranean areas. Also some cooperation and twinning activities with the poplar Chinese province of Siyang are currently under development.

Eventually the CNP is also supporting the IUFRO research activites on poplars, for instance in the organisation of this current session held in Orvieto and last one, held in Nanjing in June 2006

Keywords: Poplars, Willows, National Poplar Commission, Italian State Forest Service, FAO, International Poplar Commission

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A comprehensive database of poplar research in North America from 1980 – 2010

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Short rotation woody crops such as *Populus* species and hybrids (hereafter referred to as poplars) are renewable energy feedstocks that can potentially be used to offset electricity generation and natural gas use in many temperature regions. Highly productive poplars grown primarily on marginal agricultural sites are an important component of the future North American energy strategy. Additionally, poplars can be strategically placed in the landscape to conserve soil and water, recycle nutrients, and sequester carbon. These purpose-grown trees are vital to reducing our dependence on nonrenewable and foreign sources of energy used for heat and power. Establishing poplar genotypes that are adapted to local environmental conditions substantially increases establishment success and productivity. In the past 30 years, there has been exponential increase in the amount of research on poplars. This research primarily falls into six general categories: genetics, growth and productivity, insects and diseases, physiology, phytotechnologies, and silviculture. We constructed a comprehensive, annotated database of peer-reviewed literature, dating back to 1980, and compiled these papers into one readily-accessible cd-rom. The database will also be available online, with specific details to be released at the conference. All papers are listed within and cross-listed between the six categories, greatly improving the ease of literature searching and use with regards to poplar research. We will make this cd free and available at the meeting, and will have copies for distribution.

Keywords: Poplar research database, Short Rotation, Biomass production

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Effect of pollarding for stock fodder on root dynamics of conservation willow trees in New Zealand

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Poplar and willow trees have been used for soil stabilisation on pastoral hill slopes in New Zealand for 50 years. Planting in the first 25 years was done with some urgency and government financial support when it was realised that wholesale clearing of resident tree cover for pasture establishment had resulted in serious erosion and loss of valuable topsoil. No advice was given on tree management. Early plantings are now very large trees and the thought of dealing with large trees has deterred modern farmers from continuing with a planting programme. A management program that utilises the trees for multiple purposes has been encouraged by local authorities and scientists, supported by case studies of innovative farmer practices. In particular, this management program has advocated for tree willows pollarding to manage size and utilise them as a fodder source (particularly during periods of summer drought) replacing the trees when necessary, and for poplars either form pruning to produce a timber resource on a 15-25 year replacement cycle, or pollarding for fodder and size management. Current research projects are measuring the effect of pollarding on tree root development in both poplars and tree willows, as a means of evaluating this management effect on the primary role of the trees, soil stabilisation. Techniques used for investigating root development are whole tree root excavation, and root coring. A field study of the effect of pollarding on fine root development of tree willow (Salix matsudana × alba Tangoio) was carried out measuring fine coarse root ratio for unpollarded (UP), pollarded (IP) and repollarded trees (RP) at three monthly intervals over a period of one year following pollarding. The IP trees had been pollarded once only, three years before the study. The RP trees were pollarded twice, three years prior to the study and at the commencement of the study. Soil cores were taken at 150 mm depth intervals down to 600 mm at three angles and at two distances, 2 m and 3 m, from the trunk. Willow tree roots were extracted from each soil core and fine (<2 mm diameter) and coarse root length and mass determined. Three trees were sampled for each treatment. Initial mean DBH for the UP trees, IP and RP trees was 40.6 cm, 36.3 cm and 37.9 cm respectively. Seasonal changes in fine:coarse root ratio of the UP trees were measured as a baseline, and differences due to pollarding, and the immediate effect of pollarding on fine root parameters were determined. Findings from this field study will be presented.

Keywords: willow, pollarding, soil stabilisation, fine root, hill slope

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Introduction of a new class of Italian poplar clones for China

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Over the last 50 years, poplar plantations throughout much of China have relied heavily on Italian cultivars including 'I-214', 'I-45', 'I-63', 'I-69', 'I-72', 'I-107' (synonym 'Neva'), 'I-108' (synonym 'Guariento') etc., with good success for a wide variety of uses including timber production, agro-forestry, and ecological protection. Although the propagation of many of these genotypes continues, a priority has been placed on the development of new cultivars to support major plantation programs with an increasing emphasis on broadening genetic diversity to reduce the risk of unforeseen biotic and abiotic challenges. In 2007, seven Italian poplar cultivars were introduced from Alasia Franco Vivai and established in nurseries at Beijing under close quarantine and pest monitoring. These were used in establishing three regional clone trials at Zhengzhou in Henan Province, Shijiazhuang in Hebei Province, and at Beijing in 2008. Check clones from the Chinese poplar program were included in these trials. Two-year growth variation was evaluated as a function of the main effect of clone and the clone×site interaction effect. Results showed wide variation among clones at each site. For example, at the Beijing clone trial, height, breast-height diameter and stem volume varied from 8.8 m to 10.8 m, 6.3 cm to 10.9 cm, and 0.0121 m^3 to 0.0419 m^3 respectively, and their coefficients of phenotypic variation among clones were 6.1%, 13.8% and 28.8%. Variance analyses and estimates of genetic parameters demonstrated that clone differences were highly significant for height, diameter and volume at each site; clone repeatability ranged from 0.871 to 0.978, indicating the strong genetic effect on growth. Combined variance analyses demonstrated significant differences among sites, clones and clone×site interaction effects. Superior clones characterized by both rapid growth rates and high phenotypic stability were identified using: 1) an analysis of multiple contrast (LSD method), 2) effective clone value (Ci), 3) coefficient of variation for interaction effect variance (iCV), and 4) regression coefficient (bi). Trials are being measured annually to substantiate two-year results.

Keywords: Italian poplar cultivars, genetic variation, clone-by-site interaction

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Review of the research conducted by the Poplar Technology Center

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There are several reasons to consider poplar cultivation for developing an intensive clonal forestry in Chile. We expect that developing poplar plantings will allow many land owners to use their land for forest cultivation and provide wood to a more diversified wood processing industry in the future. The biomass production from poplar plantings and its conversion to energy is a good option to supply 5% of the energy produced by electric companies starting 2010, and 10% at 2024. Poplar hybrids have a promising role in the disposal of sewage sludge and the treatment of lecheates coming from municipal landfills in many cities of the country. There are tens of mining tailings in the north of Chile, as a byproduct of the largest copper industry in the world, that require being phytostabilized in order to avoid their collapse and the dispersion of pollutants in the air. Plantings with poplars using biosolids could solve these environmental problems.

The University of Talca has been involved in research with poplars since 1998 and the Poplar Technology Center (PTC) was created on 2003. Between 1999 and 2002, the PTC introduced more than 2500 hybrids of thirteen pedigrees, which combine five species, originated by the former Poplar Molecular Genetics Cooperative (University of Washington). A small number of commercial hybrids were also introduced from Europe. Between years 2002 and 2003, twelve nursery tests were planted in several regions of the center of Chile. Unrooted cuttings of more than 1000 hybrids were planted during winter. Initial growth (height and diameter of the stem), sprouting ability, and presence of pests and diseases were recorded annually. At the end of 2005, approximately 100 hybrids were selected from the nursery tests to determine their capability for producing high wood quality products. Cuttings from each hybrid were planted in four candidacy tests during 2006. Few European commercial clones were also included as genetic controls. The experimental design was a randomized block design, with two cuttings per hybrid in each one of ten blocks. Growth and resistance to pests and diseases has been recorded annually. On 2008, one cutting per hybrid and block was fell from each trial and wood properties were measured. The study of the genetic variation of growth and wood properties (primarily from juvenile wood), within and between trials, is currently going on. Future assessments of wood properties will investigate genetic changes from juvenile to mature wood. Between 2010 and 14, the PTC will establish new clonal trials aimed to measure genetic differences in biomass production and chemical properties among tested hybrids. Several silviculture practices will also be included in the field experimental design and their interaction with the clonal performance will be measured.

Here, we present a summary of the main results obtained from the study of nursery and candidacy trials. The research is confronting data correlated at multiple levels and the use of the traditional approach for measuring statistical differences between clones, or the interaction between clones and any sort of environmental condition, is not recommended. Therefore, we discuss the use of the Generalized Linear Mixed Model theory as a robust methodology to work with repeated measures over time, space or both, obtained from clonal trials.

Keywords: Poplar technology, Chile, Poplar growth, Pest and diseases, Nursery practices

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Euphrates Poplar's (*P. euphratica Oliv.*) natural distribution in Turkey environmental threats and conservation priorities

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The most important region for Euphrates poplar's natural distribution in Turkey is where known as Mesopotamia, where is located in between of Euphrates and Tigris-Botan Rivers. In recent researches, new natural distribution sites, other than the region between Euphrates and Tigris-Botan Rivers, have been found for Euphrates poplar.

This study investigates the current conditions and inventory of Euphrates poplar naturally distributed along the Euphrates and Tigris-Botan Rivers. The environmental factors threatening Euphrates poplar are explored in this area. Besides, the information about the researches are presented, which have been carried out to protect Euphrates poplar plantations from these environmental factors. Also the suggestions were made, which relate to the research priorities towards conservation and inbreeding of natural areas of this species.

Keywords: *Populus euphratica,* Poplar natural distriburions, Euphrates River, Tigris River

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Current and future use of poplar and the impact on breeding and selection

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Poplars are potentially the best option to increase biomass production in temperate zones, but there is a need to identify clear objectives on how to use this material in the forestry wood chain. In the past growth, disease resistance and adaptation to climatic conditions have been the major drivers for selection and breeding of poplars. The evolving wood industry and other potential end uses of biomass calls for focussing new hybrid poplar on either specific end uses or envisage multiple purpose applications. Improved material characterisation as well as high throughput methodology to support selection and breeding will allow for future strategic decisions on this. Some examples and options will be detailed in the paper.

Keywords: Biomass production, Poplar end-uses, Breeding selection

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Session 7: Poster

Poplar improvement at Wimco and its role in sustaining culture on farmland in India

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Wimco – manufacturer of safety matches in India, faced with acute shortage of matchwood during 1960's and 70's for its north Indian unit, earnestly started promoting poplar (*Populus deltoides*) farming on farmer's fields around three decades back. The company, based on its initial success on poplar farming in indo-gangetic plains near Himalayan foothills through its introduced G3 and G48 clones, initiated organized research on its culture and improvement during 1982 to provide further impetus to poplar farming. The company has maintained continuity in its poplar improvement program for the last 28 years during which more than a dozen clones, developed through multiphase screening of individuals raised from manipulated crosses and half-sib populations, are now finding favor among the growers throughout the poplar growing region. Wimco employs a simple and realistic but exhaustive screening process for full-sib and half-sib seedling populations, which are developed from selected parents each year. Poplar improvement program, for each year developed seedling population, spanned around 20 years is divided in three main screening phases viz., nursery screening phase (1-4 years), local level field trials (5-12 years) and multilocational trials (13-20 years), most of which are conducted, on farmer's fields, inside commercial grown plantations integrated with agricultural crops. The nursery level screening cull over 97% of the seedling/sapling population based on mainly susceptibility to pests and diseases, growth form, stem sprouts etc.; local level field trials screen out around 90% of the remaining population based on mainly growth, yield and tree architecture; and only exceptionally good performing individual(s) are finally picked up (based on site specific & wide scale adaptability) for operational multiplication and supply to the growers. Some local level experiments i.e., site specific field trials against localized pests and disease and tests i.e., wood suitability testing for manufacturing end products are also conducted periodically. Poplar improvement is presently concentrated on *P. deltoids*, it being well adapted in locations, which are not suitable for the indigenous poplar species. Most of the seedlings raised from crosses of *P. deltoides* made with *P. ciliata*, *P. suaveolens*, and *P. nigra* failed to tolerate the hot and dry conditions those exist in our operational area. Some hybrids developed between *P. ciliata* and *P. suaveolens*; and *P. nigra* and *P. suaveolens* are neither well adapted nor productive in these locations. Some of these are maintained to attain reproductive phase for making backcrosses with *P. deltoides*. During 2008-9, some seed were produced in crosses made between *P. gamblei* and *P. deltoides*, which, however, failed to germinate.

This multiphase and integrated poplar improvement, a major part of which is executed on farmer's fields, has proved useful with a few negative exposures like premature harvesting and trading the planting stock of good performing clones by the growers. The program has increased the availability of clones for replacing exiting clones due to excessive disease and pest susceptibility, increased poplar production and enhanced clonal biodiversity in production system. The presentation includes detailed experimental data to illustrate the ongoing poplar improvement at Wimco.

Keywords: Poplar clones, Agroforestry, Poplar seedlings, Nursery practices

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Growth and production of poplar and willow SRC plantations in China

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In the future, the renewable energy from poplar and willow biomass will play more and more roles for the energy need in the world. Two short rotation coppice(SRC) plantations of poplar and willow were established in the middle and north of China in the spring of 2006 and 2007, respectively. Five poplar clones and four willow clones were selected for the growth and biomass production under three planting densities and at most three-year harvest rotation. Annual biomass production was shown to be higher with biennial harvesting than with annual harvesting. The growth and biomass of poplar clones are more than that of willow clones in the short rotation coppice plantation in the middle of China. The annual dry matter of poplar aboveground biomass at the planting density of 10000 plants hm^2 is 8-13 t, the annual dry matter of willow aboveground biomass at the planting density of 15000 plants hm^2 is 7-11t. The growth and biomass production of willow in the north of China is much lower than that of willow in the middle of China. Due to the severe harm of willow disease and insects, it will be important to improve the willow resistance to disease and insects by the way of biotechnology.

Keywords: Poplar, Willow, Short Rotation Coppice, Biomass production

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Euphrates Poplar's (*Populus euphratica Olivier*) production and application techniques in Turkey

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The best known feature of *Populus euphratica* in its areas of occurrence is its strong liking for high temperatures, which it tolerates remarkably well, as it also does a high degree of salinity. Southeastern Anatolia, region that has the highest temperature and drought in Turkey, switched to irrigated agriculture by building a dam on Euphrates and Tigris-Botan Rivers, where Euphrates poplar naturally distributed. However, over irrigation in agriculture caused saltiness in heavy and limy soil. Existence of Euphrates poplar in these salty lands aided region by increasing soil productivity and by supplying wood material.

In this study, production possibilities of Euphrates poplar, which has decreasing natural distribution area because of threatening environmental factors, are studied. Production technique for this species is root cutting. However, it is hard to get root cutting materials because of Euphrates poplar's distributions, Euphrates poplar exist in a narrow lane along riverside. That is why alternative propagation techniques seed and vegetative elaborated on. Also, plantation of obtained seeds along Southeastern Anatolia's irrigation canals and farm sides, especially lands with rich alluvial soil, how these plantation effects region's economics and wood need of locals are studied.

Keywords: *Populus euphratica*, Production techniques

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Price Index trend of timber of fast growing plantations in Iran

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Global demand for wood products is growing rapidly due to the rising standard of living of people and increase in the countries population. Woods from existing fast growing plantations have become an important player in wood products markets. Review is showing that price index of wood products are increasing in world. Therefore, the main questions are whether, how price index of wood of fast growing plantations and the annual rates of change in price are trend? Since, the research methodologies are analytic and approximate and forecasting methods (for example; trend and regression formulation) were used. Our results with low percent error of predicted (10.07%) showed that the products price index will increasing in next years (2010:398% and 2009:370%) but rate of inflation will lower (2007 to 2016:6 % and 1997 to 2006:13%) and also market situation will better than years ago. Wood and its products freedom is affected on the price control.

Keywords: Fast growing plantations, poplar wood prices

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Effect of heat treatment of poplar plies on physical and mechanical properties of laminated veneer lumbers

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One of the original reasons to heat-treat wood is to improve the physical behavior, but the mechanical properties of wood increases through heat treatment. In this study, the mechanical properties of LVL manufactured from heat-treated and untreated veneers (control samples) were tested, and air-dry density, and swelling properties were determined. Laminated wood samples were produced from heat treated wood materials in the seven ply form (3 mm each) from poplar (*Populus nigra*) wood by using UF adhesive. Significant difference was determined ($p = 0.05$) between physical and mechanical properties for three temperatures and three durations of heat treatment for veneers used in LVL. In order to determine bending strength and modulus of elasticity values, the samples were subjected to tests perpendicular and parallel to the glue line in accordance with the ISO 16978 standard. It was observed that the lowest air-dry density was determined for LVL manufactured from treated at 180 °C for 5h. The lowest weight increment as 57.53%, the lowest radial swelling as 9.74%, the lowest tangential swelling as 4.1% and the lowest longitudinal swelling as 0.44% for LVL manufactured from heat treated veneers at 180 °C for 5 and 3 h were determined. The highest flatwise and edgewise MOR (107.67 N/mm^2 and 102.1 N/mm^2) and MOE (6190 N/mm^2 and 6017 N/mm^2) were obtained in control sample manufactured from untreated veneers.

Keywords: Poplar wood technology, Heat treatments, Laminated veneer lambers

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Effect of thermal treatment on some mechanical properties of poplar (*Populus Alba*) wood

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Thermal treatment is often used for improve the dimensional stability of wood. The objective of this study was to evaluate effects of thermal treatment on some mechanical properties of Poplar wood (*Populus Alba*). Samples were exposed to temperature levels of 110˚C, 140˚C, and 170˚C for time spans ranging from 3 to 7 h. Mechanical properties including compression strength, modulus of elasticity, modulus of rupture and shear strength of heat-treated samples were determined. Results showed that treated samples had lower mechanical properties than those of the control samples.

Keywords: Poplar wood technology, Heat treatments, *Populus alba*

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The SunGrant poplar development Program in the United States

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The SunGrant Initiative Feedstock Partnership is a national research program aimed at maximizing yield and production efficiency of energy crops in the United States. This effort is supported by the U.S. Department of Energy, Office of Biomass Programs for a five year period and is the predominant federally-funded program to improve yields and genetic diversity of *Populus* throughout the country. This research effort is structured as a unique collaboration of industry and university researchers. This program brings together both knowledge of silviculture and stand yield as well as large collections of *Populus* genetic resources. The latter is composed of extensive *ex situ* collections of *Populus deltoides*, *P. nigra*, *P. trichocarpa* and *P. maximowiczii* that together provide a platform for the future coordinated national breeding effort. These collections will be described along with a presentation of breeding plans and field testing strategies designed to increase growth, adaptability, wood quality, and disease resistance of *Populus* and its hybrids. The review will also include treatment of the nationwide field testing infrastructure and research plans to study: (1) potential yields under high-density, short rotation coppiced management, (2) fertilization, (3) wood quality and, (4) stand dynamics. The presentation will conclude with a discussion of current yields in regions of the U.S. and the gains expected through breeding and silvicultural research.

Keywords: Poplar yields, Breeding, Growth

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Development of hybrid poplar plantations in Saskatchewan: what have we learned in 15 years?

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Plantations of poplar were established in the early 1900s on a research basis and hybrid poplar has been an integral species in shelterbelt management on the prairies; however, it is in the last 15 years that hybrid poplar has been grown in plantations on agricultural land for fibre and as a means of providing additional income for farmers. Several studies have been established in Saskatchewan investigating the impact of planting stock type, fertilization and spacing on the productivity of hybrid poplar. Results from planting stock types have indicated that rooted material as either rooted cutting or plugs provide better establishment and survival than cuttings, especially in drought prone years. Fertilzation with nitrogen and phosphorus has had no impact on tree growth, and repeated fertilization for 2 years up to 150 kg ha^{-1} N has still showed no impact on growth for four different clones. Spacing trials at 1.2, 2 and 2.4 m have shown mean annual growth averaging up to 9 m^3 ha^{-1} yr^{-1} after 13 years of growth. Growth models using 3PG were developed for hybrid poplar and have predicted yields of 210 m^3 ha^{-1} yr^{-1} after 20 years. Weed management is an issue and few herbicides are registered for hybrid poplar use. Herbivory of plantations planted near forest zones is an issue and the most pressing disease to date is the septoria canker. An improving economic climate in forestry may further stimulate the development of hybrid poplar plantations close to mills but socio-economic factors related to farming must still be addressed to move this industry forward.

Keywords: Poplar hybrids, Production systems, Weed management

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Opportunities and challenges for a research partnership network on plantation of fast growing tree species : The Réseau Ligniculture Québec

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On a global scale, the plantation of fast growing trees is increasing. By 2050, FAO predicts that that 75% of the wood supply will come from such plantations which will occupy 5 to 10 % of forested lands. Fast growing tree plantation will be restricted to a small proportion of the landscape because they need good growing conditions and because of pressures to set aside land for conservation or for extensive forestry. Because of this and because of an increasing demand for wood products, fast growing forest plantations are required to be highly productive. In the province of Quebec, Canada, the Quebec government proposed to maintain 70% of the forest areas in extensive forestry, 12% in conservation and that 15% would be devoted to a more intensive forestry. Maintaining or improving wood supplies while respecting this forest zoning represents a challenge that the RLQ is contributing to help achieving. The network involves 6 universities, governmental research organisations, seven private industries and private organisations involve in forestry. The research program includes more than 60 projects. Knowledge is required at a wide breath of levels: from forest genetics, to landscape management, including subject as diverse as the preservation of soil and of biodiversity to the selection of appropriate growing stocks and the development of management techniques. The involvement of stakeholders with various interests as permitted a forum of information exchange that has proven successful. The successes and future challenges of the network will be illustrated.

Keywords: fast growing trees, management, networking

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